Effect of *Alstonia scholaris* (Linn.) R.Br. on stress and cognition in mice

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Effect of stress and its modulation by methanolic extract of bark of *Alstonia scholaris* was studied using acute restraint stress model in mice. The extract was also evaluated for nootropic and antioxidant potential to support anti-stress activity testing. Acute restraint stress resulted in significant increase of plasma corticosterone, glucose, protein, cholesterol and triglyceride levels in stress group of animals. Methanolic extract pretreatment at 100, 250 and 500 mg/kg for 7 days displayed promising anti-stress effect by normalizing these stress-induced biochemical perturbations in plasma of mice. Effect on cognitive functions was evaluated using passive avoidance model and elevated plus maze model. Pretreatment with extract at 100, 250 and 500 mg/kg augmented acquisition and retention of memory of learned task as evidenced by increased step-down and shortened-transfer latency in passive avoidance model and elevated plus maze model, respectively. Diazepam (2 mg/kg, ip) and piracetam (200 mg/kg, po) were used as standard drugs for anti-stress and nootropic activity testing. Further, the extract at 200 µg/ml showed maximum scavenging of stable radical 1,1-diphenyl, 2-picryl hydrazyl at 90.11% and nitric oxide radical at 62.77%. The present study, thus, provided scientific support for anti-stress (adaptogenic), antioxidant and nootropic activities of methanolic extract of bark of *Alstonia scholaris*.

**Keywords:** *Alstonia scholaris*, Antioxidant, Anti-stress, Nootropic

Traditional medicines are rich in non-specific anti-stress agents which are of increasing clinical significance. Among them, adaptogens are the plant-derived biologically active substances, which appear to induce a state of non-specific increase of resistance of the organism to diverse aversive assaults which threaten internal homeostasis and which improve physical endurance for doing work even in adverse circumstances and in difficult environmental conditions. Since the introduction of adaptogens, several plants have been investigated, which were once used as tonics due to their adaptogenic and rejuvenating properties in traditional medicine.

Further, the role of stress has been emphasized in diseases ranging from psychiatric and endocrine disorders to cognitive dysfunctions. A large number of medicinal plants have been reported to possess intellect promoting activity. It has been observed that several medicinal plants possess either nootropic activity or anti-stress activity, but only a few plants like *W. somnifera* possess both anti-stress as well as nootropic activity. Hence, finding drugs of plant origin that possess both anti-stress and nootropic potential could be a valuable contribution to the existing armamentarium of few agents possessing combination of psychopharmacological activities.

*Alstonia scholaris* (Linn.) R.Br. belonging to family Apocynaceae grows throughout India, in deciduous and evergreen forests, also in plains. *Alstonia scholaris* has been mentioned in Ayurveda and is known as *Saptaparna*. The plant is known to contain alkaloids (ditamine and echitamine), flavonoids and phenolic acids. Scientific studies have established the multifarious utility of this plant in wide array of pharmacological activities, including antidiarrhoal, antimicrobial, anti-fertility, immunomodulatory, and anticancer activities. However there is no report available on adaptogenic activity of the plant. Hence, the present work was undertaken to investigate the anti-stress potential of methanolic extract of *Alstonia scholaris* [AS(ME)] in vivo, in normal and stress-induced mice following a biochemical approach. In addition, nootropic and antioxidant potential of the extract was evaluated to support the anti-stress activity testing.

**Material and Methods**

**Animals**—Swiss Albino male mice (20-25 g) were used for the study. The animals were housed into

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groups of six in a photoperiod of 12/12 hr light and dark cycle, at 25°±2°C and fed with standard pelleted diet and water ad libitum. All the experiments were carried out between 0900 and 1600 hr in the laboratory. The experimental protocols for the study were approved by the Institutional Animal Ethics Committee.

Preparation of extract—The barks of Alstonia scholaris were purchased in bulk from local market and the identity of the plant was authenticated by Dr. Ganesh Iyer, Ramnarayan Ruia College, Mumbai. A voucher specimen (No. 4287) was deposited in pharmaceutical science and technology department, University Institute of Chemical Technology, Mumbai. Dried powdered barks were defatted with petroleum ether (60-80°C) and extracted with methanol using Soxhlet extractor. The extract so obtained was dried at 40°C using a vacuum evaporator.

Source of chemicals—The drugs used in the study were obtained from various sources—diazepam (Calmipose®, Ranbaxy, India), piracetam (Nootropil®, UCB India Pvt Ltd, Gujarat), 1,1-diphenyl, 2-picyrl hydrazyl (DPPH) and scopalamine hydrobromide (Sigma, USA). The biochemical kits used for biochemical analysis were obtained from Merck India Limited, Mumbai. All chemicals and solvents were of analytical grade.

Acute toxicity—In accordance with OECD guidelines, acute oral toxicity study of AS(ME) was performed in mice. The extract was found to be safe at 2000 mg/kg, po; hence, doses of 100, 250 and 500 mg/kg were selected for in vivo pharmacological studies.

Treatment and acute restraint stress—Mice were divided randomly into 6 groups, each group containing 6 mice. Group I - mice received saline orally, served as vehicle control; Group II - mice received saline orally and stress, served as negative control; Group III - mice received standard drug, diazepam (2 mg/kg, ip) and stress, served as positive control; Group IV - mice were treated with AS(ME) (100 mg/kg, po) and stress; Group V - mice were treated with AS(ME) (250 mg/kg, po) and stress; and Group VI - mice were treated with AS(ME) (500 mg/kg, po) and stress.

All the animals were pretreated for 7 days with the respective test drug or standard. After 45 min of pretreatment with test drug/standard/vehicle on day 7, mice of different groups were exposed to stressful stimuli. Fore and hind limbs of mice were tied separately and then together, and finally secured with adhesive tape, thereby, immobilizing them for 2 hr. After induction of stress, blood was collected and plasma corticosterone, glucose, triglyceride, cholesterol and total protein levels were estimated.

Passive shock avoidance—Mice were divided into 6 groups of 10 mice each and given treatment as follows—Group I - saline-treated animals, served as vehicle control; Group II - animals received scopolamine (1mg/kg, ip), served as negative control; Groups III - animals received piracetam 200 mg/kg orally, served as positive control; and Groups IV, V and VI were fed orally with AS(ME) at doses 100, 250 and 500 mg/kg, respectively. Treatment schedule consisted of treating all groups with their respective test drug/standard/vehicle for six consecutive days. Experiment was conducted on day 7 and 8, after 45 min of administration of test drug/standard/vehicle to respective group.

The apparatus consisted of a box (27 × 27 × 27 cm) having three walls of wood and one wall of plexiglass, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 × 7 × 1.7 cm) in the center of the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform, set in the center of the grid-floor. When the mouse stepped down and placed all its paws on the grid-floor, shock of (20VAC) was delivered for 15 sec, and step-down-latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from wooden-platform to the grid-floor, with all its paws on the grid-floor. Animals showing SDL in the range of 2-15 sec during the first test were used for the second session and the retention test. The second-session was carried out 90 min after the first test. When the animals stepped down before 60 sec, electric shocks were delivered for 15 sec. During the second test, animals were removed from the shock-free-zone, if they did not step down for a period of 60 sec. Retention was tested after 24 hr in a similar manner, except that the electric shocks were not applied to the grid floor. Each mouse was again placed on the platform, and the SDL was recorded with an upper cut-off time of 300 sec.

Elevated plus maze—Treatment groups and treatment schedule were similar to passive avoidance test described above. Measure of transfer latency (TL) on elevated plus maze was essentially same as
described by Itoh\textsuperscript{21}. In the first trial, the mouse was allowed to explore the maze for 20 sec after the measurement of TL. TL was defined as time taken by the animal to enter fully (with all four paws) any of the enclosed arms from open arm end. The retention of memory was tested after 24 hr of first trial. TL measured on day 1 and 2 of the trial served as parameters for acquisition and retrieval, respectively\textsuperscript{22}.

**DPPH radical scavenging assay**—DPPH radical scavenging activity was measured by spectrophotometric method. To a methanolic solution of DPPH (100 \( \mu \text{M} \), 2.95 ml), 0.05 ml of test compounds dissolved in methanol was added at different concentration (25-200 \( \mu \text{g/ml} \)). Equal amount of methanol was added to the control. Absorbance was recorded at 517 nm at regular intervals of 30 sec for 5 min\textsuperscript{23}.

**Nitric oxide scavenging assay**—Nitric oxide scavenging activity was measured by using a spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of extract (25-200 \( \mu \text{g/ml} \)) dissolved in methanol and incubated at 25°C for 30 min. A control without test compound but with equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of Griess reagent (1%, sulphanilamide; 2%, phosphoric acid; 0.1%, naphthylethelene diamine hydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with phosphoric acid; 0.1%, naphthylethelene diamine was measured at 546 nm\textsuperscript{24}.

**Statistical analysis**—The results were subjected to statistical analysis using one-way ANOVA with post Bonferroni test.

**Results**

Pretreatment of mice with AS(ME) at 100, 250 and 500 mg/kg (po) significantly decreased stress-induced elevations in plasma corticosterone, glucose, triglyceride, cholesterol and total protein levels compared to stress control group (Table 1).

In passive shock avoidance model, the extract pretreatment at 100, 250 and 500 mg/kg (po) significantly increased SDL compared to scopolamine treated group after 24 hr of treatment (Table 2). However, pretreatment of the extract at all three doses significantly decreased TL compared to scopolamine treated mice on day 1 and 2 of trial in elevated plus maze model (Table 3).

In DPPH assay, scavenging capacity of methanolic extract was found to be 90.11% with \( \text{IC}_{50} \) being 96.80 \( \mu \text{g/ml} \) (Table 4). Scavenging of nitric oxide by extract in nitric oxide scavenging assay was concentration dependent. Maximum percentage of inhibition was 62.77%. \( \text{IC}_{50} \) value was recorded at 138.70 \( \mu \text{g/ml} \) (Table 4).

**Discussion**

Stress response is sub-served by a complex system with subsequent involvement of hypothalamus–pituitary–adrenal (HPA) axis\textsuperscript{25-27}. Stresses, both physical and emotional, act via neural pathways to hypothalamus and lead to increase in corticotrophin releasing hormone (CRH) secretion and hence

**Table 1**—Effect of AS(ME) pretreatment on acute restraint stress-induced changes in biochemical levels in plasma of mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Plasma corticosterone (( \mu \text{g/dl} ))</th>
<th>Plasma glucose (mg/dl)</th>
<th>Plasma total protein (g/dl)</th>
<th>Plasma cholesterol (mg/dl)</th>
<th>Plasma triglyceride (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.4 (± 25.55)</td>
<td>99.27 (± 10.59)</td>
<td>4.56 (± 0.81)</td>
<td>69.02 (± 12.06)</td>
<td>41.12 (± 9.1)</td>
</tr>
<tr>
<td>Vehicle +stress</td>
<td>181.0 (± 29.89)</td>
<td>150.65</td>
<td>7.5 (± 1.67)</td>
<td>140.88</td>
<td>72.22</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg) + stress</td>
<td>92.10 (± 21.60) ***</td>
<td>101.50</td>
<td>4.824 (± 0.95)</td>
<td>71.25 (± 22.06) #</td>
<td>29.63</td>
</tr>
<tr>
<td>AS(ME) (100 mg/kg) + stress</td>
<td>101.80 (± 10.67) ***</td>
<td>106.27</td>
<td>4.48 (± 0.85)</td>
<td>68.71 (± 18.51) ***</td>
<td>30.16</td>
</tr>
<tr>
<td>AS(ME) (250 mg/kg) + stress</td>
<td>93.90 (± 9.19) ***</td>
<td>99.97</td>
<td>4.348 (± 0.77)</td>
<td>62.06 (± 17.45) ***</td>
<td>30.31</td>
</tr>
<tr>
<td>AS(ME) (500 mg/kg) + stress</td>
<td>91.80 (± 7.67) ***</td>
<td>99.03</td>
<td>4.1685 (± 14.33)</td>
<td>60.25 (± 21.67) ***</td>
<td>25.98</td>
</tr>
</tbody>
</table>

P values: *<0.001: significant as compared to control; **<0.05; ***<0.01; ****<0.001: significant as compared to stress control. Statistical test employed is ANOVA followed by post Bonferroni test.
Release of ACTH in stress stimulates adrenals to increase production of hormones epinephrine, norepinephrine and corticosteroids. These hormones have profound effect on metabolic functions. Increased plasma cortisol influences mobilization of stored fat and carbohydrate reserve which in turn increase blood glucose, protein, cholesterol and triglyceride levels.

One of the best explored models of stress in rats/mice is forced immobilization. As painful stimuli are not directly involved in restraint stress, this form of stress is probably more akin to physiological stress, combining emotional stress (escape reaction) and physical stress (muscle work), resulting in both restricted mobility and aggression. In acute restraint stress model, pretreatment of mice with AS(ME) at 100, 250, 500 mg/kg body wt have been found to inhibit stress-induced rise in plasma corticosterone levels with consequent reduction in plasma glucose, cholesterol, triglycerides and total protein levels compared to stress control group, suggesting adaptogenic effect.

Results of antioxidant activity showed that AS(ME) scavenged DPPH radical in a dose-dependent manner. The bleaching of DPPH absorption is representative of the capacity of AS(ME) to scavenge free radicals independently from any enzymatic activity. In nitric oxide assay, nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH reacts with oxygen to form nitrite ion which can be estimated using Griess reagent. The extract in a dose dependent manner inhibited nitrite formation by competing with the oxygen atom to react with nitric oxide.

Nootropic drugs belong to the category of psychotropic agents with selective facilitatory effect on intellectual performance, learning and memory. Increase in SDL and decrease in TL in AS(ME) treated mice in passive shock avoidance model and elevated plus maze model respectively, gives an indication of cognitive enhancer effect of the extract. Increase in SDL time is considered as evidence for successful acquisition, and retention of unpleasant experience, while decrease in TL on both days of trial clearly indicates improvement in learning as well as memory, in accordance with the hypothesis of Itoh. Further, anti-stress and antioxidant activities of AS(ME) can be correlated with nootropic potential of...
the extract since, the role of stress and free radicals have been implicated in the loss of memory, concentration and also in Alzheimer’s disease.\(^{37,38}\)

In conclusion, psychopharmacological profile of methanolic extract of bark of *Alstonia scholaris* suggested potential anti-stress (adaptogenic), antioxidant and nootropic activities. Further investigations are required to characterize the active constituent(s) responsible for observed activities of the bark extract.

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