Effect of poly herbal formulation, EuMil, on neurochemical perturbations induced by chronic stress

A Bhattacharya¹, A V Muruganandan²*, Vikas Kumar² & S K Bhattacharya¹

¹Neuropharmacology Laboratory, Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, India
²R&D Centre, Indian Herbs Ltd., Saharanpur 247 001, India

Received 16 August 2001; revised 9 August 2002

EuMil, a polyherbal formulation consisting of standardised extracts of Withania somnifera (L) Dunal, Ocimum sanctum L., Asparagus racemosus Willd and Emblica officinalis Gaertn., is used as an anti-stress agent to attenuate the various aspects of stress related disorders. In the present study, the neurochemical mechanisms underlying the anti-stress activity of EuMil were evaluated by measuring the rat brain monoamine neurotransmitter levels and tribulin activity. Chronic electroshock stress (14 days) significantly decreased the nor-adrenaline (NA) and dopamine (DA) levels in frontal cortex, pons medulla, hypothalamus, hippocampus and striatal, hypothalal region, respectively, and increased the 5-hydroxytryptamine (5HT) level in frontal cortex, pons medulla, hypothalamus and hippocampus. Chronic stress, also increased the rat brain tribulin activity. EuMil (100 mg/kg, p.o., 14 days) treatment normalized the perturbed regional NA, DA, 5HT concentrations, induced by chronic stress. EuMil also significantly attenuated the stress-induced increase in the rat brain tribulin activity. The amelioration of chronic stress-induced neurochemical perturbations by EuMil explains the neurochemical mechanisms underlying the observed putative anti-stress activity of the product.

EuMil is a polyherbal formulation based on classical Ayurvedic literature and comprises of standardized extracts of Withania somnifera (L) Dunal, Ocimum sanctum L., Emblica officinalis Gaertn. and Asparagus racemosus Willd. All these herbs are classified in Ayurveda, the traditional Indian system of medicine as Rasayanas, which are claimed to improve physical and mental health, increase the resistance of body to infection and other external factors tending to perturb homeostasis of the human system and promote revival of physiological functions after debilitating diseases and to augment intellect¹.

Anti-stress activity of the major constituents of EuMil namely Withania somnifera, Ocimum sanctum are well documented²–⁴. Earlier, we have reported the putative anti-stress activity of EuMil in a wide spectrum of stress paradigms, viz. cold swim stress test, swim endurance test, immobilization stress-induced gastric ulcers and anoxia tolerance test⁵. In the present study the neurochemical mechanisms underlying the anti-stress activity of the EuMil were evaluated by estimating the monoamine neurotransmitters levels and tribulin activity in rat brain.

¹Correspondent author: E-mail: ihsee@vsnl.com; Fax: (0132) 726288

Materials and Methods

Animals—Male wister rats (210 – 240 g), were group housed in colony cages at an ambient temperature of 25 ± 2°C and 45-55% relative humidity, with a 12 hr light/dark cycle. The animals had free access to standard pellet chow (Hindustan Lever) and drinking water.

Drugs treatment—Rats were divided into four groups of six animals each. Group 1 served as a normal control and received vehicle (0.3% carboxy methyl cellulose, CMC, p.o.) only. Group 2 served as a stressed control and received vehicle and electroshock. Group 3 received EuMil (100 mg/kg, p.o.) only. Group 4 received EuMil (100 mg/kg, p.o.) for 14 days and Group 3 received EuMil (100 mg/kg, p.o.) for 14 days and electroshock (60 min after the administration of EuMil).

Induction of stress—Initially, the hanging stress model of stress was used. However, 2 weeks duration of hanging stress did not appear to induce significant degree of stress, as indicated by insignificant effects on gastric ulceration and plasma corticosterone levels. As such, an alternate chronic stress model was then adopted⁶. In this model, the rats were subjected to chronic electroshock stress by means of a convulsimeter (Techno), using pinnal electrodes. Increasing intensity of shock was given for a fixed duration of
0.2 sec each morning according to the following protocol: Day 1 - 50 mV, Day 2 - 60 mV, Day 3 - 70 mV, Day 4 - 80 mV, Day 5 - 90 mV, Day 6 - 100 mV, Day 7 - 14 - 100 mV. Experiments were conducted on Day 14, 1 hr after the last electroshock.

Estimation of monoamine and monoamine metabolites—Rats were sacrificed by decapitation on day 14th after the last electroshock. The brains were quickly dissected out, ice-cooled and the different brain regions hypothalamus, hippocampus, corpus striatum, brain stem and frontal cortex were dissected out. The tissues were weighed, frozen in dry ice and stored at -80°C until assay. The estimation of noradrenaline (NA), dopamine (DA) and 5-hydroxytryptamine (5-HT) was performed simultaneously by high performance liquid chromatography (HPLC) with electrochemical detector.

Estimation of brain tribulin activity—The brains were dissected out on day 14th after the last electroshock. The brains were homogenized (20% w/v) in cold 2 M HCl and centrifuged in cold (0-2°C) for 15 min. Tribulin activity was estimated by following the method of Bhattacharya et al.

Statistical analysis—Results were analysed by one way ANOVA and posthoc group comparisons were made by applying Newman-Keuls test.

Results and Discussion
The results indicate that chronic (14 days) electroshock stress had a significant effect on rat brain monoamine concentrations, which appears to be region dependent. Chronic stress significantly decreased the NA concentrations in frontal cortex, pons medulla (brain stem), hypothalamus and hippocampus, but had little effect on NA concentrations in striatum (Table 1). EuMil (100 mg/kg, p.o.) had little per se effect on brain NA but tended to normalize perturbed regional NA concentrations induced by chronic stress (Table 1).

Chronic stress significantly reduced striatal and hypothalamic DA concentrations, but had insignificant effect on DA concentrations in frontal cortex, brain stem and hippocampus (Table 1). EuMil (100 mg/kg, p.o.) had insignificant per se effect on all brain region, but normalized the decrease in striatal and hypothalamic DA concentrations induced by chronic stress (Table 1). Literature reports have also

Table 1—Effect of subchronic administration of EuMil on chronic stress induced alterations in rat brain noradrenaline, dopamine, 5-hydroxytryptamine concentration

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Control</th>
<th>Stress</th>
<th>EuMil</th>
<th>EuMil + Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noradrenaline</td>
<td>Dopamine</td>
<td>5-Hydroxytryptamine</td>
<td></td>
</tr>
<tr>
<td>Fronal cortex</td>
<td>0.42 ± 0.03</td>
<td>0.32 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46 ± 0.06</td>
<td>0.38 ± 0.05</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.41 ± 0.02</td>
<td>0.38 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42 ± 0.04</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.81 ± 0.04</td>
<td>0.56 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.84 ± 0.07</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>2.64 ± 0.82</td>
<td>1.84 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.74 ± 0.84</td>
<td>2.29 ± 0.41</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.41 ± 0.03</td>
<td>0.26 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.39 ± 0.04</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>Fronal cortex</td>
<td>0.51 ± 0.04</td>
<td>0.43 ± 0.08</td>
<td>0.59 ± 0.08</td>
<td>0.52 ± 0.05</td>
</tr>
<tr>
<td>Striatum</td>
<td>8.64 ± 0.36</td>
<td>6.68 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.04 ± 0.39</td>
<td>8.36 ± 0.29</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.46 ± 0.04</td>
<td>0.30 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.05</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.31 ± 0.02</td>
<td>0.26 ± 0.06</td>
<td>0.34 ± 0.04</td>
<td>0.32 ± 0.04</td>
</tr>
<tr>
<td>Fronal cortex</td>
<td>0.49 ± 0.06</td>
<td>0.69 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38 ± 0.06</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.06</td>
<td>0.69 ± 0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38 ± 0.06</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>3-Hydroxytryptamine</td>
<td>0.74 ± 0.09</td>
<td>0.86 ± 0.06</td>
<td>0.66 ± 0.06</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>3-Hydroxytryptamine</td>
<td>0.84 ± 0.06</td>
<td>1.08 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.92 ± 0.04</td>
<td>0.88 ± 0.06</td>
</tr>
<tr>
<td>3-Hydroxytryptamine</td>
<td>1.01 ± 0.04</td>
<td>1.44 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.92 ± 0.08</td>
<td>0.98 ± 0.08</td>
</tr>
<tr>
<td>3-Hydroxytryptamine</td>
<td>0.46 ± 0.04</td>
<td>0.79 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42 ± 0.06</td>
<td>0.44 ± 0.06</td>
</tr>
</tbody>
</table>

n = 6; p<sup>a</sup> < 0.05, <sup>b</sup> < 0.01, <sup>c</sup> 0.001 different from control (vehicle-treated) values. Data without superscripts are non-significant (p > 0.05) in comparison to control values.
Groups | Brain weight (gm) | Percent inhibition | MAO A | MAO B
---|---|---|---|---
Control (vehicle) | 2.02 ± 0.22 | 19.6 ± 1.6 | 28.4 ± 1.8 | 
Stress | 1.99 ± 0.16 | 29.4 ± 2.6 | 36.8 ± 2.9 | 
EuMil | 2.04 ± 0.19 | 17.2 ± 1.8 | 24.2 ± 2.8 | 
EuMil + Stress | 2.05 ± 0.16 | 22.8 ± 1.6 | 30.5 ± 1.4 | 

n = 6; *P < 0.05, †< 0.01, ‡0.001 different from control (vehicle treated) values. Data without superscripts are non-significant (P>0.05) in comparison to control values.

suggested the attenuated levels of NA and DA in stressful and depressive conditions9. Thus, normalization of the chronic stress-induced decreased levels of NA by EuMil explains its putative, observed, anti-stress activity.

Chronic stress significantly increased 5HT concentrations in frontal cortex, brainstem, hypothalamus and hippocampus but had insignificant effect on striatal 5HT. EuMil (100 mg/kg, p.o), had insignificant per se effect on brain 5HT concentrations, but could normalize the stress induced increases in regional brain 5HT concentrations (Table 1). Increased brain 5HT activity has been linked to anxiety10. Reports also indicate that raised 5HT function is involved in attenuated cognitive performance partially due to decrease in the acetylcholine release11. Chronic stress induced increase in 5HT function may be responsible for the stress associated anxiety and altered cognitive performance. Therefore, normalization of augmented serotonergic function, by EuMil may attenuate the anxiety and thereby producing anti-stress activity.

Chronic stress induced significant increase in rat brain tribulin activity as indicated by increase in the engodogenous MAO-A inhibitor (50%) and MAO-B inhibitor (29.6%) activity (Table 2). Although EuMil per se had insignificant effects on rat brain tribulin activity, it significantly decreased the stress effects. The increases in MAO-A and MAO-B inhibitor activity induced by stress in EuMil treated rat was 16.3% and 7.4%, respectively (Table 2). Brain tribulin activity has been postulated to function as a marker of stress and anxiety5. Recent studies indicated that the MAO-A inhibitory component of tribulin may be more important in this context than the MAO-B inhibitor moiety12. Anti-stress drugs, including benzodiazepines and non-benzodiazepine agents, like ondansetron, are known to reduce raised MAO-A inhibitor activity following stress situations or administration of anxiogenic agents13.

The perturbations in behavioural effects induced by chronic electroshock stress and their amelioration following subchronic treatment with EuMil, can be rationalized to a large extent by the present findings.

Acknowledgement
The authors are grateful to the Indian Herbs Ltd., Saharanpur for providing the financial assistance to carry out the present study.

References