Introduction

Stress is a common phenomenon in the present scenario of life. It has become an integral part of life. Extreme stressful conditions results in modulation of homeostasis, which may lead to pathogenesis of many diseases like diabetes, hypertension, anxiety, peptic ulcer, etc (Desai et al., 2011). To overcome this stress many drugs like diazepam, amphetamine, anabolic steroids are widely used. But problems associated with these drugs are; development of drug dependence and toxicity. Since ancient times, herbal plants are used as remedy to combat stress. Literature report reveals that many poly herbal formulations such as Siotone (Bhattacharya et al., 2000), AVM (Shaik et al., 2006) and Ranahamsa Rasayanaya (Indrajith et al., 2010) have been reported to possess significant antistress activity. Some of the ingredients of this formulation namely *Tribulus terrestris* (Shivakumar et al., 2006), *Emblica officinalis* (Nirmala et al., 1999) and *Coriandrum sativum* (Koppula S and Choi. 2012) have been reported to possess significant antistress activity. *Cocos nucifera* is one among the various ingredients of Ranahamsa Rasayanaya, whose antistress activity has not been scientifically validated till date.

Hence, the present research aimed to explore the antistress potential of ethanolic extract of endocarp *Cocos nucifera* in immobilization stress model.

Materials and Methods

Plant material

The *Cocos nucifera* endocarp was collected from local market after identification and authentication by Dr. M. B.Mulimani, Professor of Botany, S.B Arts and K.C.P. Science College, Bijapur, Karnataka. A voucher specimen (CN03) has been deposited at the herbarium of Dept. of Pharmacology, HSK College of Pharmacy, Bagalkot.

Fresh endocarp was air dried, pulverized to a coarse powder. Investigation on anti-stress activity of endocarp *Cocos nucifera*

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Abstract

Objective: *Cocos nucifera* is one among the ingredients of polyherbal formulation used for the management of stress by Ayurvedic practitioners. Hence, the present study was undertaken to scientifically validate antistress effect of *Cocos nucifera* in immobilization stress model. Materials and Methods: Rats were administered graded doses of ethanolic extract of *Cocos nucifera* endocarp for 10 days. All the animals were subjected for immobilization stress and its antistress efficacy was assessed by estimating various parameters such as hematology, biochemicals like serum glucose, cholesterol, triglycerides and blood urea nitrogen (BUN), brain neurotransmitters, organs weight and body weight. Results: Stressed rats demonstrated altered values of all these parameters. Ethanolic extract of *Cocos nucifera* endocarp exhibited significant anti-stress activity by restoration of all the altered values. Conclusion: Present findings validate its use in Ayurvedic system of medicine.

Keywords: Antistress, *Cocos nucifera*, immobilization stress, phenolic content, flavonoid content

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by using grinder and passed through a 40-mesh sieve. Then the powdered material was packed into Soxhlet column and extracted with ethanol. After this, the extract was concentrated using rotary flash evaporator.

**Phytochemical investigations**

**Preliminary phytochemical screening**

Test extract was subjected to preliminary phytochemical screening for the detection of various phytoconstituents (Idris Bello et al., 2016).

**Total phenolic content**

The total phenolic content of *Cocos nucifera* extract was determined UV spectrophotometrically using folin-Ciocalteu method. 0.5 ml of folin-Ciocalteu reagent was mixed with aliquots (0.1 ml) of *Cocos nucifera* extract and made up to 3 ml with distilled water. After 3 min, 2 ml of sodium carbonate (20%) was added and mixed thoroughly. The sample was then incubated at 50°C for 5 min and then cooled. The absorbance of sample was measured at 650 nm against the blank. The total phenolic content of extract was expressed as mg gallic acid equivalent per gm of extract. The coefficient of determination was $r^2 = 0.9968$.

**Total flavonoid content**

The total flavonoid content of *Cocos nucifera* extract was estimated using aluminium chloride method. 0.5 ml of sample solution was mixed with 0.5 ml of AlCl$_3$, ethanol solution (2%). This mixture was kept for incubation at room temperature for 1 hr. Then absorbance of sample was measured at 420 nm. Extract samples were evaluated at a final concentration of 0.1 mg/ml. The total flavonoid content was calculated as mg quercetin equivalent per gm of extract. The coefficient of determination was $r^2 = 0.9965$ (Stankovic, 2011).

**Determination of Acute toxicity (L.D$_{50}$)**

Study protocol was approved from Institutional Animal Ethics Committee (IAEC) before initiation of the experiment (Ref No. BPC/82/2014 dated 09/06/2014).

In this study, the animals were fasted for 18 hr before the experiment. After dosing, food but not water was withheld for further 1 hr. Mortality and general behavior of the animals observed individually at least once during the first 30 minutes, periodically during the first 24 hr, with special attention given during the first 4 hr and daily thereafter, for a total of 14 days. Fixed dose method of OECD Guideline No. 423 was followed for toxicity study.

Based on the results of the study, 1/5$^a$, 1/10$^a$ and 1/20$^a$ of L.D$_{50}$ cut off value were selected as screening doses for investigation (Saeed et al., 2017).

**Evaluation of antistress activity of ethanolic extract of Cocos nucifera endocarp in immobilization stress**

**Experimental design**

Adult albino rats of either sex weighing 150 – 200 g were distributed into six groups ($n=6$):

- **Group I:** Normal control (NC: untreated);
- **Group II:** Stress control (SC: received vehicle);
- **Group III:** Std (WS: received *Withania somnifera* 100 mg/kg, p.o (per oral));
- **Group IV:** EECNE 125 mg/kg, p.o;
- **Group V:** EECNE 250 mg/kg, p.o;
- **Group VI:** EECNE 500 mg/kg, p.o.

The treatment was made as stated above for 10 days 1hr before the exposure of stress. Stress was induced by immobilizing rats with head down, supine position by fixing the forelimbs and hind limbs to a wooden board inclined at an angle of 60° daily 2 hr for a period of 10 days, except normal control rats (Brajnandan, 2017).

**Hematological and biochemical estimations**

On the last day 1 hour after drug treatment, the blood was collected from retro-orbital puncture under mild anaesthesia in sodium citrate tubes. Thus collected blood was utilised for estimation of hemoglobin (Hb), RBC, WBC, differential leucocytes count (DLC) and platelets and also for the estimation of biochemical parameters such as, serum glucose (Kulkarni and Juvekar., 2008), cholesterol (Allain et al., 1974), triglycerides (McGowan et al., 1983)and BUN (Sibi and Sajid, 2013).

The rats were then euthanized by decapitation for collection of organs like brain, liver, spleen and adrenal glands. Liver, spleen and adrenal glands weight was recorded after washing with alcohol per 100 g body weight (Tsigos and Chrousos, 2002).

**Estimation of brain neurotransmitters**

Norepinephrine (NA) and serotonin (5-HT) levels of brain of all rats were estimated using high-performance liquid chromatographic (HPLC) technique (Tache et al., 1976).

**Statistical analysis**

The data were demonstrated as mean ± Standard error of mean (SEM). Analysis of variance followed by a performance of Tukey's Kramer multiple comparison test, as a $p<0.05$ being significant. GraphPad Prism® 5.0 software was used to measure the level of significance.

**Results**

**Phytochemical analysis**

The total phenolic content of the extract was found to be
48.37 ± 2.61 gallic acid equivalents/g and the total flavonoid content was 39.72 ± 2.85 rutin equivalents/g.

**Anti-stress activity of ethanol extract of Cocos nucifera endocarp in immobilization stress model**

**Effect of EECNE on haematological parameters**

In the present study, stress control rats shows a decreased Hb level, percentage of lymphocytes and eosinophils, however elevated RBC, WBC, platelets count and percentage increase in neutrophils and monocytes monitored over normal control. Rats pre-treated with test extract at different doses (250 and 500 mg/kg) significantly restored these altered haematological parameters compared to stress control group. Whereas, extract at lowest dose i.e.125 mg/kg also restored these values but it was found to be statistically non-significant when compared with control group (Table 1).

**Effect of EECNE on biochemical parameters**

It was observed that the exposure of rats to immobilization stress caused significant elevation of serum triglycerides, glucose, total cholesterol and BUN levels over normal control rats. Animals pre-treatment with graded doses (250 mg/kg and 500 mg/kg except 125 mg/kg) of EECNE demonstrated significant attenuation of these altered biochemical parameters (Table 2).

**Effect of EECNE on organs weight**

The immobilization stress induced a marked change in weight of organs i.e. significant gain in liver and adrenal glands weight and reduction in weight of spleen when compared to normal control. Rats pre-treatment with EECNE at 250 mg/kg and 500 mg/kg significantly reversed the altered weight of organs (Table 3).

**Effect of EECNE on body weight**

The rats subjected to immobilization stress shown slow body weight accumulation as compared to normal control animals, where normal body weight accumulation observed. It was observed that the test extract at different doses significantly (250 mg/kg and 500 mg/kg) slow down the stress mediated loss of body weight (Table 4).

**Effect of EECNE on brain neurotransmitters**

HPLC method of determination of brain neurotransmitters indicates that, rats exposed to stress shown a significant reduction of NA and 5-HT levels compared to normal control. Pre-treatment of rats with EECNE demonstrated significant restoration of these altered neurotransmitters level except lowest dose i.e. 125 mg/kg (Table 5).

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**Table 1. Effect of EECNE on haematological parameters**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Hb g %</th>
<th>RBC millions/cmm</th>
<th>Platelets lakhs/cmm</th>
<th>WBC thousands/cmm</th>
<th>Differential leucocytes count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>--</td>
<td>13.48±0.21</td>
<td>6.61±0.38</td>
<td>8.48±0.67</td>
<td>9.69±0.38</td>
<td>N: 45.68±0.87 45.80±0.41 2.64±0.23</td>
</tr>
<tr>
<td>II</td>
<td>SC</td>
<td>Vehicle</td>
<td>9.42±0.37</td>
<td>10.94±0.56</td>
<td>13.38±0.45</td>
<td>15.80±0.41</td>
<td>L: 66.04±0.67 12.76±0.66 5.75±0.55 0.89±0.52</td>
</tr>
<tr>
<td>III</td>
<td>WS</td>
<td>100</td>
<td>12.96±0.24</td>
<td>6.98±0.73</td>
<td>8.89±0.57</td>
<td>10.81±0.84</td>
<td>E: 47.72±0.43 30.38±0.82 4.92±0.40 2.52±0.26</td>
</tr>
<tr>
<td>IV</td>
<td>EECNE</td>
<td>125</td>
<td>10.03±0.32</td>
<td>9.35±0.78</td>
<td>11.77±0.46</td>
<td>14.56±0.67</td>
<td>N: 63.66±0.46 15.65±0.36 5.59±0.36 1.22±0.63</td>
</tr>
<tr>
<td>V</td>
<td>EECNE</td>
<td>250</td>
<td>11.58±0.38</td>
<td>8.38±0.82</td>
<td>10.20±0.65</td>
<td>12.72±0.55</td>
<td>L: 57.87±0.50 24.23±0.39 5.23±0.32 1.90±0.45</td>
</tr>
<tr>
<td>VI</td>
<td>EECNE</td>
<td>500</td>
<td>12.56±0.40</td>
<td>7.21±0.54</td>
<td>9.47±0.94</td>
<td>10.33±0.84</td>
<td>M: 48.67±0.75 29.52±0.68 4.99±0.28 2.45±0.51</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, (n=6), where *Non-significant, p<0.05, **p<0.01, ***p<0.001 as compared to control. L: Lymphocytes, E: Eosinophils, N: Neutrophils, M: Monocytes

**Table 2. Effect of EECNE on serum biochemical changes**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Glucose (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>BUN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>--</td>
<td>93.87±6.88</td>
<td>54.48±3.92</td>
<td>58.09±4.73</td>
<td>17.68±1.75</td>
</tr>
<tr>
<td>II</td>
<td>SC</td>
<td>Vehicle</td>
<td>142.13±6.30</td>
<td>127.56±5.76</td>
<td>94.76±3.24</td>
<td>35.22±3.38</td>
</tr>
<tr>
<td>III</td>
<td>WS</td>
<td>100</td>
<td>94.65±6.38</td>
<td>50.06±5.55</td>
<td>50.30±2.35</td>
<td>18.03±1.75</td>
</tr>
<tr>
<td>IV</td>
<td>EECNE</td>
<td>125</td>
<td>132.78±7.43**</td>
<td>123.45±5.19***</td>
<td>90.07±3.20*</td>
<td>32.45±2.61**</td>
</tr>
<tr>
<td>V</td>
<td>EECNE</td>
<td>250</td>
<td>120.07±7.25**</td>
<td>97.23±5.72***</td>
<td>74.65±2.48*</td>
<td>26.06±2.39*</td>
</tr>
<tr>
<td>VI</td>
<td>EECNE</td>
<td>500</td>
<td>98.58±6.27**</td>
<td>61.64±5.91***</td>
<td>62.72±4.53***</td>
<td>20.36±1.37***</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, (n=6), where *Non-significant, p<0.05, **p<0.01, ***p<0.001 as compared to control. NC: Normal control, SC: Stress control, WS: Withania somnifera, EECNE: Ethanolic extract of Cocos nucifera endocarp.
Discussion

The literature survey reveals that, the immobilization stress is considered as the most severe type of stress and one of the most widely used experimental animal models. The results of the present study indicate that, the stress has altered the haematological parameters and animals pre-treated with test extract for the period of 10 days, significantly ameliorated the adverse effect of stress on these haematological changes. This might be due to anti-oxidant or anti-lipid peroxidation effect of EECNE.

Table 3. Effect of EECNE on organs weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Liver (g/100 g b.w.)</th>
<th>Spleen (g/100 g b.w.)</th>
<th>Adrenal glands (g/100 g b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>--</td>
<td>3.79 ± 0.54</td>
<td>0.189 ± 0.04</td>
<td>0.168 ± 0.04</td>
</tr>
<tr>
<td>II</td>
<td>SC</td>
<td>Vehicle</td>
<td>7.09 ± 0.65</td>
<td>0.061 ± 0.01</td>
<td>0.483 ± 0.06</td>
</tr>
<tr>
<td>III</td>
<td>WS</td>
<td>100</td>
<td>3.98 ± 0.44&quot;***</td>
<td>0.179 ± 0.03&quot;***</td>
<td>0.178 ± 0.03&quot;***</td>
</tr>
<tr>
<td>IV</td>
<td>EECNE</td>
<td>125</td>
<td>6.48 ± 0.82&quot;ns</td>
<td>0.077 ± 0.01&quot;ns</td>
<td>0.426 ± 0.06&quot;ns</td>
</tr>
<tr>
<td>V</td>
<td>EECNE</td>
<td>250</td>
<td>5.27 ± 0.38&quot;**</td>
<td>0.116 ± 0.03&quot;**</td>
<td>0.230 ± 0.04&quot;**</td>
</tr>
<tr>
<td>VI</td>
<td>EECNE</td>
<td>500</td>
<td>4.33 ± 0.69&quot;***</td>
<td>0.163 ± 0.03&quot;**</td>
<td>0.191 ± 0.03&quot;**</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, (n=6), where "Non-significant, p< 0.01, "p< 0.001 as compared to control. NC:Normal control, SC:Stress control, WS:Withania somnifera, EECNE:Ethanolic extract of Cocos nucifera endocarp.

Table 4. Effect of EECNE on body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial Body weight (g)</th>
<th>Last day Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>NC</td>
<td>--</td>
<td>153.58 ± 5.20</td>
<td>182.73 ± 8.39</td>
</tr>
<tr>
<td>II</td>
<td>SC</td>
<td>Vehicle</td>
<td>152.27 ± 6.32</td>
<td>157.41 ± 6.74</td>
</tr>
<tr>
<td>III</td>
<td>WS</td>
<td>100</td>
<td>155.71 ± 9.04</td>
<td>186.62 ± 9.47&quot;***</td>
</tr>
<tr>
<td>IV</td>
<td>EECNE</td>
<td>125</td>
<td>156.68 ± 6.06</td>
<td>159.76 ± 6.55&quot;ns</td>
</tr>
<tr>
<td>V</td>
<td>EECNE</td>
<td>250</td>
<td>154.31 ± 6.69</td>
<td>167.61 ± 6.27&quot;</td>
</tr>
<tr>
<td>VI</td>
<td>EECNE</td>
<td>500</td>
<td>157.82 ± 7.79</td>
<td>175.43 ± 7.45&quot;**</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, (n=6), where "Non-significant, p< 0.05, "p< 0.01; "p< 0.001 as compared to control. NC:Normal control, SC:Stress control, WS:Withania somnifera, EECNE:Ethanolic extract of Cocos nucifera endocarp.

Table 5. Effect of EECNE on brain neurotransmitters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Brain neurotransmitters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nor-adrenaline (µg/g)</td>
</tr>
<tr>
<td>I</td>
<td>NC</td>
<td>--</td>
<td>0.182 ± 0.008</td>
</tr>
<tr>
<td>II</td>
<td>SC</td>
<td>Vehicle</td>
<td>0.047 ± 0.005</td>
</tr>
<tr>
<td>III</td>
<td>WS</td>
<td>100</td>
<td>0.154 ± 0.007&quot;***</td>
</tr>
<tr>
<td>IV</td>
<td>EECNE</td>
<td>125</td>
<td>0.052 ± 1.67&quot;ns</td>
</tr>
<tr>
<td>V</td>
<td>EECNE</td>
<td>250</td>
<td>0.94 ± 0.45&quot;**</td>
</tr>
<tr>
<td>VI</td>
<td>EECNE</td>
<td>500</td>
<td>0.139 ± 0.41&quot;***</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, (n=6), where "Non-significant, p< 0.05; "p<0.01; "p<0.001 as compared to control. NC:Normal control, SC:Stress control, WS:Withania somnifera, EECNE:Ethanolic extract of Cocos nucifera endocarp.
gluconeogenesis and lipogenesis. Immobilization stress leads to hyper-activation of adrenal cortex, which in turn releases excessive amount of cortisol, which influences mobilization of carbohydrate reserves and stored fat that causes hyperglycaemia and increased triglyceride levels (Tom et al., 2017). In the present research work we found that, the title plant has significantly reduced stress induced hyperglycaemia. This effect may be due to reduction in the cortisol level probably by preventing hyperactivation of adrenal cortex (Brajnandan et al., 2017).

The stress causes over-activation of hypothalamo-hypophyseal axis (HPA) which in turn releases excess catecholamines and corticosteroids; this could lead to rise in blood cholesterol level since adrenaline is known for mobilization of lipids from adipose tissues. This release of excess catecholamines also leads to rise in triglycerides and blood urea nitrogen levels (Kannur et al., 2016). From our research it was found that the extract significantly restored all these levels probably due to inhibition of stress induced catecholamines release by regulating hyperactive hypothalamo-hypophyseal axis.

Exposure of animals to stress resulted in significant reduction of weight of spleen with concomitant gain in liver and adrenal glands weight. The previous reports suggest that rise in weight of liver is attributed to increased cortisol which in turn enhances mRNA levels in liver. Whereas adrenal gland weight is increased because stress stimulates adreno–medullary response leads to release of adrenaline brings about excessive release of ACTH, which in turn stimulates the adrenal medulla and cortex results in rise of adrenal gland weight. Stress reduces the weight of spleen; this is due to constriction of spleen to release more RBCs under stressful conditions (Schimmer and Parker, 2006). However, the animals pre-treated with extract significantly reverted the altered weight of organs and this may be due to normalization of cortisol release (maintains liver weight), regulation of adreno–medullary response (maintains adrenal gland weight) and reduced constriction of spleen (maintains spleen weight).

In our study, it was observed that the normal control rats showed normal weight gain, whereas the stressed rats exhibited decrease in the body weight accumulation. The decrease in body weight in stressed rats may be due to reduced food intake or increase in metabolic demands, reduced digestion and increased adrenal steroid secretion. The animals pre-treated with EECNE have exhibited prevention in the weight loss induced by immobilization stress. This observation is probably may be due to increase in food intake, decrease in metabolic demands, increased digestion process or decreased adrenal steroid secretion (Cristina and Suzanne, 2016).

Reports suggest that, the neurotransmitters such as nor-adrenaline (NA) and serotonin (5-HT) plays a vital role in tackling of stress (Gonzalo and Louis, 2003). Under severe stress conditions these neurotransmitters levels are significantly decreased. In our study also, brain levels of NA and 5-HT were found to be reduced significantly in immobilization stress model. Animals pre-treated with extract demonstrated significant improvement of levels of these neurotransmitters indicates the adaptogenic potential of EECNE.

Earlier reports on the chemical constituents of plants and their pharmacology suggest that plant containing phenolic compounds, flavonoids, possess significant activity against many CNS disorders (Tache et al., 1976). Investigations on the phytochemical screening of EECNE revealed the presence of phenolic compounds and flavonoids. It is possible that the mechanism of antistress action of plant could be mediated by these phytochemicals (Suaib et al., 2012; Catarino et al., 2015).

**Conclusion**

*Cocos nucifera* endocarp extract has shown significant improvement in stress-induced alterations of hematological and biochemical parameters, body weight, organs weight and brain neurotransmitters levels, indicating its protective effect against stress. However, further experiments are needed to establish the mechanism behind the adaptogenic potential of plant.

**Conflict of interest statement**

The authors declared that there is no conflict of interest.

**Acknowledgement**

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**References**


