Indian Journal of Experimental Biology
Vol. 48, January 2010, pp. 53-60

Adaptogenic and *in vitro* antioxidant activity of flavanoids and other fractions of *Argyreia speciosa* (Burm.f) Boj. in acute and chronic stress paradigms in rodents

P V Habbu [1*], K M Mahadevan [2], P V Kulkarni [3], C Daulatsingh [3], V P Veerapur [4] & R A Shastry [1]

[1*]Post graduate Department, Division of Pharmacognosy, S.E.T's College of Pharmacy, Dharwad 580 002, India
[2]Post graduate Department in Chemical Sciences and Research, Kuvempu University, Shankarghatta, Shimoga, India
[3]Post graduate Department, Division of Pharmacology, S.E.T's College of Pharmacy, Dharwad 580 002, India
[4]Post graduate Department, Division of Pharmaceutical Chemistry, S.E.T's College of Pharmacy, Dharwad 580 002, India

Received 20 March 2009; revised 16 Septemper 2009

Argyreia speciosa (sweet) (Burm.f.) Boj. is an Ayurvedic rasayana plant used as an adaptogen. The present study reports the investigations done on the adaptogenic property of ethanol (EtAS; 100 and 200 mg/kg; po), ethyl acetate (EAAS; 100 and 200 mg/kg; po) fraction and flavanoids such as quercetin and kaempferol (25 mg/kg; po) of the root. Immobilization induced acute stress (AS; 3 days) and chronic stress (CS; 7 days) and swimming induced stress models were used to screen the anti-stress effect of the plant fractions and isolated flavanoids. The tested doses of EtAS and isolated flavanoids were able to produce significant effects in normalizing altered serum biochemical parameters and the severity of ulcer in both AS and CS models. Higher dose of EtAS, quercetin and kaempferol (25 mg/kg; po) were found to be significant in restoring the hypertrophy of adrenal gland and atrophy of spleen and thymus gland only in CS model. Greater swimming time was noted in the mice pretreated with tested doses of flavanoids and EtAS. In addition, levels of adrenal ascorbic acid and cortisol were restored compared to stress control group. EtAS exhibited significant scavenging effect of DPPH, hydroxyl radical and LPO. Thus, EtAS, quercetin and kaempferol are capable of increasing the capacity to tolerate non-specific stress in experimental animals, as evident from restoration of large number of parameters in the stress models studied. Bioactivity of EtAS may be due to the synergetic action of isolated flavanoids. Improvement in stress markers may be due its prolong effect of resistance to stress and partly due to free radical scavenging activity.

Keywords: Adaptogen, Antioxidant activity, *Argyreia speciosa* root, Flavanoids, Immobilization stress, Swimming endurance test, *Vruddhadaruka*

Stress disturbs the normal physiological condition and result in a state of threatened homeostasis. Stress has been postulated to be involved in the etiopathogenesis of a diverse variety of diseases ranging from psychiatric disorders such as anxiety and depression, immunosuppression, endocrine disorders including diabetes mellitus, male sexual dysfunction, cognitive dysfunctions, peptic ulcer, hypertension and ulcerative colitis[1]. Benzodiazepines and anxiolytics, despite having significant anti-stress activity, have not proved effective against chronic stress induced adverse effects on immunity, behavior cognition, male sexual function, during pregnancy and lactation. Additionally, the problem of tolerance and physical dependence on their prolonged use, limits the clinical utility of these drugs. Therefore there is a need for an effective herbal anti-stress agent in the therapy of stress induced disorders[2]. Rasayanas of ayurveda may be effective anti-stress 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 exhaustion[3]. Plant adaptogens like *Panax ginseng*[4], *Elutherococcus senticosus*[5], *Withania* somnifera[6,7], *Bacopa monnieri*[8] fractions and their constituents have been extensively studied for anti-stress/adaptogenic activity.

Argyreia speciosa (Burm.f) Boj. (Convulvulaceae) is a rasayana plant, commonly known as *Vrudhadaruka* in Indian system of medicine. Roots of *A. speciosa* are used traditionally as aphrodisiac, rejuvenating, intellect promoting, brain tonic, in the treatment of infected wounds, bronchitis, syphilis and pulmonary tuberculosis[9,10]. The plant has been screened for anti-inflammatory[11], immuno-

*Correspondent author
Telephone : +91836-2448540, +919448224894 (Mobile)
Fax: +91836-2467190
E-mail: prasannahabbu@ymail.com

modulatory[12], and hepatoprotective[13], antimicrobial and antitubercular[14] and nootropic[15] activities. Phytochemical investigations on the roots of *A. speciosa* have resulted in the isolation of a range of biologically active substances like flavonoid sulphates[16], stigmasteryl p-hydroxycinnamate, coumarin[17] and many phenolic compounds. Although the roots are used as an ingredient in traditional formulations, there is no scientific data available on adaptogenic properties of *Argyreia speciosa* root fractions and its isolated components. Therefore, we attempt to investigate the anti-stress potentiality of different fractions and isolated flavanoids of *A. speciosa* using different stress models in rodents.

Materials and Methods

Drugs and chemicals — Diagnostic kits were purchsed for estimation of glucose, triglycerides, aspartate aminotransferase (AST), alamine aminotransferase (ALT) from ERBA Diagnostic Mannheim Ltd. (Germany), cholesterol (Span Diagnostics Ltd, India) and creatinine kinase (Agappe Diagnostics Ltd.) A gift sample of standardized *Withania somnifera* (WS) extract was obtained from Natural Remedies, Bangalore, India. 1, 1-diphenyl-2 picrlhydrazyl (DPPH) was obtained from Sigma Chemical Co. (St Louis, MO USA). DNPH (SD-Fine Chemicals, India), Mannitol, Thio urea, ascorbic acid, deoxy ribose (HiMedia, India), oestradiol valerate injection (German Remedies, India) were also used.

Plant material and fractionation — Roots of *Argyreia speciosa* were collected from hilly areas (900 m) surrounding Dharwad, Karnataka province, India and authentication of the plant was done by Dr. G.R. Hedge, Department of Botany, Karnatak University, India. A herbarium specimen of the plant was kept in the Department of Pharmacognosy (SETCPD/Ph.cog/herb/33/2006), SET's College of Pharmacy, Dharwad, India. The collected material was washed with running water. The roots were chopped into small pieces and dried under shade. Dried roots were coarsely powdered and used for fractionation. Coarse plant material was cleaned by passing the powder material through 120 mesh sieve to remove any fine dust or powder, and coarse powder was used for extraction. Dried powder of root was exhaustively extracted successively using ethyl acetate (EAAS), and ethanol (95%) (EtAS), respectively in a Soxhlet apparatus. Both the fractions were concentrated by rotary flash evaporator, under reduced pressure and controlled temperature, followed by freeze drying and stored in a descicator.

Isolation of flavanoids — Flavanoidal aglycones were isolated by the method as reported earlier[16]. The structures of flavanoids were confirmed by spectral studies and co-TLC with reference compounds.

Preparation of drugs — EAAS and EtAS and isolated flavanoids were suspended in 0.5% gum acacia, and a fine emulsion was made having uniform particle distribution. The emulsion of both the fractions and flavanoids was administered orally by intragastric (ig) administration daily for three days in acute stress (AS) and for 7 days in chronic stress (CS). Freshly prepared emulsions were administered during the study period.

Animals — Albino rats of either sex weighing 150-200 g and Swiss albino mice of 15-20 g were used in the study. They were housed three to four per cage at 22°±2°C and 12 h light/dark under controlled environment. Animals were fed standard laboratory food and water was given *ad libitum*. Rats and mice were kept for 7 days in laboratory for habituation. All the experiments were performed in light period, and were conducted according to the CPCSEA regulations, India (Breeding and experiments on animals, control and supervision, 1998) and the Institutional Animal Ethics Committee (SETCP/-IAEC/07-08/02) approved the experimental protocol.

Stress protocol — Among the methods employed, immobilization has been used extensively and accepted widely for studying the stress induced physical and psychological alterations and consequences of the stress[19]. In our experiments, the stress was produced by restraining the individual inside an acrylic hemicylindrical plastic tube (4.5 cm diam. 12 cm long) for a period of 150 min once daily for 3 days in AS and once daily for seven consecutive days in CS as described earlier[18]. Freshly prepared emulsion of both the fractions and flavanoids were administered orally (po) daily for 3 days in AS and for 7 days in CS. After the stress protocol, blood was collected via retro-orbital plexus and serum was separated for biochemical estimations. The rats were sacrificed immediately under ether anesthesia. The abdomen and thorax were cut open, and the organs (adrenals, spleen and thymus) were dissected out and weighed after removing the adhering tissues.

Chronic stress induced sexual behavior -—Male rats were used in this paradigm. Animals were divided

in to normal control (unstressed), stress control, WS (100mg/kg), and test groups (EAAS, EtAS-100 and 200 mg/kg, quercetin and kaempferol 25 mg/kg, respectively). A male rat was placed in a cage for 10 min with six oestronized (sequentially treated with oestradiol valerate 5 mg/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[18].

Swimming endurance test — Swiss albino mice (15-20 g) were selected and divided into seven groups of six animals each. Animals were divided into normal control (unstressed), stress control, WS (100 mg/kg), and test groups (EAAS, EtAS-100 and 200 mg/kg; po, quercetin and kaempferol 25 mg/kg; po, respectively). Treatment was given to mice for 7 days. On day 7, after drug administration for 1 h, the animals were forced to swim in glass chambers (30 × 30 × 15 cm) containing water at room temperature. The mice were allowed to swim till they got exhausted and the moment they drowned was considered as the endpoint. Mean swimming time for each group was noted. Adrenal glands are weighed and ascorbic acid and cortisol from the adrenal gland were estimated [20].

***In vitro* free radical scavenging activity[21]**

Reaction with DPPH radical — The scavenging effect of fractions (1-100 µg/ml) against DPPH stable radical was determined using ascorbic acid (ASC) as standard.

Reaction with hydroxyl radical — Steady state hydroxyl radical ($^{\bullet}$OH) scavenging activity of fractions (6-500 µg/ml) was measured by degradation of deoxy-D-ribose method. Mannitol was used as standard.

Lipid peroxidation (LPO) assay — Lipid peroxidation assay was performed as per standard method. Trolox was used as standard.

Statistical analysis — All the results obtained from the different tests are presented as mean ± SE and compared against the stress control group using analysis of variance (ANOVA) followed by a *post hoc* comparison Tukey's test. Statistical significance was set at $P < 0.05$.

Results

IR spectrum of quercetin showed characteristic absorption band at 3406 cm^{-1} due to hydroxyl group. Another band at 1609 cm^{-1} attributed to stretching frequency of carbonyl group. The CH=CH stretching peak appeared at 2912 cm^{-1}. The ^{1}H NMR spectrum revealed the presence of a hydrogen bonded hydroxyl signal with C=O (C_5- OH) at δ 12.48. A singlet at δ 10.77 was assigned to C_3-OH proton. A singlet due to C_7-OH proton resonated at δ 9.58. A broad singlet at δ 9.36 which integrated for two protons was assigned to C'_3 and C'_4 hydroxyl groups. Two singlets at δ 6.18 and δ 6.40 which integrated for a proton each were due to protons present on phenyl ring of flavanoid nucleus (H-6 and H-8). Two doublets at δ 6.88 and δ 7.67 were attributed to H_5' and H_6' protons. A peak due to C'_2 proton appeared as singlet at δ 7.67. The Mass spectrum of quercetin showed molecular ion peak at m/z at 303.0 which corresponds to its molecular formula (C_{15} H_{10} O_7) and molecular weight.

IR spectrum of kaempferol exhibited a characteristic absorption band at 3247 cm^{-1} due to hydroxyl groups. Another band at 1612 cm^{-1} attributed to stretching frequency of carbonyl group. The CH=CH stretching appeared at 2925 cm^{-1}. The ^{1}H NMR spectrum revealed the presence of a hydrogen bonded hydroxyl signal with C=O (C_5- OH) at δ 12.47. A singlet at δ 10.78 was assigned to C_3-OH proton. A singlet due to C_7-OH proton resonated at δ 10.10. A singlet at δ 9.39 was assigned to C'_4 hydroxyl group. Two singlets at δ 6.18 and δ 6.43 which integrated for a proton each were due to protons present on phenyl ring of flavanoid nucleus (C_6-H and C_8-H). Doublet at δ 6.90 was attributed to C'_3 and C'_5 protons. Doublet due to C'_5 proton and C'_6 protons appeared at δ 8.05(2H). The Mass spectrum of kaempferol showed molecular ion peak at m/z at 287.0 which corresponds to its molecular formula (C_{15} H_{10} O_6) and molecular weight. All the above spectral details were consistent with the earlier reported data[22-25].

Effect of EtAS, EAAS and isolated flavanoids in acute stress (AS) and chronic stress (CS) induced alterations in biochemical parameters (Tables 1 and 2). AS and CS resulted in a significant increase in the serum glucose, total cholesterol and triglyceride compared to AS and CS control. Pretreatment with EAAS (100 and 200 mg/kg), EtAS (100 and 200 mg/kg), quercetin, kaempferol and WS (100 mg/kg) significantly reduced the elevated levels of glucose, total cholesterol and triglyceride level in AS and CS.

Exposure to AS and CS resulted in the significant increase in serum AST, ALT and CK level as compared to respective control. Pre-treatment with EtAS (100 and 200 mg/kg), quercetin and kaempferol (25 mg/kg) and WS (100 mg/kg) significantly decreased AST, ALT and CK level in AS and CS.

Acute and chronic immobilization stress resulted in a significant increase in score of ulcer index. Pre-treatment with test doses of EtAS, quercetin and kaempferol, and WS (100 mg/kg), significantly decreased in ulcer index compared to AS and CS control group (Fig. 1).

Effect of EtAS, EAAS and isolated flavanoids on weight of adrenal gland, spleen and thymus in AS and CS model have been represented in Table 3. Rats exposure to AS and CS resulted in significant increase in adrenal gland weight. Pre-treatment with EtAS, quercetin and kaempferol (25 mg/kg) and WS (100 mg/kg) significantly restored the adrenal weight in CS. A significant decrease in spleen weight was observed on exposure to AS and CS. The spleen weight was increased by EtAS, quercetin and kaempferol and WS (100 mg/kg) in AS and CS. Rats exposure to CS, resulted in significant decrease in the weight of thymus. In AS model, only isolated flavanoids showed restoration of thymus weight,

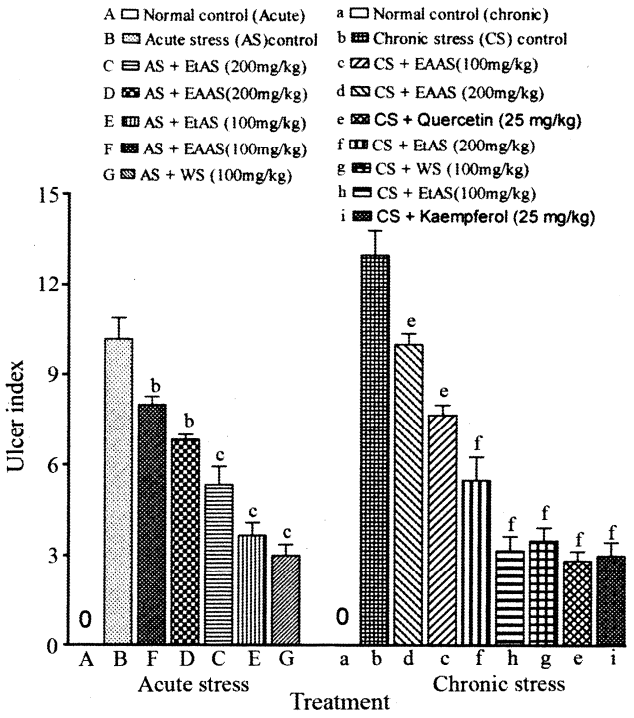

Fig.1 — Effect EtAS, EAAS and flavanoids on ulcer index in Acute (AS) and Chronic (CS) immobilization induced stress in rats. Results are represented as mean ± SE (n=6). $^{b}P < 0.01$and $^{c}P < 0.001$ as compared with stress control group for AS. $^{e}P < 0.01$and $^{f}P < 0.001$as compared with stress control group for CS

Table 1 — Effect of EtAS, EAAS and flavanoids on serum biochemical parameters in acute immobilization induced stress in rats [Values are mean ± SE of 6 rats]

Groups/dose(mg/kg)	AST(IU/L)	ALT(IU/L)	TG(mg/dl)	TC(mg/dl)	CK(IU/L)	Glucose(mg/dl)
Normal control	38.33±2.20^{c}	24.45± 0.99^{c}	31.38±2.52^{c}	43.88±3.91^{c}	136.0±4.62^{c}	82.92±1.22^{c}
Acute stress control	162.7±5.92	44.20±0.88	77.50±5.04	75.55±5.71	250.5±10.63	183.3±7.98
EAAS 100	135.01±12.45	37.83±3.28	56.59±5.92	66.97±2.43	227.3±10.81	149.6±8.55
EAAS 200	125.4±12.71	37.36±2.06	44.74±3.02	60.42±5.27	222.0±6.51	102.6±5.46^{c}
EtAS 100	77.72±3.14^{c}	30.03±1.50^{a}	43.86±3.78	51.31±2.23^{b}	179.5±5.38^{c}	102.0±3.01^{c}
EtAS 200	57.94±3.39^{c}	26.60±2.05^{c}	42.82±3.78^{a}	52.69±6.26^{b}	162.2±6.77^{c}	90.75±2.03^{c}
WS100	66.01±3.28^{c}	27.60±2.78^{b}	42.23±1.99^{a}	55.15±3.03^{a}	160.8±6.39^{c}	91.01±1.57^{c}
Quercetin 25	55.64±4.36^{c}	27.62±3.25^{c}	39.22±3.55^{b}	51.43±7.16^{b}	158.2±4.63^{c}	95.57±3.47^{c}
Kaempferol 25	59.45±6.49^{c}	29.50±4.65^{c}	41.52±4.58^{b}	55.43±5.05^{a}	166.54±3.33^{c}	92.13±4.55^{c}

$^{a}P < 0.05$ and $^{b}P < 0.01$ $^{c}P < 0.001$as compared with acute stress control group

Table 2 — Effect of EtAS, EAAS and flavanoids on serum biochemical parameters in chronic immobilization induced stress in rats [Values are mean ± SE of 6 rats]

Groups/dose (mg/kg)	AST(IU/L)	ALT(IU/L)	TG(mg/dl)	TC(mg/dl)	CK(IU/L)	Glucose(mg/dl)
Normal control	37.21±3.45^{e}	26.84±4.32^{e}	36.18±5.03^{f}	33.61±3.92^{f}	147.0±5.69^{e}	85.75±4.26^{f}
Chronic stress control	86.78±20.76	41.89±2.59	88.09±15.98	67.49±3.22	202.7±8.79	137.1± 4.23
EAAS 100	76.69±4.19	39.33±2.66	65.63±10.66	54.06±2.47	197.3±16.16	134.4±10.50
EAAS 200	72.79± 7.23	30.12±2.87	56.73±8.54	44.13±3.41	170.3±11.12	107.4± 5.15
EtAS 100	36.67±3.89^{e}	27.98±2.08^{d}	43.23±3.27^{e}	48.49±1.95^{d}	156.7±5.98^{d}	98.73± 13.24^{d}
EtAS 200	34.53±4.02^{f}	27.50±2.89^{e}	38.13±7.56^{f}	46.56±3.64^{d}	158.2±4.28^{d}	90.56±12.98^{e}
WS100	42.98±5.52^{d}	28.66±1.59^{d}	36.82±0.10^{f}	45.17±2.46^{e}	147.7±4.25^{e}	87.41±6.01^{f}
Quercetin 25	32.67±4.32^{f}	25.58±5.45^{e}	32.93±1.23^{f}	45.35±2.88^{f}	168.0±4.64^{d}	98.71±3.21^{f}
Kaempferol 25	43.98±5.78^{d}	28.21±1.65^{d}	31.66±1.47^{f}	48.76±1.00^{e}	162.2±6.77^{e}	96.30±2.75^{f}

$^{d}P < 0.05$ and $^{e}P < 0.01$ $^{f}P < 0.001$as compared with chronic stress control group

whereas thymus weight was restored significantly by the higher dose of EtAS, quercetin and kaempferol (25 mg/kg) and WS in CS model.

Rats exposed to CS significantly inhibited the male sexual response indices, inducing decrease in latencies in licking female genitalia, mounting and intromission, number of mounts and intromissions. Pre-treatment with quercetin (25 mg/kg) and WS (100 mg/kg) reversed these changes, whereas none of the other test drugs showed significant results (data not shown).

In forced swimming endurance test, the survival time of swimming mice increased significantly in dose dependent manner by pre-treatment with EtAS (100 mg/kg) EtAS (200 mg/kg) and flavanoids compared to normal control group. Exposure to swimming stress caused hypertrophy of adrenal gland which was associated with significant depletion of adrenal content ascorbic acid and cortisol contents compared to non swimmer group (Fig. 2A and B). Pre-treatment with tested doses of EtAS, quercetin, kaempferol and WS prevented significantly the altered adrenal weight. Furthermore, a significant ($P < 0.01$; $P < 0.001$) increase in adrenal ascorbic acid and cortisol contents were observed. *In vitro* antioxidant studies demonstrated that EtAS was a potent free radical scavenger of DPPH, OH radical and LPO with IC_{50} value of 36.54, 121.18 and 41.83 µg/ml, respectively. (Fig. 3, A, B and C).

Discussion

Immobilization model used in our study found to cause long term desensitization of hypo-thalamicpituitary-adrenal axis (HPA) response which affected both peripheral and central components of the HPA axis[26]. Immobilization has been the ideal choice for the induction of stress responses in animals and more specifically for the investigation of drug effects on typical stress-related gastrointestinal, neuroendocrine, and immunological pathology. The

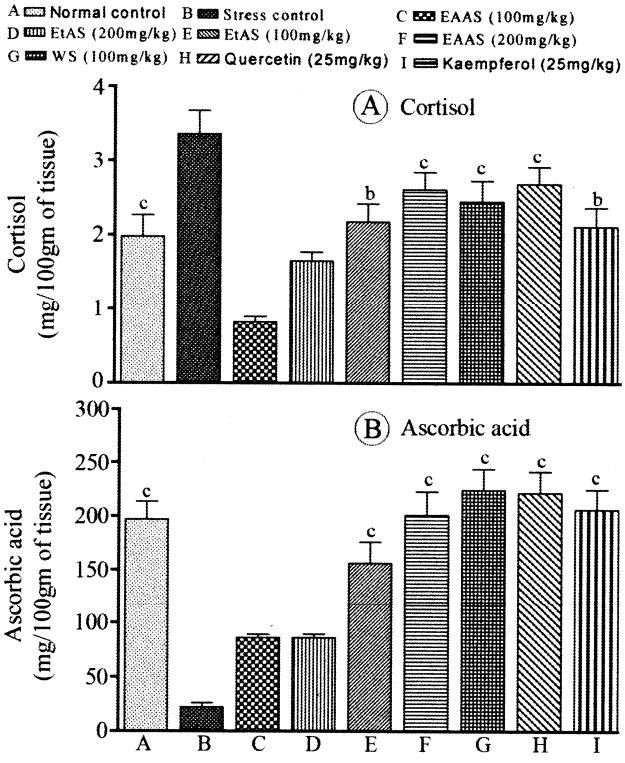

Fig.2 — Effect EtAS and EAAS and flavanoids on level of [A] Cortisol (mg/100 g of tissue) [B] Ascorbic acid (mg/100 g of tissue) in adrenal gland on swimming induced stress in mice. Results are represented as mean ± SE (n=6). $^{b}P < 0.01$; $^{c}P < 0.001$ as compared with stress control group

Table 3 — Effect of EtAS, EAAS and flavanoids on the weight of adrenal gland, spleen and thymus on immobilization [Acute and chronic] induced stress in rats
[Values are mean ± SE of 6 rats]

Groups/dose (mg/kg)	Acute immobilization stress (AS)			Chronic immobilization stress (CS)		
	Weight of adrenal gland (mg)	Weight of spleen (mg)	Weight of thymus gland (mg)	Weight of adrenal gland (mg)	Weight of spleen (mg)	Weight of thymus gland (mg)
Normal control	14.00±0.36^{c}	644.5±15.05^{b}	667.7±9.28^{c}	17.00±0.93^{e}	664.0±16.08^{f}	651.5±19.33^{f}
Acute stress control	23.17±1.25	473.7±33.59	619.3±27.93	----	-------	-----
Chronic stress control	---	--	----	21.33±0.91	444.0±39.21	386.3±10.48
EAAS 100	20.67±0.95	488.5±5.23	578.2±22.97	18.17±1.11	459.2±18.47	446.3±8.81
EAAS 200	19.83±0.79	554.8±20.97	570.0±7.64	20.17±0.94	533.0±22.50	437.7 ±8.82
EtAS 100	20.50±0.92	605.0±6.90	563.0±12.68	17.67±0.76^{e}	630.3±57.76^{e}	445±6.33
EtAS 200	19.00±1.31	612.7±7.67^{a}	583.5±15.40	15.17±0.47^{f}	603.8±48.79^{d}	615.0±17.23^{f}
WS100	19.50 ±1.17^{a}	620.0±14.17^{a}	608.3±8.11^{b}	15.67± 0.76^{f}	599.7±10.92^{d}	623.8±19.37^{f}
Quercetin 25	17.43 ±1.45 ^{b}	610.5±6.68^{a}	615.3±5.21^{a}	14.83 ±0.30^{f}	632.3±23.91^{e}	611.5±16.07^{f}
Kaempferol 25	18.65±3.25 ^{b}	618.0±10.17^{a}	598.4±12.97^{a}	15.17±0.47^{f}	623.5±18.02^{e}	617.5±20.13^{f}

$^{a}P < 0.05$, $^{b}P < 0.01$ $^{c}P < 0.001$ as compared with stress control group for AS. $^{d}P<0.05$, $^{e}P < 0.01$and $^{f}P < 0.001$as compared with stress control group for CS

distinct advantage of using immobilization as a stressor lies in the fact that it produces both physical as well as inescapable psychological stress[27].

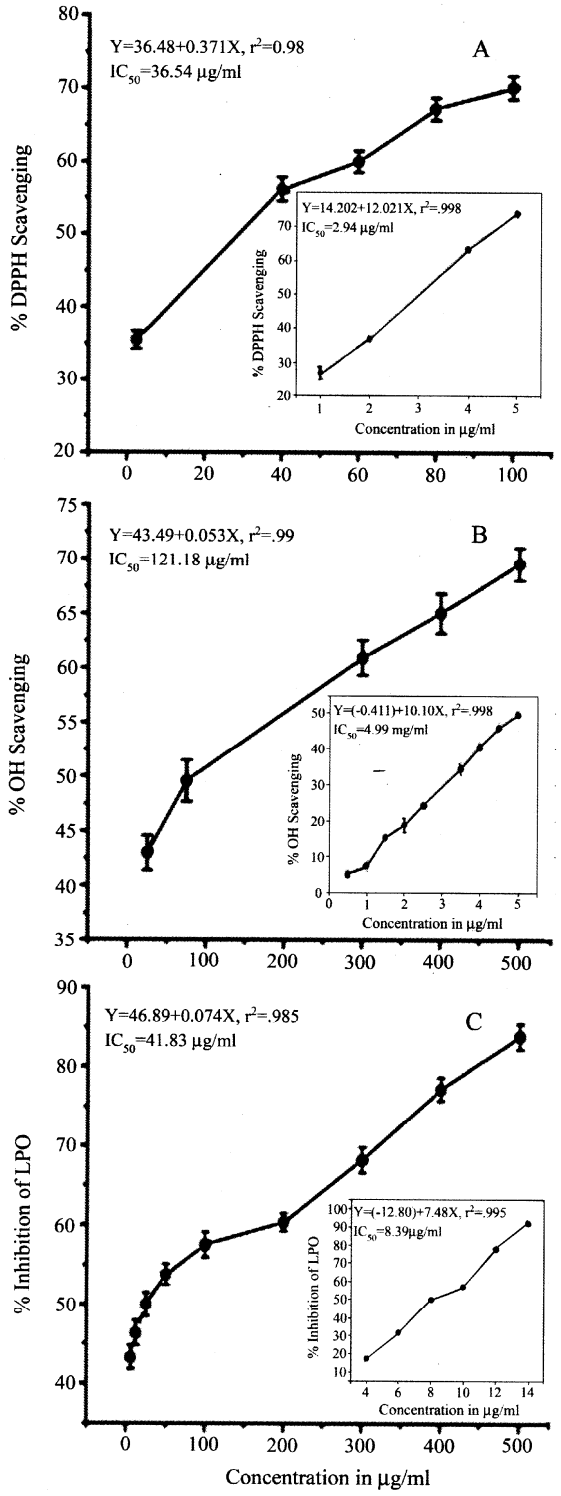

Fig.3 — Scavenging effect of ethanolic fraction (EtAS) of *Argyreia speciosa* on A) DPPH radical; B) hydroxyl radical and C) LPO

Blood sugar level in response to stress is highly contradictory and studies related to stress in rats shows fluctuations in blood sugar level ranging from slight decrease, relative increase or no change[28]. Rats exposure to AS in our study showed elevated level of glucose as compare to CS. The hyperglycemic response in AS is due to release of glucocorticoids, as a result of HPA axis stimulation to compensate initial demand of energy[18]. The acute demand of glucose is fulfilled by the increase in glucogenolysis from liver during AS. During CS, this available source depletes. Thus, it seems to have a direct action on peripheral metabolism. EtAS, flavanoids and WS at the tested doses in acute stress decreased the elevated blood glucose, which might be due to the suppression of HPA response.

AS and CS raises the serum cholesterol level through enhanced activity of hypothalamo-hypophyseal axis resulting in increased liberation of catecholamine and corticosteroids. Effect of AS and CS on serum triglyceride has been shown to be variable probably due to mobilization of fats from adipose tissue by catecholamine. The suppression of stress induced triglyceride level by *A. speciosa* fractions and flavanoids might be due to the suppression of stress-induced lipolysis.

AS induced significant increase in ALT, AST, and CK might be the outcome of AS induced secretion of corticosterone from cortex, epinephrine from medulla, and epinephrine from sympathetic nerve terminals. In contrast to ALT, which is found primarily in liver, AST is present in many tissues, including the heart, kidney, brain, and skeletal muscles. As a result of transamination, amino acid can enter the citric acid cycle and then function in the intermediary metabolism of carbohydrate and lipids[27]. The CK system is important in stabilizing the ATP levels and energy metabolism of the myocardium and other skeletal muscles of rats during stress. A maximum increase in CK activity was observed after AS exposure when compared to CS, may be due to partial adaptation. A reduced CK activity in CS as compared to AS is due to partial habituation. Reduction in ALT and AST by EtAS, flavanoids in our study may be due to direct action on the peripheral metabolism and of CK might be due to decrease in energy demand.

Stressful events activate autonomic and endocrine responses responsible for gastric ulceration. This can be attributed to the stimulation of paraventricular nucleus of hypothalamus, increased intestinal

motility, acid secretion and group of other factors[29]. Gastric damage induced by CS and AS was reduced by tested doses of EtAS, isolated flavanoids and WS as reflected by decreased mean ulcer severity score indicating their protective effects on gastric mucosa during stressful conditions.

Stress-induced adrenal hypertrophy observed both in AS and CS is due to the activation of HPA axis, which is highly responsive to stress and is one of the principal mechanism by which an organism mobilizes its defense against stress events[30]. The prolonged activation of HPA axis resulted in an increase in the adrenal hypertrophy in CS as compared to AS. During stress, nerve terminals accelerate recruitment of lymphocytes to blood from spleen, which is a major storage pool of lymphocytes. This result in the squeezing of the spleen causing reduction in weight observed in AS and CS exposures. Atrophy of thymus was found only during CS exposure and not during AS. The transient activation of HPA axis and release of neurochemicals do not have profound impact on thymocytes as in AS, but persistent high level of corticosterone during CS causes apoptosis and necrosis in immature T and B cells resulting in the decline of thymus weight. Prevention of adrenal hypertrophy and atrophy of spleen and thymus was observed by pre-treatment with EtAS, flavanoids and WS in both AS and CS models.

Stress alters the normal functioning of the body. In a special contrivance, when an animal forced to swim becomes immobile after an initial period of vigorous activity. This resembles a state of mental depression. The adrenal glands contain relatively large amount of ascorbic acid and cortisol which are markedly reduced by stress and causes hypertrophy when they are stimulated by stress[29-31]. Our results revealed that pre-treatment with EtAS, isolated flavanoids and WS increased labor efficiency and increase of swimming performance. Moreover, prevented the depletion of ascorbic acid, cortisol and hypertrophy of adrenal glands.

Stress may also impair antioxidant defenses, leading to oxidative damage, by changing the balance between oxidant and antioxidant factors. Both immobilization and variable stress are followed by an increase in lipid peroxidation, measured in plasma and in brain. Quercetin and kaempferol are widely distributed polyphenolic flavanoid compounds in nature. These flavanols possess anti-inflammatory[32], analgesic[33], cytotoxic[34] antioxidant and antimicrobial[35,36] and many wide range of activities. Although phytotoxic hexadecanyl p-hydroxy cinnamate and scopoletin were isolated from the plant, this is the first report on adaptogenic activity of flavanols of the plant. Several theories have been suggested to explain the effects of adaptogenic substances. One theory argues that adaptogens function primarily due to their antioxidant effect which is found to be partially accurate[37]. In our earlier report, ethanolic fraction of *Argyreia speciosa* has been shown to exert significant antioxidant activity induced by augmented activity of oxygen free radicals scavenging enzymes, superoxide dismutase, catalase and peroxidase *invivo* [13].

The present study established the adaptogenic/anti-stress activity of *Argyreia speciosa* in rodent models of stress. Further, the observed activity might be due to the prevention of desensitization of both peripheral and central components of the hypothalamicpituitary-adrenal axis (HPA) and partly due to antioxidant activity.

Acknowledgement

The authors are thankful to President, Soniya Education Trust and Principal SET's College of Pharmacy, Dharwad, for support and encouragement. The authors acknowledge Natural Remedies, Bangalore, India, for providing a gift sample of standardized *Withania somnifera* extract. Dr Hebbar, Department of Botany is also acknowledged for his help in identification and collection of plant material.

References

1 Elliott G R & Eisdorf C, *Stress and Human healt*, (Springer Verlag, Berlin, Heidelberg, New York) 1982.

2 Muruganandam A V, Vikas K & Bhattacharya S K, Effect of polyherbal formulation EuMil on chronic stress induced homeostatic perturbation in rats, *Indian J Exp Biol*, 40 (2002)1151.

3 Rege N N, Thatte U M & Dhanukar S A, Adaptogenic properties of six rasayana herbs used in ayurvedic medicine, *Phytother Res,* 13 (1999) 275.

4 Sang H L, Byung H J, Sun Y K, Eunjo H L & Bong C C, The anti-stress effect of ginseng total saponin and ginsenoside Rg3 and Rb1 evaluated by brain polyamine level under immobilization stress, *Pharmacol Res,* 54 (2006) 46.

5 Goulet E & Dionne I, Assessment of the effects of *Elutherococcus senticosus* on endurance performance. *Int J Sport Nutr Exer Metab,* 15(1) (2005) 75.

6 Bhattacharya A, Ghosal S & Bhattacharya S K, Antioxidant effect of *Withania somnifera* glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum, *J Ethnopharmacol,* 74 (2001) 1.

7 Singh B, Chandan B K & Gupta D K, Adaptogenic activity of a novel withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun, Part II, *Phytother Res,* 17(5) (2003) 531.

8 Deepak R, Bhatia G, Palit G, Pal R, Singh S & Singh H K, Adaptogenic effect of *Bacopa monniera* (Brahmi), *Pharmacol Biochem Behavr,* 75 (2003) 823.

9 Warrier P K, Nambiar V P & Ramankutty C, *Indian medicinal plants-a compendium of 500 species,* Vol. I (Orient Longman New Delhi) 1994, 191.

10 Sharma P C, Yelne M B & Dennis T J, *Database on medicinal plants used in Ayurveda.* (Central Council for Research in Ayurveda and Siddha, New Delhi) 2004 550.

11 Gokhale A B, Damre A S, Kulkarni K R & Saraf M N, Preliminary evaluation of anti-inflammatory and anti-arthritic activity of *S.lappa, A.speciosa and A.aspera. Phytomedicine,* 9 (2002) 433.

12 Gokhale A B, Damre A S & Saraf M N, Investigations in to the Immunomodulatory activity of *Argyreia speciosa, J Ethnopharmacol,* 84 (2003) 109.

13 Habbu P V, Shastry R, Mahadevan K M, Joshi H K & Das S K, Hepatoprotective and antioxidant effects of *Argyreia speciosa* in rats, *Afr J Trad Compl Altern Med* 5(2) (2008) 158.

14 Habbu P V, Shastry R A, Mahadevan K M & Manjunath H, Antimicrobial activity of flavanoids sulphates and other fractions of *Argyreia speciosa* (Burm.f.) Boj. *Indian. J Exp Biol,* 47 **(**2009) 121.

15 Habbu P V, Shastry R A, Mahadevan K M & Sneha C, Antiamnesic activity of *Argyreia speciosa* in mice. 2009 *Int J Green Pharm,* (in Press).

16 Petra M, Britta T, Macki K & Eckart E, Flavanoid sulphates from Convolvulaceae, *Phytochemistry,* 50 (1999) 267.

17 Srivastava A & Shukla Y, Aryl esters and a coumarin from *Argyreia speciosa, Indian J Chem,* 37B **(**1998) 192.

18 Deepak R, Gitika B, Tuhinadri S & Gautam P, Anti-stress effects of *Ginkogo biloba* and *Panax ginseng*: A comparative study, *J Pharmacol Sci*, (2003) 458.

19 Al-Mohaisen M, Cardounel A & Kalimi M, Repeated immobilization stress increases total cytosolic glucocorticoid receptor in rat liver, *Steroids,* 65 (2000) 8.

20 Kannur D M, Hukkeri V I & Akki K S, Adaptogenic activity of *Caesalpinia bonduc* seed extracts in rats, *J Ethnopharmacol,* 108 (3) (2006) 327.

21 Veerapur V P, Prabhakar K R, Vipin K P, Machendar R K, Ramakrishana S & Mishra B *et al*, *Ficus Racemosa* stem bark extract: A potent anti-oxidant and a potent natural radioprotecter, *Evidenc Comple Alter Med (eCAM),* (2007) 1.

22 Gülnur T, Esra K, Merve M & Erdem Y, Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden), *J Ethnopharmacol,* 116 (1) (2008) 120.

23 Anil K & Richa G, Quercetin Protects Against Acute Immobilization Stress-Induced Behaviors and Biochemical Alterations in Mice, *J Med Food* 11(3) (2008) 469.

24 Geetha G N, Daniel M & Sabnis S D, Chemosystematics of *Ipomonea* Linn and some related Taxa, *Curr Sci*, 55 (1986) 961.

25 Mansoor A, Neeru J, Shafiullah, Sohrab K M & Mohammed I, Two new flavone glycosides from the leaves of *Argyreia speciosa*, *J Chem Res,* 7 (1993) 248.

26 Singh B, Gupta D K & Chandan B K, Adaptogenic activity of a glycol-peptido-lipid fraction from the alcoholic extract of *Trichopus zeylanicus* Gaerth, *Phytomedicine,* 8 (4) (2001) 283.

27 Henke P G, The hypothalamus–amygdala axis and experimental gastric ulcer, *Neurosci Biobehav Rev,* 3 (1979) 75.

28 Krupavaram B, Venkatrao N, Nandakumar K, Gowda T S, Shalam M D & Shantakumar S M, A study on adaptogenic activity of root extracts of *Boerharvia diffusa* Linn, *Indian Drugs,* 44 (4) (2007) 264.

29 Singh B, Chandan B K, Sharma N, Singh S, Khajuria A & Gupta D K, Adaptogenic activity of a glycol-peptido-lipid fraction from the alcoholic extract of *Trichopus zeylanicus* Gaerth Part II, *Phytomedicine,* 12 (2005) 468.

30 McEwen B S, The neurobiology of stress: From serendipity to clinical relevance, *Brain Res,* 886 (2000) 172.

31 Dhir A, Naidu P S & Kulkarni S K, Protective effect of naproxen (non-selective COX-inhibitor) or rofecoxib (selective COX-2 inhibitor) on immobilization stress-induced behavioral and biochemical alterations in mice, *Eur J Pharmacol,* 535 (2006) 192.

32 Eun-Ju L, Eun-Jin C, Choong-Il C, Jong-Sug P & Mi-Kyung Sung, Effects of Anti-inflammatory Quercetin and Kaempferol on Cell Growth and the Production of Angiogenic Factors in HT-29 Human Colon Cancer Cells, *FASEB* 21(2007) 847.22.

33 Palanichamy S & Nagarajan S, Analgesic activity of *Cassia alata* leaf extract and Kaempferol 3-O-Sophoroside, *J Ethnopharmacol,* 29 (1) (1990) 73.

34 Dimas K, Demetzos C, Mitaku S, Marselos M. Tzavaras T & Kokkinopoulos D, Cytotoxic activity of kaempferol glycoside against human leukaemic cell lines *in vitro, Pharmacol Res,* 41(1) (2000) 83.

35 Lim Y H, Kim I H & Seo J J, *In vitro* activity of kaempferol isolated from the *Impatiens balsamina* alone and in combination with erythromycin or clindamycin against *Propioni bacterium* acnes, *J Microbiol,* 45(5) (2007) 473.

36 Panizzi L, Catalano S, Miarelli C, Cioni P L & Campeol E, *In-vitro* antimicrobial activity of extracts and isolated constituents of *Geum rivale, Phytother Res,* 14 (2000) 561.

37 Panosian A, Adaptogens tonic herbs for fatigue and stress. *Alter Comple Thera,* 9(6) (2003) 327.