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Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress

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Abstract

Withania somnifera (WS) *Dunal* is classified in Ayurveda, the ancient Hindu system of medicine, as a *rasayana*, a group of plant-derived drugs reputed to promote physical and mental health, augment resistance of the body against disease and diverse adverse environmental factors, revitalise the body in debilitated conditions and increase longevity. These attributes are remarkably similar to the properties ascribed to adaptogens like *Panax ginseng* (PG) in contemporary medicine. As such, the adaptogenic activity of a standardised extract of WS roots was investigated against a rat model of chronic stress (CS). The stress procedure was mild, unpredictable footshock, administered once daily for 21 days to adult male Wistar rats. CS induced significant hyperglycaemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression. These CS induced perturbations were attenuated by WS (25 and 50 mg/kg po) and by PG (100 mg/kg po), administered 1 h before footshock for 21 days. The results indicate that WS, like PG, has significant antistress adaptogenic activity, confirming the clinical use of the plant in Ayurveda.
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Keywords: *Withania somnifera*; *Panax ginseng*; Chronic unpredictable footshock stress; Antistress adaptogenic activity

1. Introduction

Withania somnifera (WS) *Dunal* (family, Solanaceae), known as *ashwagandha* in Ayurveda, the ancient Hindu system of medicine, has been in use for more than 2500 years. The roots of the plant are categorised as rasayanas, a group of plant-derived drugs that are reputed to promote health and longevity by augmenting defence against disease, arresting the aging process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well-being (Weiner and Weiner, 1994). The properties ascribed to Ayurvedic *rasayanas* are remarkably similar to those said to be present in adaptogens, such as *Panax ginseng* (PG), which appear to increase nonspecific resistance of the body against diverse stressors and help to promote physical and mental states of the individual (Brekhman and Dardymov, 1969). While reviewing the clinical uses of WS in Ayurveda, Weiner and Weiner (1994) conclude that they include several diseases postulated to be induced by stress.

Several earlier investigations have indicated that WS has a profile of activity that is consonant with putative antistress and antioxidant activity. Thus, WS, or its major active principles, have anti-inflammatory (Begum and Sadique, 1987; Al-Hindawi et al., 1992), antitumour and radio-sensitizing actions (Devi, 1996) and suppress cyclophosphamide toxicity (Davis and Kuttan, 1998). Likewise, the active principles of WS, comprising sitoindosides VII–X and Withaferin-A, have been shown to have significant antistress activity against acute models of experimental stress (Bhattacharya et al., 1987), immunomodulatory actions (Ghosal et al., 1989), inhibition of cognitive deficits in animal models of Alzheimer's disease (Bhattacharya and Kumar, 1997; Bhattacharya et al., 1995a,b), antioxidant activity in rat brain areas (Bhattacharya et al., 1997a) and anxiolytic–antidepressant action in rats (Bhattacharya et al., 2000a). Similarly, these compounds attenuated iron-overload-induced hepatotoxicity (Bhattacharya et al., 2000b) and streptozotocin-induced hyperglycaemia (Bhattacharya et al., 1997b) in rats, which was concomitant with augmented oxidative free radical scavenging activity in the liver and pancreas, respect-

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ively. WS was also reported to have significant effects on rat brain neurotransmitter functions (Schliebs et al., 1996). A recent study, using the same experimental model of chronic stress (CS) used in this investigation, reported that there was significant depletion of oxidative free radical scavenging enzymes and an increase in lipid peroxidation in rat frontal cortex and striatum that could be reversed by subchronic administration of WS glycowithanolides (Bhattacharya et al., 2001).

Recent studies (Bhattacharya, 1998; Bhattacharya et al., 2000c; Muruganandam et al., 2002) have shown that chronic unpredictable stress, similar to the one used in the present investigation, can induce glucose intolerance, gastric ulcerations, increase in plasma corticosterone levels, behavioural depression, cognitive deficits, male sexual dysfunction and immunosuppression, associated with increased oxidative stress (Bhattacharya et al., 2001) and significant perturbations in monoamine levels in different rat brain areas (Bhattacharya et al., 2002). These physiological and biochemical effects of CS were inhibited by a polyherbal formulation containing WS (Bhattacharya et al., 2002; Muruganandam et al., 2002).

Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases, ranging from psychiatric disorders such as depression and anxiety, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis (Elliott and Eisdorfer, 1982). The benzodiazepine anxiolytics, despite having significant antistress activity against acute models of stress, have not proved effective against CS-induced adverse effects on immunity, behaviour, cognition, peptic ulcer and hypertension (Elliott and Eisdorfer, 1982). Furthermore, these drugs have adverse effects on the fetus during pregnancy, and on the neonate during lactation (Trevor and Way, 2001). PG has been widely used as an adaptogen for the therapy of stress disorders. However, it is now known to induce several adverse effects like "Ginseng abuse syndrome" on prolonged use (Dennehy and Tsourounis, 2001). Given the increasing recognition that CS, particularly when the individual is unable to cope with the stressor, may underlie the increasing incidence of stress-related physical and mental disorders, there is need for an effective antistress adaptogen. The answer probably lies in the Ayurvedic rasayanas, the most promising of which is WS (Weiner and Weiner, 1994). This formed the premise of the present study.

2. Materials and methods

2.1. Plant material

WS has several chemotypes as ascertained by systemic chemical analysis (Ghosal, 1999); the Indian chemotype, rich in withanolide glycosides (Ghosal, 1999), was used in the present study. A herbarium specimen of the plant has been preserved at the R&D Centre, Indian Herbs, Saharanpur, India. Freshly harvested 2-year-old thin roots of WS were dried, coarsely powdered and then exhaustively extracted with aqueous ethanol (1:1) at 55 ± 5 °C. The extract was concentrated under reduced pressure to remove ethanol and the aqueous concentrate was exhaustively extracted with chloroform to remove fatty material and free withanolides. The chloroform-insoluble (water-soluble) fraction was spray-dried to give a free-flowing colourless powder (yield 12–15%, wt/wt). Earlier studies (Bhattacharya et al., 2001) have shown that this fraction contains sitoindosides (= withanolide glycosides) VII–IX and withaferin A as the major bioactive entities, the relative abundance of these compounds in the extract being 28–30%. Crude PG root powder (Biological Evans, Hyderabad, India) was used as the standard drug for comparison of adaptogenic activity. WS and PG were suspended in 0.3% carboxymethyl cellulose in distilled water. WS was used at two dose levels, 25 and 50 mg/kg, and PG at the dose of 100 mg/kg. The drugs were administered orally (po) for 21 days, 1 h before footshock. Control animals received only the vehicle in the same volume used for drug administration (2.5 ml/kg po).

2.2. Pharmacological methods

Adult male Wistar strain rats (180–220 g) were used. They were housed in colony cages (four rats per cage) at an

Table 1
Effects of WS and PG on CS-induced hyperglycaemia and perturbed glucose tolerance in rats (data are mean ± S.E.M.)

Treatments * (mg/kg, po)	n	Blood glucose (mg%), Day 21	Glucose tolerance (glucose load 1 g/100 g po), blood glucose (mg%), Day 21			
			0 h	0.5 h	1 h	3 h
Vehicle (V)	8	76.8 ± 4.2	79.3 ± 4.4	178.0 ± 7.7	138.4 ± 5.6	88.6 ± 6.0
CS	12	144.2 ± 8.4[a]	139.4 ± 8.6[a]	242.0 ± 9.8[a]	196.6 ± 8.6[a]	134.2 ± 7.6[a]
WS (25) + CS	8	111.6 ± 6.2[b]	114.9 ± 5.4[b]	202.2 ± 4.9[b]	156.2 ± 4.9[b]	112.3 ± 6.2[b]
WS (50) + CS	8	98.4 ± 3.8[b]	102.4 ± 3.0[b]	182.0 ± 5.4[b]	134.7 ± 5.2[b]	106.2 ± 4.8[b]
PG (100)	8	122.4 ± 5.2[b]	119.3 ± 4.2[b]	208.4 ± 6.6[b]	169.0 ± 5.6[b]	118.4 ± 4.9[b]

WS and PG had no significant per se effect on the experimental indices used.

[a] $P < .05$ different from group V.

[b] $P < .05$ different from group CS.

* WS, PG and V were administered once daily for 21 days 1 h before CS.

ambient temperature of 25 ± 2 °C and 45–55% relative humidity, with 12-h light/dark cycle. The animals had free access to standard pellet chow and drinking water. Drug administrations and sacrifice of the animals was done between 0900 and 1400 h. Recommended guidelines for the care and use of the animals was strictly followed ("Guide for the Care and Use of Laboratory Animals," NIH publication No. 85-23, revised 1985).

2.2.1. Footshock-induced stress

The method of Conti et al. (1990) was adopted with some modifications required to add the element of unpredictability to the procedure. The rats were subjected to once daily 1-h footshock through a grid floor. The duration of each shock (2 mA) and the intervals between the shocks was randomly programmed between 3–5 and 10–110 s, respectively. Footshock stress was administered for 21 days.

2.3. Experimental methods

2.3.1. Gastric ulceration

On Day 21, rats were killed by decapitation, the stomachs were split open along the greater curvature and the number of discrete ulcers were noted by means of a magnifying glass. The severity of ulcers was scored after histological confirmation as 0 = no ulcers, 1 = changes limited to superficial layers of mucosa with no congestion, 2 = half the mucosal thickness shows necrotic changes, 3 = more than two thirds of mucosal thickness shows necrotic changes and 4 = complete destruction of mucosa with haemmorhage. Thereafter, the pooled ulcer score was calculated (Bhattacharya et al., 1987).

2.3.2. Behavioural depression

CS is known to induce endogenous depression. The following methods were used to assess depressive behaviour.

(a) *Swim stress-induced "behavioural despair."* Rats were made to swim individually in a polypropylene vessel (45 × 40 × 30 cm) with a water level of 20 cm. This ensured that the rat's feet did not touch the floor of the vessel and that it could not climb out of it. Each rat was allowed to swim for 10 min. Thereafter, during the next 5 min, the periods of total immobility, characterized by complete cessation of swimming with the head floating just above water level, was noted. This immobility period, after the initial frenzied attempts to escape, is postulated to represent behavioural despair as an experimental model of endogenous depression (Porsolt et al., 1978).

(b) *Learned "helplessness" test.* On Day 19 of the investigation, rats were subjected to footshock (60 scrambled shocks, 15 s duration, 0.8 mA, every minute) in a two-compartment jumping box (Techno) with the escape door to the unelectrified adjoining compartment closed. The exercise continued for 1 h. On Day 21, 48 h afterwards 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 intertrial interval of 30 s. During the first 3 s of the trial, a buzzer stimulus (conditioned stimulus) was presented, followed by electroshock (unconditioned stimulus) (0.8 mA) through the grid floor for the next 3 s. The avoidance response, characterized by escape to the adjoining "safe" chamber during conditioned stimulus, was noted. Failure to escape during unconditioned stimulus within 15 s was assessed as "escape failure,"

Table 2
Effects of WS and PG on CS-induced increase in plasma corticosterone levels in rats (data are mean ± S.E.M.)

Treatments * (mg/kg, po)	*n*	Plasma corticosterone (μg/dl)
Vehicle (V)	8	14.6 ± 1.9
CS	12	21.6 ± 2.1[a]
WS (25) + CS	8	15.8 ± 1.6[b]
WS (50) + CS	8	13.7 ± 2.0[b]
PG (100)	8	17.6 ± 1.4[b]

WS and PG had no significant per se effect on the experimental index used.
[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* WS, PG and V were administered once daily for 21 days 1 h before CS.

Table 3
Effects of WS and PG on CS-induced gastric ulceration in rats (data are mean ± S.E.M.)

Treatments * (mg/kg, po)	*n*	Ulcer incidence (%)	Number of ulcers	Severity of ulcers
CS	12	100	20.4 ± 3.6	34.6 ± 2.2
WS (25) + CS	10	40[a]	12.4 ± 2.6[a]	18.2 ± 3.9[a]
WS (50) + CS	10	20[a]	8.4 ± 1.6[a]	10.4 ± 1.4[a]
PG (100)	10	40[a]	11.9 ± 2.0[a]	20.2 ± 2.8[a]

[a] $P < .05$ different from group CS.
* WS, PG and V were administered once daily for 21 days 1 h before CS.

Table 4
Effects of WS and PG on CS-induced increase in swim stress-induced immobility in rats (data are mean ± S.E.M.)

Treatments * (mg/kg, po)	*n*	Duration of immobility (s)
Vehicle (V)	8	122.4 ± 4.9
WS (50) + V	6	98.4 ± 4.2[a]
PG (100) + V	6	119.4 ± 5.5
CS	12	248.4 ± 3.9[a]
WS (25) + CS	8	172.2 ± 5.9[a]
WS (50) + CS	8	134.6 ± 4.4[b]
PG (100) + CS	8	184.9 ± 6.2[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* WS, PG and V were administered once daily for 21 days 1 h before CS.

Table 5
Effects of WS and PG on CS-induced increase in "learned helplessness" in rats (data are mean ± S.E.M.)

Treatment groups * (mg/kg, po)	*n*	Escape failures (*n*)	Avoidance response (*n*)
Vehicle (V)	12	14.2 ± 0.8	5.4 ± 0.6
WS (50)	6	10.4 ± 0.6[a]	7.9 ± 0.8[a]
PG (100)	6	12.2 ± 0.6[a]	7.0 ± 0.5[a]
CS	10	26.2 ± 0.9[a]	1.2 ± 0.4[a]
WS (25) + CS	8	18.4 ± 1.1[b]	2.9 ± 0.8[b]
WS (50) + CS	8	15.4 ± 0.9[b]	4.1 ± 0.6[b]
PG (100) + CS	8	19.4 ± 0.8[b]	3.0 ± 0.8[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered once daily for 21 days in the unstressed group or 1 h before stress.

which is postulated to indicate depression (Thiebot et al., 1992).

2.3.3. Cognitive functions

The following parameters were used to assess effects on learning and memory:

(a) *Transfer latency on the elevated plus-maze.* The elevated plus-maze consisted of two opposite open arms (50 × 10 cm) crossed with two closed arms of the same dimension, with 40-cm-high walls. The arms were connected with a central square, 10 × 10 cm, to give the maze the shape of a plus sign. The maze was elevated 50 cm above the floor and kept in a dimly lit room. Rats were placed individually on one far end of an open arm and the time taken to enter one of the closed arms was recorded as transfer latency. On Day 1, before giving stress or drug treatment, the rat was given five trials at 10-min intervals. The transfer latency usually stabilized by this time. Transfer latency was again recorded on Day 21 in order to assess the retention of learning (Itoh et al., 1990).

(b) *Passive avoidance test.* 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 center of the floor. A rat was placed on the platform and allowed to step down. Twenty-four hours later, on Day 1 of the experiment, the rat was again placed on the platform and on stepping down it received foot shock (0.75 mA, 2 s) through the grid floor. The rat was given three more trials until the latency of the step down had stabilized. The test was repeated on Day 21. Retention of memory for each animal was calculated by determining the "inflexion ratio" in seconds (cutoff point 300 s) by the formula:

$$\text{Inflexion ratio} = L_{21} - L_1/L_1$$

where L_1 is the step down latency on Day 1 and L_{21} is the step down latency on Day 21, in seconds (Jaiswal and Bhattacharya, 1996).

2.3.4. Sexual behaviour

Male rats were used in this paradigm. A male rat was placed in a cage for 10 min with six oestronized (sequentially treated with oestradiol valerate 5 μg/rat, followed 48 h later by hydroxyprogesterone 1.5 mg/rat sc) female rats (120–150 g), in a dimly lit room. The parameters observed included latency (in minutes) to lick female genitals, mounts and intromissions and the number of mounts and intromissions (Morishita et al., 1993).

2.3.5. Immune function

The following methods were used:

(a) *Cell-mediated immune response.* Rats were immunized with sheep RBC (SRBC) (1×10^8 cells sc) on the dorsum, on Day 1. Rats were challenged with 1×10^8 SRBC on Day 21, injected into the left hind paw, and saline was injected into the right hind paw. The differences in the footpad thickness (left − right) was measured 24 h later by mercury displacement technique (Sen et al., 1991).

(b) *Humoral immune response.* The peritoneal macrophage (PM) and PM phagocytosis assay technique was adapted for use in rats. PM was collected by washing the peritoneal cavity with 5 ml of Hank's balanced salt solution (HBSS) + bovine serum albumin (BSA) 10%. Fluid was recovered by gentle aspiration and aliquots (2×10^6/μl) were used for phagocytic assay. PM were incubated on glass plates (22 × 22 mm) at 37 °C for 30 min in a humified chamber. The glass-adherent cells were incubated with live yeast cells of *Candida albicans* previously opsonized in autologous serum (5×10^6 in 600 μl of HBSS + autologous serum 50%) at 37 °C for 30 min (ingestion time), then washed with HBSS and again incubated for 30 min with autologous serum 50% (digestion time). The plates were

Table 6
Effects of WS and PG on CS-induced suppression of sexual behaviour in male rats (data are mean ± S.E.M.)

Treatment groups * (mg/kg, po)	*n*	Latency (min)			Number of actions	
		Licking	Mounting	Intromission	Mounting	Intromission
Vehicle (V)	10	2.8 ± 0.9	6.4 ± 0.5	8.2 ± 0.6	5.4 ± 0.8	4.2 ± 0.6
CS	12	7.6 ± 0.8[a]	12.2 ± 1.0[a]	16.4 ± 1.9[a]	1.6 ± 0.6[a]	0.9 ± 0.2[a]
WS (25) + CS	8	5.2 ± 0.6[b]	9.4 ± 0.8[b]	12.0 ± 0.8[b]	3.1 ± 0.9[b]	2.6 ± 0.6[b]
WS (50) + CS	8	3.6 ± 0.8[b]	6.9 ± 0.6[b]	9.8 ± 0.7[b]	4.4 ± 0.8[b]	3.6 ± 0.8[b]
PG (100) + CS	8	5.8 ± 0.8[b]	9.9 ± 0.8[b]	13.1 ± 0.9[b]	2.8 ± 0.6[b]	2.2 ± 0.6[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered for 21 days 1 h before CS. WS and PG had no per se effect on any of the experimental indices.

stained with Wright and observed on a light microscope. A total of 300 cells were counted and the results expressed as phagocytosis percent, phagocytosis index and digestion index (Stossel, 1974).

2.3.6. Biochemical investigations

(a) Blood glucose and glucose tolerance test (Tietz, 1970).

(b) Plasma corticosterone estimation (Varley, 1967).

Blood was collected in rats sacrificed for assessment of gastric ulceration.

2.4. Statistics

The data were expressed as mean ± S.E.M. and statistically evaluated using analysis of Duncan's multiple range test. The values were considered significantly different when the P value was less than 0.05.

3. Results

3.1. Biochemical investigations

CS adversely affected blood glucose concentration and the glucose tolerance test. The stress-induced hyperglycaemia and perturbed glucose tolerance were attenuated dose dependently by WS (25 and 50 mg/kg), and by PG (100 mg/kg) (Table 1). CS induced marked increase in plasma corticosterone levels, which was inhibited dose dependently by WS and by PG (Table 2).

3.2. Gastric ulcers

The CS induced marked increase in ulcer incidence, the number and severity of gastric ulcers. These effects were significantly attenuated dose dependently by WS (25 and 50 mg/kg) and by PG (100 mg/kg) (Table 3).

Table 7
Effects of WS and PG on CS-induced learning retention (memory) deficit in the transfer latency test in rats (data are mean ± S.E.M.)

Treatment groups * (mg/kg, po)	n	Transfer latency (s)			
		Day 0	Day 7	Day 14	Day 21
Vehicle (V)	12	56.4 ± 5.2	48.4 ± 3.9	46.4 ± 5.8	42.8 ± 5.4
WS (50)	6	48.6 ± 3.9	40.2 ± 5.2	36.4 ± 2.8[a]	28.4 ± 2.6[a]
PG (100)	6	51.4 ± 4.4	46.2 ± 3.8	44.7 ± 3.2	40.2 ± 2.9
CS	10	50.2 ± 3.0	66.4 ± 2.2[a]	74.3 ± 2.9[a]	88.4 ± 2.0[a]
WS (25) + CS	8	48.4 ± 4.2	58.4 ± 4.4	60.4 ± 3.2[b]	49.4 ± 2.8[b]
WS (50) + CS	8	52.4 ± 3.6	52.3 ± 3.8[b]	54.8 ± 2.8[b]	44.1 ± 2.2[b]
PG (100) + CS	8	49.2 ± 4.6	60.4 ± 3.8	58.2 ± 3.4[b]	54.2 ± 3.9[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered for 21 days once daily in the unstressed or 1 h before CS.

Table 8
Effects of WS and PG on CS-induced learning retention (memory) deficit in the passive avoidance test in rats (data are mean ± S.E.M.)

Treatment groups * (mg/kg, po)	n	Step-down latency on Day 21 (inflexion ratio)
Vehicle (V)	10	7.8 ± 0.9
WS (50)	6	9.2 ± 0.6[a]
PG (100)	6	8.1 ± 0.8
CS	10	2.8 ± 0.4[a]
WS (25) + CS	8	4.6 ± 0.8[a]
WS (50) + CS	8	6.2 ± 0.6[b]
PG (100) + CS	8	4.4 ± 0.8[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered once daily for 21 days in the unstressed group or 1 h before stress.

3.3. Behavioural depression

CS induced significant increase in the immobility period in the Porsolt's swim stress-induced behavioural despair test. This increase was significantly decreased by WS (25 and 50 mg/kg) and PG (100 mg/kg) (Table 4). Likewise, CS induced significant increase in escape failures, concomitant with decrease in avoidance responses. WS (25 and 50 mg/kg) and PG (100 mg/kg) treatments reversed these effects (Table 5).

3.4. Sexual behaviour

CS significantly inhibited the male sexual response indices, inducing an increase in latencies in licking female genitalia, mounting and intromission, with decrease in the number of mounts and intromissions. WS (25 and 50 mg/kg) and PG (100 mg/kg) reversed these changes (Table 6).

3.5. Cognitive functions

CS significantly and adversely affected retention of learning (memory) in the test parameters used. Thus, the transfer latency and passive avoidance tests, indicated that

Table 9
Effects of WS and PG on CS-induced suppression of peritoneal macrophage activity in rats (data are mean ± S.E.M.)

Treatment groups * (mg/kg, po)	n	% Phagocytosis	Ingestion index	Digestion index
Vehicle (V)	12	58.6 ± 9.8	3.3 ± 0.8	1.6 ± 0.9
WS (50)	6	78.2 ± 6.9[a]	4.9 ± 0.6[a]	2.2 ± 0.8
PG (100)	6	62.4 ± 7.8	3.9 ± 0.9	1.8 ± 0.9
CS	10	36.4 ± 2.6[a]	1.4 ± 0.6[a]	0.9 ± 0.2
WS (25) + CS	8	55.0 ± 3.8[b]	2.0 ± 0.8[b]	1.4 ± 0.6
WS (50) + CS	8	62.9 ± 5.8[b]	2.8 ± 0.6[b]	1.9 ± 0.4[b]
PG (100) + CS	8	54.9 ± 4.8[b]	2.2 ± 0.8[b]	1.6 ± 0.5[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered once daily for 21 days in the unstressed group or 1 h before stress.

Table 10
Effects of WS and PG on an immunologic model of pedal inflammation in rats (data are mean ± S.E.M.)

Treatments * (mg/kg, po)	*n*	Increase in paw volume (units)
Vehicle (V)	12	3.2 ± 0.8
CS	10	0.8 ± 0.2[a]
WS (25) + CS	8	1.4 ± 0.6[b]
WS (50) + CS	8	2.2 ± 0.9[b]
PG (100) + CS	8	1.9 ± 0.6[b]

[a] $P < .05$ different from group V.
[b] $P < .05$ different from group CS.
* The test drugs and the vehicle were administered once daily for 21 days 1 h before stress.

CS can attenuate retention of learned tasks, i.e., disrupted the memory engram. WS (50 mg/kg) had memory-enhancing activity per se, whereas PG was ineffective. However, both the drugs could inhibit the adverse effect of CS on retention of learned tasks (Tables 7 and 8).

3.6. Immune functions

CS had an immunosuppressive effect against both humoral and cell-mediated immune response. Thus, there was suppression of all experimental indices, namely, phagocytosis, ingestion and digestion indices, indicating inhibition of peritoneal macrophage activity. WS (50 mg/kg) but not PG (100 mg/kg) appeared to induce an increase in peritoneal macrophage activity. However, both WS (25 and 50 mg/kg) and PG (100 mg/kg) could reverse the immunosuppression activities of CS (Table 9). CS suppressed pedal edema in the immunologic model of inflammation used. This CS effect was inhibited by WS (25 and 50 mg/kg) and PG (100 mg/kg) (Table 10). An overall activity indicated of WS anti-CS effects of WS and PG showed that WS (25 and 50 mg/kg po) was approximately equi-effective as PG (100 mg/kg po) whereas WS (50 mg/kg po) exhibited a higher antistress activity.

4. Discussion

The human society has become complex and, in many ways, more demanding. However, our physiological responses designed to cope with the ever-increasing adverse situations have not evolved appreciably during the past thousand years. The failure of successful adaptation during stressful situations has resulted in stress-related illnesses that result from, or are associated with, dysregulation of the stress response (Chrousos and Gold, 1992). Various attempts have been made to counter the aversive effects of stress, ranging from yoga and meditation to antistress drugs, particularly the anxiolytic benzodiazepines (BDZ). However, despite claims to the contrary, these nonpharmacological and pharmacological methods appear to have limited utility (Mason, 1975). An answer to this perplexing problem of countering stress-induced perturbations of physiological homeostasis came from the plant kingdom. A group of plant-based drugs, the adaptogens, appears to induce a state of nonspecific resistance, enabling the organism to counteract and adapt to various stressors that can adversely affect the physiological system. The topic of adaptogens and their likely utility in stress medicine has been reviewed (Wagner et al., 1994). The general aims of adaptogen therapy appear to lie in their ability to reduce stress reactions during the alarm phase 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 (Wagner et al., 1994). Several plants have been shown to have adaptogenic activity, the most prominent being PG, which finds mention in ancient Chinese medicine (Wagner et al., 1994). Although adaptogens are not officially accepted in modern medicine, PG finds extensive use as an antistress remedy.

Ayurveda documents several plants, including WS, which are categorized as rasayanas. The properties ascribed to rasayanas in Ayurveda are remarkably similar to those of adaptogens. WS has been subjected to experimental studies using acute stress paradigms and shown to have significant stress-attenuating activity (Bhattacharya et al., 1987). In addition, WS has several properties generally associated with adaptogens (Wagner et al., 1994), including immunomodulatory (Ghosal et al., 1989), cognition-promoting (Bhattacharya et al., 1995a,b), anti-inflammatory (Al-Hindawi et al., 1992), antioxidant (Bhattacharya et al., 1997a,b, 2000b, 2001), radiosensitizing (Devi, 1996) and mood stabilizing behavioural effects (Bhattacharya et al., 2000a) in experimental animals. Limited clinical studies appear to confirm the adaptogenic antistress action of WS (Singh and Udupa, 1993).

A variety of stress situations have been employed to investigate the consequences of stress and to evaluate antistress agents, and the lack of consistency of stress protocols and their biological consequences is astounding. Acute or short-duration stress appears to have limited aversive effects on the individual since the body sets in motion an array of physiological, biochemical and endocrine responses to counter stress effects. However, chronicity and excessiveness of the stressor, and the inability of the organism to cope with the stress, appear to induce the syndromal state described by Selye in 1936 (Chrousos and Gold, 1992; Anisman et al., 1984). As such, a workable model of experimental stress has to incorporate the factors of chronicity, unpredictability and the inability to escape from the stressor. The experimental model used in this study fulfils these criteria and has been shown in earlier studies (Bhattacharya et al., 2000a,b,c, 2001) to induce significant physiological, biochemical and neurochemical perturbations that could be attenuated by putative adaptogenic agents comprising of herbal rasayana formulations (Bhattacharya et al., 2000c, 2002; Muruganandam et al., 2002) in rats.

CS induced significant hyperglycaemia and intolerance that was inhibited by WS and PG. Diabetes mellitus is a

well-accepted consequence of continued inescapable stress, given the close interrelations between stress and the endocrine and autonomic nervous systems (Chrousos and Gold, 1992). Neither WS or PG have antidiabetic activity but were effective in CS-induced perturbation of glucose homeostasis. Likewise, both WS and PG inhibited the significant increase in plasma corticosterone levels induced by CS. Activation of the hypothalamo–pituitary–adrenocortical axis during stress is a well-known phenomenon (Chrousos and Gold, 1992), and increase in plasma corticosterone in rodents and cortisol in humans have been utilized as markers of stress and its intensity (Solomon et al., 1984). However, PG ginsenosides have been shown to elevate serum levels of ACTH and corticosterone in rats, which could be blocked by dexamethasone pretreatment (Wagner et al., 1994). The so-called ginseng abuse syndrome was proposed to be a consequence of elevated corticoid levels (Wagner et al., 1994). These findings are contrary to the present observation and may be due to the use of crude PG extract rather than pure ginsenosides used by earlier investigators (Wagner et al., 1994). However, our findings are in conformity with earlier reports (Bhattacharya et al., 2000c; Muruganandam et al., 2002).

Similarly, production of gastroduodenal ulcerations appear to be an inevitable consequence of stress, the intensity of the disease depending upon the duration of stress situation and appears to involve stress-induced autonomic and neuroendocrine system activation. As a consequence, both the offensive and defensive factors involved in the etiopathogenesis of peptic ulceration are affected (Bhattacharya et al., 1987). WS has earlier been reported to attenuate acute stress-induced gastric ulcerations in rats by affecting a reduction in acid–pepsin levels and an increase in gastric mucin activity (Bhattacharya et al., 1987). PG has been reported to attenuate stress-induced gastric ulcerations (Bhattacharya et al., 2000c; Muruganandam et al., 2002).

Generalized stress, particularly if continued in nature, is known to induce melancholic depression (Gold et al., 1988). It has been suggested that the symptoms of endogenous depression represent tachyphylaxis of the mesocortical system to chronic activation of the stress system (Chrousos and Gold, 1992). The experimental paradigms used in this investigation to induce behavioural states akin to clinical depression have been subjected to extensive scrutiny and validated for inducing behavioural despair and for the evaluation of putative antidepressants (Thiebot et al., 1992). PG has earlier been found to induce significant antidepressant effect on the two test models used in this study (Bhattacharya et al., 2000c; Muruganandam et al., 2002). Ayurveda postulates that rasayanas, including WS have the ability to restore mental health by stabilizing perturbed mood of the individual (Singh and Udupa, 1993). Our results tend to support this concept. It is of interest to note that WS has been reported to have both anxiolytic and antidepressant activity in rats, supporting the contention that rasayanas are effective mood regulators (Singh and Udupa, 1993).

Sustained stress is known to induce cognitive dysfunction (Chrousos and Gold, 1992). Experimental stress was reported to have adverse effects on the memory engram in rats. The learning acquisition was minimally affected, the major action being disruption of retention of learned tasks (Bhattacharya, 1993). It has been postulated that cognitive dysfunction and behavioural depression, induced by stress, may be induced by similar neurochemical mechanisms, including depletion of monoamines by sustained stress (Anisman et al., 1984; Bhattacharya et al., 2002) A subgroup of Ayurvedic rasayanas, known as *medhyarasayanas*, which includes WS, is used to promote intellect and memory. The cognition-promoting effect of medhyarasayanas is best seen in children with memory deficits, or when memory is compromised following head injury, prolonged illness and in old age (Singh and Udupa, 1993). Earlier studies have shown that WS (Bhattacharya et al., 1995a), or WS-containing Ayurvedic formulations (Bhattacharya et al., 1995b, 1997a,b), can reverse cognitive deficits in rat models of Alzheimer's disease, induced by central administration of colchicine or ibotenic acid. The memory-facilitating action was accompanied by reversal of the decrease in frontal cortical and hippocampal cholinergic activity. WS-containing polyherbal formulations were also shown to attenuate memory deficits induced by prenatal undernutrition, postnatal environmental impoverishment and hypoxia in rats (Bhattacharya, 1994), all of which can be considered different varieties of stress. In the present study, WS induced per se improvement in retention of the learned task in both the experimental parameters used, the effects being discernible after 2 weeks of treatment in the transfer latency test. WS and WS-containing formulations have earlier been shown to have a delayed memory-augmenting effect (Bhattacharya, 1994; Bhattacharya et al., 1995a,b, 1997a,b). On the contrary, PG did not have a per se nootropic effect. However, both WS and PG were effective in reversing the memory deficit induced by CS in both the transfer latency and the passive avoidance tests.

There has long been an interest in the role of stress in production of human diseased states, at least some of them being linked to suppression of the immune response (Solomon et al., 1984). Both humoral and cell-mediated immune responses are affected, indicating that stress may have an adverse effect on normal immune surveillance (Solomon et al., 1984). In the present study, CS was shown to suppress experimental paradigms of humoral and cell-mediated immune responses, as was reported earlier using other experimental parameters (Bhattacharya et al., 2000c; Muruganandam et al., 2002). Plant adaptogens, including PG, have been shown to have immunomodulatory action, improving nonspecific immune reactivity (Wagner et al., 1994). Ayurveda records that rasayanas have the ability of protecting the body against external factors that induce disease. This implied resistance against disease may repres-

ent the modern concept of immunity (Singh and Udupa, 1993). WS has been earlier shown to inhibit acute-stress-induced immunosuppression in rats (Ghosal et al., 1989).

CS induced significant suppression of male sexual function in rats, which was reversed by both WS and PG. This effect has been reported earlier with PG- and WS-containing Ayurvedic formulations, using the same paradigm of CS in rats (Bhattacharya et al., 2000c; Muruganandam et al., 2002). Stress has been found to profoundly inhibit reproductive functions by affecting various components of the hypothalamic–pituitary–adrenal axis (Chrousos and Gold, 1992). Decrease in testosterone levels during stress, with a poststress recovery to normal levels, has been constantly noted (Solomon et al., 1984). WS-containing formulations have often been promoted by some Ayurvedic pharmaceutical concerns as aphrodisiacs, although Ayurveda makes a clear distinction between aphrodisiacs that promote normal male sexual function, like the plant *Mucuna pruriens*, and rasayanas, which are able to restore male sexual function when it is perturbed due to emotional and physical factors like prolonged illness (Singh and Udupa, 1993). The present study also indicates that neither WS nor PG can influence normal male sexual behaviour but can normalise stress-induced male sexual dysfunction.

It has been hypothesized that a large number of human illnesses reflect the consequences of the generalized stress response brought about by failure of Selye's postulated resistance phase and failure of adaptation to stress, leading to the stage of exhaustion resulting in a variety of diseases. It was predicted that pharmacological agents capable of altering the central apparatus, namely, the hypothalamo–pituitary–adrenal axis, which governs the stress response will be useful in the prevention or in the treatment of many of these illnesses (Chrousos and Gold, 1992). Adaptogens may be effective antistress agents because they appear to prolong Selye's propounded second phase of the "general adaptation syndrome," the stage of resistance to stress, and prevent the final and third phase of the state of exhaustion. The major criterion delineating the function of an adaptogen is its ability to enhance resistance against prolonged inescapable stressors of diverse nature, indicating nonspecific activity. In addition, an adaptogen should have a normalizing influence independent of the nature of the pathological state (Wagner et al., 1994). The present study supports this contention because CS of an unpredictable and inescapable nature was found to induce a wide range of physiological malfunctions, including adverse effects on glucose metabolism, cognitive, immune and male sexual functions, and behavioural, all of which were inhibited by PG, a known adaptogen (Wagner et al., 1994) and by WS, a putative adaptogen belonging to the class of Ayurvedic rasayanas. The similarity in the pharmacological actions of PG and WS prompted the nomenclature of the latter as Indian ginseng (Wagner et al., 1994; Weiner and Weiner, 1994). However, WS has a distinct clinical advantage over PG. The latter is known to induce the so-called ginseng abuse syndrome on prolonged use (Wagner et al., 1994), which has not been reported with WS or with the other rasayanas (Singh and Udupa, 1993). It appears that these rasayanas, including WS, may provide a safer alternative to PG as adaptogen for the therapy of stress-related clinical disorders.

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