Adaptogenic activity of Phyllanthus niruri Linn on Swim Endurance and Cold Stress animal model

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Abstract: Whole plant of Phyllanthus niruri Linn. (Euphorbiaceae) were screened for adaptogenic activity using Swim Endurance model and Cold Stress model. Ethyl acetate extract as well as Pet ether (40-60°C) and 70% ethanol extracts showed significant adaptogenic activity when administered orally at a dose of 200 mg/kg body weight. The extracts significantly increased the active swim endurance time. Stress induced animals exhibited hyperglycemia as well as depletion in triglyceride level, increased total leukocyte count and restored the organ weights. The extracts showed a significant action in overcoming these imbalances. It was also found that extracts proved efficient in controlling the hyperlipidemic condition due to stress.

KEYWORDS: Adaptogenic activity, Phyllanthus niruri, Swim endurance, cold stress.

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INTRODUCTION
Stress is nonspecific response of the body known to alter the physiological homeostasis of the organism resulting in various neuronal, endocrine, and visceral dysfunctions. The ability to develop and maintain resistance against a variety of stressors encountered in human life is crucial for survival. According to WHO, approximately 450 million people suffer from mental or behavioral disorders like stress. This contribute 12.3% of the global burden of disease and is predicted to rise up to 15% by 2020. It is estimated that 75–90% of visits to primary care physicians are related to stress either acutely or because of chronic problems associated with stress.

Adaptogens cause an adaptive reaction to a disease and are useful in many unrelated illness and appear to produce a state of non specific increased resistance during stress resulting in stress protection. The adaptogens not only help in coping with stress but help in enhancing the general state of well being. Studies on the mechanism of action of adaptogenic drugs revealed that they produce immunostimulation. These drugs strengthen the defense mechanism against the free radical induced damage inducing stress. Plants with potent antioxidant activity have been reviewed for their immunomodulatory and adaptogenic plenty of research to prove that these drugs potent antioxidant activity. The whole plant of Phyllanthus niruri Linn. (Euphorbiaceae) an annual herb of 30-60 cm height, generally found in Karnataka and other tropical regions. Traditionally the plant is used and to treat jaundice, digestive, diuretic, febrifuge, hepato tonic, and as stonebreaker. Scientifically the plant possesses hepatoprotective, antioxidant, antiplasmodial, lipid lowering activity and vasorelaxant. Whole plant of Phyllanthus niruri Linn found to contain various chemical constituents such as Ellagitannin, phyllanthin D, Brevifolin carboxylic acid, isouqueretin, lignans, nirantin nirtetrinal, phyltetralin, phyllanthin, hypophyslanthin, saponins, kemiferol ramnopyranoside, and eridictyol ramnopyranoside.

The whole plant of Phyllanthus niruri have a reported antioxidants and phytochemical constituents like flavanoids, Tannins, Lignans. But no reports are available in adaptogenic activity. Therefore authors
have planned to study the adaptogenic activity of *Phyllanthus niruri* Linn using animal models.

**Material and Methods**

**Collection of plant and authentication**
The *Phyllanthus niruri* Linn whole plant was collected from the local areas of Hubballi, Karnataka, and authenticated by Dr. B.D. Huddar, Head, Department of botany, Kadasiddeshwar Arts College and H.S. Kotambari Science Institute, Hubballi.

**Preparation of Extract**
The whole plant was thoroughly washed with running tap water to remove the adherent impurities. Shade dried and powdered and passed through a sieve # 44 to get uniform powder size. Stored in air tight container. The crude powdered was subjected to successive soxhlet extraction with pet ether (40-60°C), Ethyl acetate and 70% Ethanol. Excess solvent was removed by rotary film flash evaporator apparatus and residue was concentrated by using Lyotrap dryer. Final extracts were preserved in desiccator.

**Animals**
The experiment was carried out using Swiss albino mice of either sex weighing between 20-30gm for acute toxicity study and both Swiss albino mice and Wistar albino rats of either sex weighing around 20-30gm & 150-250gms respectively were used. The animals were supplied by K.L.E.S’s College of Pharmacy, Hubballi. Animals were maintained at normal laboratory conditions and were fed with standard animal feed.

**Drugs, Chemicals and Reagents**
Geriforte tablets (Himalaya drugs) 43 mg/kg was used as a standard adaptogenic drug which is multi-constituent ayurvedic drug with 35n herbal and natural constituents like Withania somnifera, Asparagus racemosus, Glycyrrhiza glabra, Centella Asiatic, Terminalia chebula, Piperlongum, Shilajit, 1% Tween 80, Erba kits for biochemical estimations etc.

**Acute Toxicity Studies**
Albino mice of either sex weighing between 20-30gm were used during investigation. The animals were fasted overnight. The OCED guideline no-42315 fixed dose method was adopted and accordingly LD₅₀ of pet ether (40-60°C) (PE), Ethyl acetate(EtoAC) and 70% Ethanolic (EtOH) extracts were found.

**Swim Endurance Test**
The animals were divided into five groups of each six animals as follows:
- **Group-I** : Control (Stress)
- **Group-II** : Standard (Stress + Standard drug 43 mg/kg b.wt orally)
- **Group-III** : Extract-I (Stress+ Pet. Ether 200mg/kg b.wt)
- **Group-IV** : Extract-II (Stress+ Ethyl acetate 200mg/kg b.wt)
- **Group-V** : Extract-III (Stress+70%Ethanol 200mg/kg b.wt)

After drug administration the animals were forced to swim in chambers containing water at room temperature. The mice were allowed to swim till they got exhausted and the moment they actively swim is consider as active time and were they floating there is no moment is consider as passive time and at last they drowned was considered as the endpoint. The active time, passive time, endpoint was noted⁶¹⁷.

**Cold Stress Method**
The animals were divided into six groups of each six animals as follows:
- **Group-I** : Normal control
- **Group-II** : Negative control (Stress + Untreated)
- **Group-III** : (Stress + Standard drug 43 mg/kg b.wt orally)
- **Group- IV** : Extract-I (Stress+ Pet ether 200mg/kg b.wt)
- **Group- V** : Extract-II (Stress+ Ethyl acetate 200mg/kg b.wt)
- **Group-VI** : Extract-III (Stress+70%Ethanol 200mg/kg b.wt)

Induction of stress is done by using the cold stress model in this the stress was induced by exposing animals to 4°C for 4 h. The animals were taken from their home cages and individually placed in polymer containers. The containers were placed inside refrigerator such that the temperature to which the animals are exposed to 4°C, they are returned to their home cages after 4 h. This procedure will be repeated for 10 days at a specific time period between 10:00 a.m. to 2:00 p.m. All the extracts were administered by oral route. The animals were treated with extracts and then stress was induced for 10 days during this period the animals were free to have food and water, on the 11th day animals were anesthetized with ether & 1ml blood
was collected into the eppindof tube by retro-orbital vein puncture. The blood collected in eppindof tube was centrifuged to separate serum and used for the determination of blood glucose, total cholesterol, triglycerides Blood urea nitrogen, HDL- Cholesterol and blood was stored in Heparin for Total Leukocyte Count & Differential cell count\textsuperscript{16,17}.

Statistical analysis

All the data are expressed in mean ± SEM. The significance of difference in means between control and treated animals was determined by One-way analysis of variance (ANOVA) followed by Dunnette multiple comparison test and p<0.05 was considered statistically significant.

Results

Table: 01. Effect of Whole plant extracts of Phyllanthus niruri L. and standard formulation (Gerifort) on swim endurance test.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Active Time(min)</th>
<th>Passive Time(min)</th>
<th>End Point(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>114.5 ± 1.532</td>
<td>5.620 ± 0.09165</td>
<td>120.1 ± 2.696</td>
</tr>
<tr>
<td>Stress+ Standard</td>
<td>239.7 ± 2.116***</td>
<td>1.510 ± 0.03120***</td>
<td>141.2 ± 53.612 ***</td>
</tr>
<tr>
<td>Stress+ PE</td>
<td>152.6 ± 0.8950***</td>
<td>1.510 ± 0.0493***</td>
<td>154.1 ± 0.881***</td>
</tr>
<tr>
<td>Stress+ EtAC</td>
<td>201.5 ± 0.6333***</td>
<td>2.610 ± 0.0493***</td>
<td>204.1 ± 0.619***</td>
</tr>
<tr>
<td>Stress+70% EtOH</td>
<td>131.3 ± 1.421**</td>
<td>5.822 ± 0.0727**</td>
<td>137.1 ± 1.353**</td>
</tr>
</tbody>
</table>

All results were expressed as Mean ± SEM; P < 0.0001***, P < 0.001**, as compared to Stress control. (PE-Petroleum Ether, EtAC-Ethyl acetate, EtOH-Ethanol)

Table: 02. Effect of Whole plant extracts of Phyllanthus niruri L. and standard formulation (Gerifort) on Cold stress induced change in the biochemical parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical parameters</th>
<th>BGL(mg/dl)</th>
<th>TG(mg/dl)</th>
<th>CHO(mg/dl)</th>
<th>HDL(mg/dl)</th>
<th>BUN(mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>89.79±2.640</td>
<td>58.53±1.338</td>
<td>72.20±1.172</td>
<td>42.53±0.728</td>
<td>20.71±0.91</td>
<td></td>
</tr>
<tr>
<td>Stress+control</td>
<td>151.8±4.396</td>
<td>45.68±0.959</td>
<td>91.90±1.731</td>
<td>51.23±0.554</td>
<td>35.23±0.801</td>
<td></td>
</tr>
<tr>
<td>Stress+standard</td>
<td>105.6±2.626***</td>
<td>58.94±1.892***</td>
<td>77.04±2.128***</td>
<td>42.45±0.353***</td>
<td>24.07±1.08***</td>
<td></td>
</tr>
<tr>
<td>Stress+PE</td>
<td>112.9±3.795***</td>
<td>54.89±1.104***</td>
<td>79.50±2.405***</td>
<td>45.39±0.610***</td>
<td>28.30±1.17***</td>
<td></td>
</tr>
<tr>
<td>Stress+EtAC</td>
<td>104.8±2.998***</td>
<td>58.89±2.438***</td>
<td>78.02±2.201***</td>
<td>43.70±0.621***</td>
<td>23.5±1.23***</td>
<td></td>
</tr>
<tr>
<td>Stress+70%EtOH</td>
<td>119.9±4.996***</td>
<td>54.78±1.087**</td>
<td>83.50±0.984*</td>
<td>47.44±0.503***</td>
<td>28.01±2.24**</td>
<td></td>
</tr>
</tbody>
</table>

All results were expressed as Mean ± SEM; P < 0.0001***, P < 0.001**, P < 0.05*, as compared to Stress control. (PE-Petroleum Ether, EtAC-Ethyl acetate, EtOH-Ethanol)

Table: 03. Effect of Whole plant extracts of Phyllanthus niruri L. and standard formulation (Gerifort) on Cold stress induced change in the organ weights.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight of organ/100gm body wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Normal</td>
<td>4.95±0.005</td>
</tr>
<tr>
<td>Stress+control</td>
<td>6.125±0.0005</td>
</tr>
<tr>
<td>Stress+standard</td>
<td>5.12±0.0005***</td>
</tr>
<tr>
<td>Stress+PE</td>
<td>5.42±0.001***</td>
</tr>
<tr>
<td>Stress+EtAC</td>
<td>5.26±0.0005***</td>
</tr>
<tr>
<td>Stress+70%EtOH</td>
<td>5.62±0.0005***</td>
</tr>
</tbody>
</table>

All results were expressed as Mean ± SEM; P < 0.0001***, P < 0.001**, P < 0.05*, as compared to Stress control. (PE-Petroleum Ether, EtAC-Ethyl acetate, EtOH-Ethanol)
Table 04: Effect of Whole plant extracts of *Phyllanthus niruri* L. and standard formulation (Gerifort) on Cold stress induced change in the TLC Cells/cumm and Differential cell count No. Cells/100 blood cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TLCCells/cumm</th>
<th>Differential cell count No. Cells/100 blood cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Normal</td>
<td>4682±90.84</td>
<td>21.15 ± 0.050</td>
</tr>
<tr>
<td>Stress+control</td>
<td>7504±29.35</td>
<td>37.45 ± 0.050</td>
</tr>
<tr>
<td>Stress++standard</td>
<td>4802±155.0***</td>
<td>25.15 ± 0.050***</td>
</tr>
<tr>
<td>Stress+PE</td>
<td>4464±807.3***</td>
<td>28.25 ± 0.050***</td>
</tr>
<tr>
<td>Stress+EtoAc</td>
<td>4993±80.67***</td>
<td>26.15 ± 0.050***</td>
</tr>
<tr>
<td>Stress+70%ETOH</td>
<td>6054±183.5**</td>
<td>30.95 ± 0.150***</td>
</tr>
</tbody>
</table>

All results were expressed as Mean ± SEM; *P < 0.0001***, *P < 0.001**, *P < 0.05*, as compared to Stress control. (PE: Petroleum Ether, EtoAC: Ethyl acetate, EtoOH:Ethanol)

**Cold stress model**

Table 2 reveals that all the biochemical parameters like Blood sugar level (89.79±2.640 mg/dl), cholesterol (91.90±1.731mg/dl), HDL (51.23±0.554mg/dl) and BUN (20.71±0.91) were increased significantly and TG (45.68±0.959 mg/dl) decreased significantly in control group as compared with normal. In contrast the standard and extract treated groups showed significant increase in TG and decrease in Blood sugar level, cholesterol, HDL and BUN levels as compared with control group. In table 3 the weight increased significantly in control group as compared with normal but the standard and extract treated groups showed significant decrease in organ weights as compared with control group. Table 4 reveals that total leukocyte count (7504±29.35) significantly increased in control group as compared with normal.

**Discussion**

All the body functions, including cellular respiration depends on the oxygen supply. Lack of any vital element will play havoc on all body mechanisms. Increase in adaptation due to the depletion of any vital elements during stress by any drug that increases the tolerance can acts as adaptogenic agent. Adaptogens produce beneficial effects in stress which are believed to act by increasing the non specific resistance. The present study is investigated for the ability of the extracts of whole plant of *Phyllanthus niruri* to supress stress induced changes in the Swim endurance test; The Petroleum Ether (40-60°C), Ethyl acetate extract of Whole plant of *Phyllanthus niruri* highly significant in increasing active swim time, whereas 70%ethanol moderately significant in increasing active swim time. The enhanced active swimming time in mice as compared to the normal animals may be attributed to the steroids and the flavonoids which are found in the Petroleum Ether (40-60°C), Ethyl acetate extracts.

Biochemical parameters and organ weights in cold stress is of psychogenic type only. Response to stress increases blood sugar levels, cholesterol, HDL-Cholesterol, BUN, total leukocyte count, differential count, organ weight and depletes triglyceride levels to some extent.

The Petroleum Ether (40-60°C), Ethyl acetate, 70%Ethanol extracts of Whole plant of *Phyllanthus niruri* produced significant reduction in blood glucose level which is compared with stress control after 10 days of treatment.

Ethyl acetate extract was found to be more effective and highly significant in lowering elevated blood glucose level (*P < 0.0001*), total cholesterol (*P < 0.0001*) levels, HDL-Cholesterol (*P < 0.0001*) levels, BUN levels (*P < 0.0001*), total leukocyte count (*P < 0.0001*), differential count (*P < 0.0001*) and increases total triglyceride (*P < 0.0001*) levels and restores organ weights in stress induced rats as that of normal. The Petroleum Ether (40-60°C) extract and 70%Ethanol was found to be moderately effective and significant in lowering total cholesterol (*P < 0.001*) levels, HDL-Cholesterol (*P < 0.001*) levels, BUN levels (*P < 0.001*), total leukocyte count (*P < 0.001*), differential count (*P < 0.001*) and increases total triglyceride (*P < 0.001*) levels and restores organ weights in stress induced rats as that of normal (Table No: 02, 03 & 04).

**Conclusion**

From the study it was concluded that Pet ether, Ethyl Acetate and 70% Ethanol extracts of whole plant of *Phyllanthus niruri* L. has shown promising adaptogenic activity and we can conclude that it may be due to presence of several antioxidant phytoconstituents like
flavonoids and lignans presents in these extracts.

References


