

# Antistress activity of *Argyrea speciosa* roots in experimental animals

Nikunj B. Patel, Varsha J. Galani, Bharatkumar G. Patel

Department of Pharmacology, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat, India

## ABSTRACT

The antistress effect of a seven-day treatment (100 and 200 mg / kg, p.o.) of the hydroalcoholic extract of *Argyrea speciosa* root (ASE) was evaluated by using the swimming endurance test, acetic acid-induced writhing test, pentylentetrazole-induced convulsion test, anoxic tolerance test, cold-restraint, stress-induced gastric ulcers, aspirin-induced ulcers, and biochemical, and histopathological changes in the cold-restraint stress test. The immunomodulatory activity was also evaluated for the same doses, and treatment of ASE was done using the hemagglutination test. Both the doses of ASE showed antistress activity in all the tested models. The ASE-treated animals showed a decrease in immobility time and an increase in anoxic tolerance time in swimming endurance and the anoxic tolerance tests, respectively. The effect of glacial acetic acid and pentylentetrazole were also reduced by decreasing the number of writhing responses and increasing the onset of convulsions, respectively. In the cold restrained stress and aspirin-induced gastric ulcer models, ASE showed a significant reduction in the ulcer index. Pretreatment with ASE significantly ameliorated the cold stress-induced variations in biochemical levels such as increased plasma cholesterol, triglyceride, glucose, total protein, and cortisol. ASE was also effective in preventing the pathological changes in the adrenal gland, due to cold restrained stress, in rats. In mice immunized with sheep red blood cells, the treatment groups subjected to restraint stress prevented the humoral immune response to the antigen. The immunostimulating activity of the ASE was indicated by an increase in the antibody titer in mice pre-immunized with sheep red blood cells and subjected to restraint stress. The findings of the present investigations indicate that the ASE has significant antistress activity, which may be due to the immunostimulating property and increased resistance, nonspecifically, against all experimental stress conditions.

**Key words:** Antistress activity, *Argyrea speciosa*, anoxic tolerance test, cold restraint stress, swimming endurance test

## INTRODUCTION

Stress is the nonspecific response of the body to any demand made upon it.<sup>[1]</sup> Normally stress-induced changes are self-limiting and adaptive until and unless events that override the 'threshold' limits become irreversible and pathological.<sup>[2]</sup> Stress is involved in the pathogenesis of

a variety of diseases including hypertension, peptic ulcer, immunosuppression, reproductive dysfunction, and behavioral disorders.<sup>[3]</sup>

According to the World Health Organization (WHO), mental well-being is a part of health. The WHO estimates that about 80% of the population living in developing countries relies almost exclusively on traditional medicine for their primary healthcare needs. India has vast ethnobotanical knowledge, since ancient times. Origin of all such knowledge in India is from the great tradition of Ayurveda, which is a living tradition of practice even today. One such plant, *Argyrea speciosa* (Linn.f.) sweet 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, revitalize the body in debilitated conditions, and increase longevity. *Argyrea speciosa*, commonly known as the elephant creeper, is found throughout India except in the dry western regions, up to a 1000-foot elevation. It is cultivated in the gardens as an ornamental plant for

### Address for correspondence:

Dr. Varsha J. Galani, Department of Pharmacology, A. R. College of Pharmacy and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar – 388 120, Gujarat, India. E-mail: vrp173@yahoo.com

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its green leaves and beautiful rose purple flowers. The plant is extensively used in the indigenous system of medicine. The roots of this plant have been regarded as tonic, aphrodisiac, and bitter. The roots of this plant are also used for rheumatism, gonorrhoea, chronic ulcer, and diseases of the nervous system.<sup>[4]</sup> Previous phytochemical studies have revealed the presence of lipids, flavanoids, triterpenes, steroids, phenylpropanoids, and coumarins in the plant. The major constituents isolated from the plant are friedelin, ergine, agroclavine, penniclavine, chanclavine, ergometrine, quercetin, kaempferol, scopoletin, and hexadecanyl p-hydroxycinnamate. Several investigations have proposed that this plant possesses nootropic, aphrodisiac, immunomodulatory, hepatoprotective, antioxidant, anti-inflammatory, analgesic, antihyperglycemic, anti-diarrheal, antimicrobial, antiviral, nematocidal, antiulcer, anticonvulsant, and central nervous system-depressant activities.<sup>[5]</sup> Roots of *A. speciosa* are also used in antistress polyherbal formulation such as Geriforte / Stresscare. Hence, the present study is designed to evaluate the antistress effect of hydroalcoholic extract of *A. speciosa* roots using various experimental models in rodents.

## MATERIALS AND METHODS

### Plant material and preparation of extract

The roots of *Argyrea speciosa* were collected from the campus of our institute and authenticated by Dr. G. C. Jadeja, Professor and Head, Department of Agriculture Botany, Anand Agricultural University, Anand, Gujarat, India. A specimen of the plant was kept in the herbarium of our institute (Voucher No. ARGH8). The plant material was completely dried under the shade and powdered. The powdered material was extracted exhaustively with 50% ethanol, by maceration for two days, at room temperature, with occasional shaking. The crude (hydroalcoholic) extract was filtered and dried under reduced pressure at 40°C (yield: 5.7 % w / w). Freshly prepared aqueous solution of the dried extract of *A. speciosa* roots (ASE), in a suitable dilution, was administered to the animals in the treatment groups.

### Preliminary phytochemical screening

The hydroalcoholic extract of the *Argyrea speciosa* roots was tested for the presence of carbohydrates, proteins, alkaloids, flavonoids, glycosides, saponins, tannins, and essential oils using the standard procedures.<sup>[6]</sup>

### Animals

Healthy adult Swiss albino mice of either sex (25 – 30 g) were used for the swimming endurance test, writhing test, immunological assay, pentylene-tetrazol-induced convulsions, and anoxic tolerance test. Healthy

Wistar albino rats of either sex (250 – 300 g) were used for the cold restrained stress test and aspirin-induced ulceration model. The animals were housed under standard conditions, with a commercial pellet diet and had free access to water. The animals were acclimatized to the laboratory environment for one hour before the experiments. The animals were randomly distributed into groups of six animals each. All experiments were conducted during the light period (08.00 – 16.00 hours). All the protocols were approved (CPCSEA/IAEC/ARCP/09-10/03) by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Drugs

Diazepam (Ranbaxy, India) was used as the standard drug (positive control) in various stress models. Pentylene-tetrazol (Sigma, USA) was used to produce convulsions in mice. Aspirin (Cadila Healthcare, India) was used to produce ulcers in the experimental animals. All the chemicals and reagents used for the biochemical studies were commercial grade analytical reagents.

### Swimming endurance test

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. The swimming test was carried out on the seventh day, after one hour of oral and 30 minutes of intraperitoneal administration of the drug, using a polypropylene vessel (45 × 40 × 30 cm) with a water level of 20 cm, and the immobility time was recorded for 30 minutes.<sup>[7]</sup>

### Anoxic tolerance test

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. On the seventh day, the mice were subjected to anoxic stress by keeping them in a confined airtight 250 ml glass jar. The time taken for the mice to exhibit the first clonic convulsion was taken as the end point. The animals were removed immediately from the vessel for recovery and resuscitated if needed.<sup>[8]</sup>

### Writhing test

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with

ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. At the end of the seventh day, writhing was induced one hour after oral and 30 minutes after intraperitoneal administration of the drug by giving 0.1 ml of 0.4% (0.4 ml / 20 mg, i.p.) glacial acetic acid. The number of writhing responses produced were recorded for 20 minutes.<sup>[7]</sup>

### Pentylentetrazol-induced convulsions

The mice were randomly divided into four groups of six animals each. The treatment groups were pretreated with hydroalcoholic extract of *A. speciosa* roots (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. At the end of the seventh day, all the animals were injected with pentylentetrazol (80 mg / kg, i.p.), one hour after oral and 30 minutes after intraperitoneal administration of the drug. The onset of convulsions, duration of convulsions, and mortality protection were recorded.<sup>[9]</sup>

### Cold restraint stress test

The rats were randomly divided into five groups of six animals each. The treatment groups were pretreated with ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group and stress control groups were pretreated with normal saline (1 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. A cold restraint stress was given to all the rats, except the control rats, by tying the limbs for two hours at 4°C on the seventh day of treatment.<sup>[10]</sup> After two hours, the animals were sacrificed by decapitation and the blood was collected in EDTA-coated propylene tubes. The blood samples were centrifuged (3000 rpm for 20 minutes at 4°C) and the plasma were separated out and stored at 20°C for biochemical and hormonal assays. These samples were used to analyze cholesterol,<sup>[11]</sup> triglyceride,<sup>[12]</sup> glucose,<sup>[13]</sup> total proteins,<sup>[14]</sup> and cortisol.<sup>[15]</sup> The adrenal glands were removed from the control, stress control, and ASE (100 mg / kg, 200 mg / kg, p.o.)-treated animals and preserved in neutral buffered 10% formalin. Microscopic sections of these adrenal glands were prepared and observed for histological changes.<sup>[14]</sup> The stomach of each stress control and ASE (100 mg / kg, 200 mg / kg, p.o.)-treated animal was dissected and cut open along the greater curvature for scoring the incidence of ulcers. The ulcer index of the glandular mucosa was calculated according to the method of Ganguly and Bhatnagar, (1973).<sup>[16]</sup>

### Aspirin-induced ulceration

The rats were randomly divided into three groups of six

animals each. The treatment groups were pretreated with ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The control group was pretreated with normal saline (1 ml / kg, p.o.) for seven days. On the seventh day, one hour after the treatment, aspirin was administered (200 mg / kg, p.o.).<sup>[17]</sup> After four hours, the animals were scarified and the stomach of each animal was removed for ulcer index determination.<sup>[16]</sup>

### Immunological assay

The mice were randomly divided into five groups of six animals each. All the mice were immunized with sheep red blood cell (SRBC), ( $0.5 \times 10^9$  cells / ml / 100 g, i.p.) on day zero. The treatment groups were then pretreated with ASE (100 mg / kg, 200 mg / kg, p.o.) for seven days. The normal control group and stress control group were pretreated with normal saline (10 ml / kg, p.o.), while the positive control group received diazepam (2 mg / kg, i.p.) for seven days. On the seventh day, after initial immunization with SRBC, the mice were subjected to restraint stress for two hours. After induction of stress, blood was collected and the serum assayed for hemagglutination (highest dilution giving hemagglutination was taken as the antibody titer).<sup>[7]</sup>

### Statistical analysis

All values are expressed as mean  $\pm$  SEM. Comparison between the stress control and drug-treated groups were made by one way ANOVA followed by he tukey test, *P* values of less than 0.05 and 0.001 were considered to be significant.

## RESULTS

### Swimming endurance test

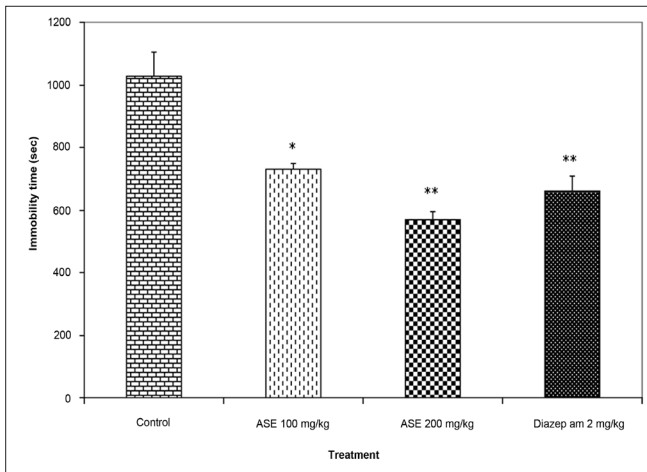
As shown in Figure 1, seven days pretreatment with ASE (100 mg / kg and 200 mg / kg) significantly reduced the immobility time of mice as compared to the control group. Similarly, positive control diazepam (2 mg / kg, i.p.) also significantly reduced the immobility time of animals.

### Anoxic tolerance test

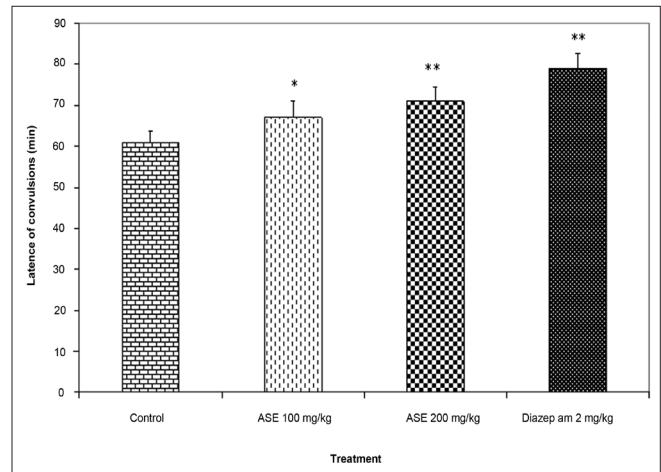
The time taken for the mice to exhibit clonic convulsions was taken as the end point in the anoxic tolerance test. Seven days pretreatment with ASE (100 mg / kg and 200 mg / kg) significantly increased the time taken for clonic convulsions as compared to the control animals. Similarly, diazepam treatment also produced significant delay in clonic convulsions [Figure 2].

### Writhing test

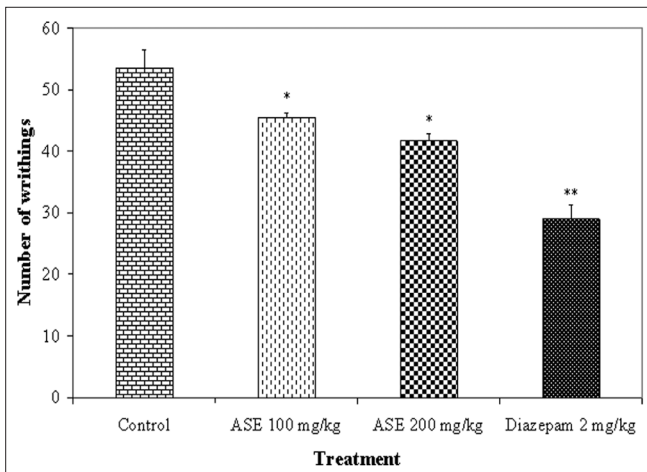
As shown in Figure 3, glacial acetic acid-induced writhing responses were significantly and dose dependently decreased by the seven-day pretreatment with ASE (100 mg / kg and 200 mg / kg). Similarly, positive control diazepam (2 mg / kg, i.p.)



**Figure 1:** Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on immobility time in the swimming endurance test; Expressed as mean  $\pm$  SEM ( $n = 6$ ), one way ANOVA followed by Tukey test; \*  $P < 0.05$ , \*\*  $P < 0.001$  when compared with the control group



**Figure 2:** Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on the latency of convulsion in the anoxic tolerance test; Expressed as mean  $\pm$  SEM ( $n = 6$ ), one way ANOVA followed by Tukey test; \*  $P < 0.05$ , \*\*  $P < 0.001$  when compared with control group



**Figure 3:** Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on the number of writhings in the writhing test; Expressed as mean  $\pm$  SEM ( $n = 6$ ), one way ANOVA followed by Tukey test; \*  $P < 0.05$ , \*\*  $P < 0.001$  when compared with control group

also significantly decreased the number of writhings in mice.

### Pentylenetetrazol-induced convulsions

As shown in Table 1, seven days of pretreatment with ASE (100 mg / kg and 200 mg / kg) increased the onset of convulsion, decreased the duration of action, and decreased the mortality rate, as compared to the control animals. The diazepam (2 mg / kg, i.p.)-treated mice did not have any convulsive episodes or mortality. When treated with pentylenetetrazole, it presented 100% protection of animals as compared to the control.

### Antilcerogenic activity

Results of cold-restraint, stress-induced ulcers and aspirin-induced ulcers are shown in Table 2. The cold restraint stress and aspirin treatment significantly increased the

ulcer incidence and the ulcer index of the glandular mucosa of the rat stomach, in stress control animals. These effects were significantly and dose dependently attenuated by the seven-day pretreatment with ASE (100 and 200 mg / kg).

### Biochemical investigations

Cold-restraint stress adversely affected the blood concentration of various biochemical parameters. The results of the biochemical parameters are summarized in Table 3. The induction of cold-restraint stress led to a rise in serum cholesterol, triglycerides, glucose, total proteins, and cortisol levels in stress control animals. Seven days pretreatment with ASE (100 mg / kg and 200 mg / kg) reduced all the biochemical parameters significantly and dose dependently, as compared to the stress control animals.

### Adrenal gland histopathology

Normal pattern of cords and cells in the zona fasciculata and zona reticularis were observed in the histological section of the adrenal gland of control rats [Figure 4a]. Normal compact arrangement of cells in the medulla of the adrenal gland was also observed in the control animals [Figure 4b]. Distortion of cords and variable-sized vacuolations in the zona fasciculata and reticularis of the adrenal gland were observed in the cold-restraint, stress-treated animals [Figure 5a]. Vacuolations in the cytoplasm of the medullary cells of the adrenal gland of stress animals were also observed [Figure 5b]. Seven days of pretreatment with ASE (200 mg / kg) showed an antistress effect on the adrenal gland, as indicated by the marked reduction of the distortion of cords and vacuolations in the cortex of the adrenal glands. Seven days of pretreatment with ASE (200 mg / kg) also reverted

**Table 1: Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on pentylenetetrazole-induced convulsions**

Treatment	Dose (mg / kg)	Onset of convulsions (seconds)	Duration of convulsions (seconds)	Mortality protection
Control	-	69.50 ± 3.06	244.67 ± 9.75	2 / 6
ASE	100	81.33 ± 3.18*	182.50 ± 17.53*	3 / 6
ASE	200	89.67 ± 2.5**	175.83 ± 13.27*	5 / 6
Diazepam	2	Absent	Absent	6 / 6

Expressed as mean ± SEM (n = 6). One way ANOVA followed by Tukey test; \* P < 0.05, \*\* P < 0.001 when compared with the control group.

**Table 2: Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on the antiulcerogenic activity**

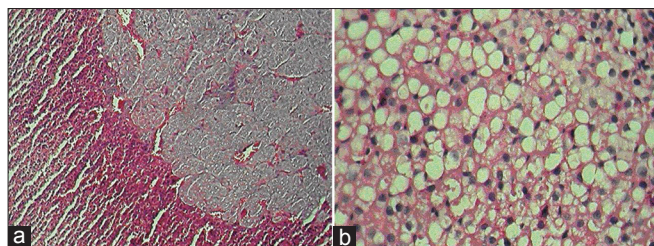
Treatment	Cold-restraint stress-induced ulcers			Aspirin-induced ulcers		
	Ulcer incidence (%)	Ulcer index	Protection	Ulcer incidence (%)	Ulcer index	Protection
Stress control	100	0.67 ± 0.05	-	100	0.41 ± 0.10	-
ASE (100 mg / kg)	66.67	0.48 ± 0.10*	28.36	50	0.32 ± 0.08*	21.95
ASE (200 mg / kg)	33.33	0.22 ± 0.05**	67.16	33.33	0.24 ± 0.06**	41.46

Expressed as mean ± SEM (n = 6). One way ANOVA followed by Tukey test; \* P < 0.05, \*\* P < 0.001 when compared with the control group.

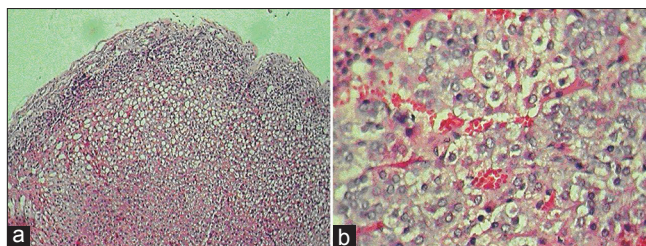
**Table 3: Effect of hydroalcoholic extract of *A. speciosa* root (ASE) on biochemical parameters**

Treatment	Cholesterol (mg / dl)	Triglyceride (mg / dl)	Glucose (mg / dl)	Total proteins (g / dl)	Cortisol (µg / dl)
Control	103.3 ± 6.49	94.98 ± 8.65	95.38 ± 7.18	4.12 ± 0.85	4.43 ± 1.14
Stress control	197.2 ± 15.4**	189.8 ± 16.2**	135.5 ± 8.5**	6.78 ± 0.31**	11.1 ± 1.1**
ASE (100 mg / kg)	102.2 ± 11.6##	103.5 ± 8.4##	100.5 ± 8.2##	4.88 ± 0.3##	3.97 ± 1.44##
ASE (200 mg / kg)	76.95 ± 6.00##	85.67 ± 6.08##	95.03 ± 6.33##	4.42 ± 0.27#	3.2 ± 0.76##
Diazepam (2 mg / kg)	69.8 ± 5.20##	75.55 ± 4.95##	90.82 ± 5.72##	4.37 ± 0.93##	2.8 ± 0.25##

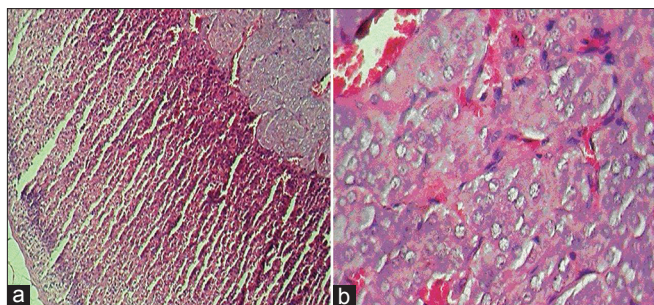
Expressed as mean ± SEM (n = 6). One way ANOVA followed by Tukey test; \*\* P < 0.001 when compared with control group.; # P < 0.05, ## P < 0.001 when compared with the stress control group.



**Figure 4:** Histopathology sections of the adrenal gland of the control group; (a) Adrenal gland section (zona fasciculata and zona reticularis) of control rat; (b) Adrenal gland section (medulla) of control rat



**Figure 5:** Histopathology sections of the adrenal gland of the stress control group. (a) Adrenal gland section (zona fasciculata and zona reticularis) of stress control rat. (b) Adrenal gland section (medulla) of stress control rat

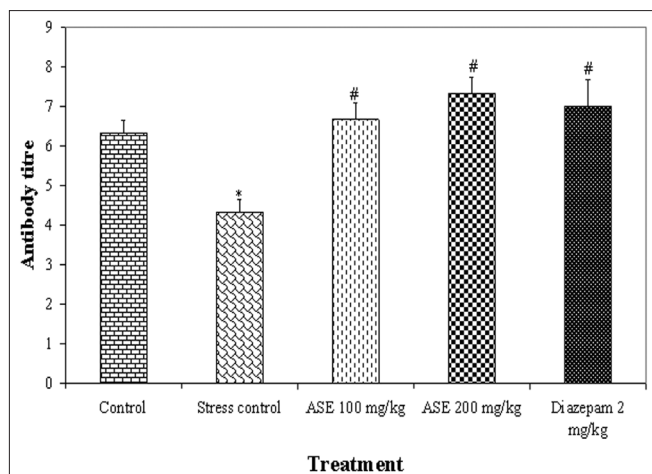


**Figure 6:** Histopathology sections of the adrenal gland of the treatment group (200 mg / kg); (a) Adrenal gland section (zona fasciculata and zona reticularis) of treated rat; (b) Adrenal gland section (medulla) of treated rat

the normal compact arrangement of cells in the medulla [Figure 6b].

**Immunological assay**

Stress control animals had an immunosuppressive effect, as indicated by a decrease in antibody titers. Seven days of pretreatment with ASE (100 mg / kg and 200 mg / kg) showed significant and dose-dependent inhibition of stress-induced reduction in antibody titers. Similarly, diazepam (2 mg / kg, i.p.)-treated animals also had significant protection against stress-induced reduction of antibody titers. [Figure 7].



**Figure 7:** Effect of hydroalcoholic extract of the *A. speciosa* root (ASE) on the immunological assay; Expressed as mean  $\pm$  SEM ( $n = 6$ ), one way ANOVA followed by Tukey test; \*  $P < 0.05$  when compared with control group; #  $P < 0.05$  when compared with stress control group

## DISCUSSION

In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models.<sup>[18]</sup> Since the introduction of adaptogens, several plants that had once been used as tonics have been investigated in Ayurvedic medicine, due to their adaptogenic and rejuvenating properties.<sup>[19]</sup> In the present study, the antistress activity of the hydroalcoholic extract of *A. speciosa* roots (100 mg / kg, 200 mg / kg) has been evaluated using various acute stress experimental models. The swimming endurance test and anoxic tolerance test are known physical stress models for the evaluation of antistress activity. In the swimming endurance test, the mice are forced to swim in a restricted space from which they cannot escape. This induces a characteristic behavior of immobility. It has been well-demonstrated that drugs with antistress activity increase swimming endurance and latency of post-anoxic convulsions.<sup>[20-22]</sup> Results of the swimming endurance test and anoxic tolerance test indicate clearly that the hydroalcoholic extract of *A. speciosa* roots (100 mg / kg, 200 mg / kg) have the properties, whereby, they increase the physical endurance as well as the overall performance in mice. Glacial acetic acid-induced writhings and a chemical-induced stress test caused hyperalgesic effects on the pain pathway.<sup>[23]</sup> The results of glacial acetic acid-induced writhings indicate that the hydroalcoholic extract of *A. speciosa* root (100 mg / kg and 200 mg / kg) can play a significant role in the inhibition of pain and inflammatory processes. Pentylentetrazol has an inhibitory function of the GABAergic system in the brain.<sup>[24]</sup> Gamma amino butyric acid *Gabapentin* (GABA) plays a major role in the central integration of the hypothalamic-pituitary-

adrenocortical (HPA) stress responses. GABAergic neurons in the bed nucleus of the stria terminalis, preoptic area, and hypothalamus can directly inhibit paraventricular nuclei outflow, and thereby, reduce adrenocorticotrophic hormone secretion. Thus, GABA produces a marked inhibitory effect on HPA axis activity.<sup>[25,26]</sup> Stress also causes a rapid decrease in GABA receptor binding in the central nervous system.<sup>[27]</sup> The antistress activity of ASE against pentylentetrazol is observed by increasing the latency for the onset of convulsions, mortality protection, and decreasing the duration of convulsions. The inability of the ASE to completely abolish the convulsions may be due to the low dose used for the protection of the animals against pentylentetrazol.

Stress situations are known to produce gastric ulcer disease, which has been reported to have multifactorial pathophysiology.<sup>[28]</sup> Exposure to acute stress results in adrenal hypertrophy and gastric ulceration, indicating the active involvement of the hypothalamic-pituitary-adrenal (HPA) axis, which is highly responsive to stress.<sup>[29,30]</sup> The hyperactivation of the paraventricular nuclei of the hypothalamus during stress, causes a decrease in mucosal blood flow and hypercontractility, through the descending projections that induce the pathogenesis of gastric ulcers.<sup>[31]</sup> The cold restraint gastric ulcer model is used to evaluate agents that can inhibit the development of gastric ulcers by their anti-ulcer and / or anti-stress effect.<sup>[32]</sup> It has been recorded that Aspirin causes mucosal damage via several factors such as inhibiting prostaglandin synthesis, enhancing acid secretion, increasing back diffusion of  $H^+$  ions, decreasing mucin secretion, and breaking of the mucosal barrier.<sup>[33]</sup> *A. speciosa* is reported to possess gastric anti-ulcer effect due to its cytoprotective action.<sup>[5]</sup> Hence, it is possible that the reduction of gastric ulcers by ASE may be due to its anti-stress as well as cytoprotective effect. Chemical stress (Aspirin 200 mg / kg, p.o.)-induced ulcer was significantly reduced by both doses of *A. speciosa*.

In response to stress, the Adrenocorticotrophic hormone (ACTH) is released, which acts on the adrenal cortex to stimulate the synthesis and release of cortisol.<sup>[7]</sup> Increase in plasma cortisol influences the mobilization of stored fat and carbohydrate reserves,<sup>[34]</sup> which in turn increases the blood glucose level, total proteins, cholesterol, and triglycerides. Pretreatment with the ASE significantly ameliorated the stress-induced variations in these biochemical levels.

There has long been an interest in the role of stress in the production of human diseased states, as at least some of them are linked to suppression of the immune response. Both humoral and cell-mediated immune responses are affected, indicating that stress may have an adverse effect on normal immune surveillance.<sup>[35]</sup> *A. speciosa* has been

shown to have an immunomodulatory action, improving nonspecific immune reactivity.<sup>[5]</sup> Ayurveda records that the *rasayanans* have the ability to protect the body against external factors that induce disease. This implies that resistance against disease may represent the modern concept of immunity.<sup>[36]</sup> The hydroalcoholic extract of the *A. speciosa* root prevents the effect of various types of stress non-specifically by various mechanisms and by increasing resistance against various types of stress. Diazepam is reported to possess a nonspecific antistress activity in experimental animals.<sup>[7]</sup>

The efficacy of most herbal remedies is attributed to various active principles, in combination. Results of phytochemical screening showed the presence of lipids, flavanoids, triterpenes, steroids, phenylpropanoids, and coumarins in the root. It is therefore probable that the components that are present in abundance in the extracts might contribute in part to the observed antistress effect.

In conclusion, our results provide evidence that the seven-day treatment with the hydroalcoholic extract of *A. speciosa* roots shows antistress (adaptogenic) activity in various acute stress models. The observed antistress activity may be due to the prevention of desensitization of both the peripheral and central components of the hypothalamic-pituitary-adrenal axis (HPA) and due to the non-specifically increased resistance produced by the *A. speciosa* root extract. This study provides significant evidence of the medicinal and traditional uses of *A. speciosa* root in stress disorders.

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