Phytochemical estimations and antihypoxic effect of ethanol leaf extract of *Milicia excelsa* (Moraceae) in mice

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Abstract

*Milicia excelsa* (Moraceae) is used to treat mental illnesses, among other traditional uses in Africa, but no scientific supports for its use. Hence, this study investigated the antihypoxic potential of the ethanol leaf extract of *Milicia excelsa* in mice, as well as determined quantitatively the phytoconstituents present in the extract. Hypoxia was induced by sodium nitrite (360 mg/kg, i.p., a haemic hypoxic model) and sodium fluoride (150 mg/kg, i.p., a circulatory hypoxic model) in mice. The phytocompounds were estimated using standard methods. The extract at 500 and 1000 mg/kg, per oral significantly (p<0.05) prolonged the death latency in the haemic hypoxic mouse model while the extract at all the doses (250, 500 and 1000 mg/kg, p.o.) significantly (p<0.05) prolonged the death latency in the circulatory hypoxic mouse model suggesting antihypoxic effect. Total alkaloid was the most abundant of the phytochemicals assayed. This study therefore, concluded that the extract has an antihypoxic effect. The observed antihypoxic effect might be due to the abundance of total alkaloids which may either in synergy or additive with other plant secondary metabolites in the extract be responsible for the observed effect.

Keywords: *Milicia excelsa* leaf extract; Haemic hypoxia; Circulatory hypoxia; Total alkaloids.

1. Introduction

Hypoxia according to an earlier definition is the inadequate supply of oxygen to the body tissues, which can result in impairment of body functions and may cause an array of physiological aberration [1]. Hypoxia-induced neurodegeneration is one of the prime pathological states in clinical practice [2]. Low level of oxygen (hypoxia) is also associated with the pathologies of stroke [3] and medicinal plants with antihypoxic effects have been suggested to be suitable candidate for the treatment of stroke [2]. The most common symptom caused by hypoxia is acute mountain sickness (AMS) or counter high altitude sickness which often occurs when people travel to high altitudes [4]. Acetzolamide is the only drug approved by the United States Food and Drug Administration to attenuate AMS, but with numerous side effects [5].

*Milicia excelsa* (welw.) C.C. Berg also known as Chlorophora excelsa belongs to the family Moraceae popularly known as Iroko tree or African teak in Africa traditional medicine is a large deciduous tree 30 to 50 m high occurring naturally in...
humid forests of West Africa [6]. Its latex, leaf, stem bark, root, fruit, and ashes are used in African traditional medicine to treat malaria [7], mental illnesses [8], sexual dysfunction [9], and rheumatism [10] among other remedial uses. The antibacterial [11] [12], anti-amoebic [13], wound healing [14], antipsychotic [15], anti-stress [16] anticonvulsant [17], anti-amnesic and cognitive enhancing effects [18] of the plant have been reported. Ursolic acid and lupeol acetate are among the isolated compounds from the leaf of *Milicia excelsa* [19]. This study was designed to investigate the antihypoxic effects of the ethanol leaf extract of *Milicia excelsa* in mice, to provide a scientific basis for the ethnomedicinal uses of the leaf in the management of various brain-related disorders.

### 2. Material and methods

#### 2.1. Plant identification and authentication

*Milicia excelsa* leaves were collected within the campus of the Obafemi Awolowo University (OAU). It was identified and authenticated by Mr. G. A. Ademoriyo of the Herbarium Unit, Department of Botany, Faculty of Science, OAU, Ile-Ife and herbarium number Ife 17482 was obtained.

#### 2.2. Preparation of the plant extract

The leaves of *Milicia excelsa* plant were air-dried for two weeks at room temperature. The air-dried leaves were ground into powder and 1 kg of the powder was extracted with 3 liters of seventy percent (70%) ethanol for 72 h. The marc was re-extracted once and the combined extract was concentrated in vacuo at a temperature of 40 °C to yield 70 g (7.0%) coded EME. The EME was prepared by dissolving with 2% Tween 20 and made up to the required volume with normal saline before administration to mice [15].

#### 2.3. Animals

The animals used for this experiment were male mice. All the animals were bred and housed in a well lit and aerated room in the Animal House, Faculty of Pharmacy, OAU, Ile-Ife. They were maintained under natural daylight/night conditions. All animals had free access to drinking water and a standard commercial diet (Guinea feeds brand, Bendel Feeds Nigeria). The animals were used in groups of four (n=6) per dose of the plant extract, per drugs in the positive and negative controls.

#### 2.4. Chemicals and drugs

Sodium nitrite, sodium fluoride (Meck, Germany), Tween 20 (Sigma Aldrich, St. Louis, Missouri, U.S.A.) and physiological saline (Unique Pharmaceutical Limited, Lagos, Nigeria) were used. EME was dissolved with 2% Tween 20 and made up to the required volume with normal saline.

#### 2.5. Preliminary phytochemical quantifications

Preliminary phytochemical quantifications of EME were performed as previously carried out: total alkaloids [20] [21], total phenol [22] [23], total flavonoids [24] [25] and tannin content [26] [27]. n=3.

#### 2.6. Anti-hypoxic activity

##### 2.6.1. Haemic hypoxia

Twenty four mice were randomised into 4 groups (n=6). Group 1 (Control group) was orally administered with 2% Tween 20 in normal saline (10 mL/kg, p.o.). Thirty minutes post oral ingestion of EME (250, 500 and 1000 mg kg⁻¹), NaNO₂ (360 mg kg⁻¹) was intraperitoneally injected to each mouse. The latency in minutes before the evidence of hypoxia was recorded [28]. Antihypoxic potential of EME was expressed relative to the control group.

##### 2.6.2. Circulatory hypoxia

Twenty four mice were randomized into 4 groups (n=6). Group 1 (Control group) was orally administered with 2% Tween 20 in normal saline (10 mL/kg, p.o.). Thirty minutes post oral ingestion of EME (250, 500 and 1000 mg kg⁻¹), NaF (150 mg kg⁻¹) was intraperitoneally injected to each mouse. The latency in minutes to the evidence of hypoxia was recorded [29]. Antihypoxic potential of EME was expressed relative to the control group.
2.7. Statistical analysis

Experimental results are expressed as mean ± SD. The data were analyzed by an analysis of variance (ANOVA) followed by Dunnett's post hoc test. Results were considered significant at p<0.05.

3. Results

3.1. Results of quantitative phytochemical estimations of EME

The results of phytochemical estimation of EME (Table 1) showed that EME total alkaloid > total phenols > tannin content > total flavonoids. The result is presented in Table 1.

Table 1 Quantitative phytochemical analysis of ethanol leaf extract of M. excels

<table>
<thead>
<tr>
<th>Content</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total alkaloid content</td>
<td>144.02 ± 1.62 mg of AE/g of extract</td>
</tr>
<tr>
<td>Total phenols</td>
<td>22.52 ± 0.76 mg GAE/g extract</td>
</tr>
<tr>
<td>Tannin content</td>
<td>19.88 ± 0.5 mg of GAE /g of extract</td>
</tr>
<tr>
<td>Total flavonoid content</td>
<td>3.01 ± 0.01 mg QE/g extract</td>
</tr>
</tbody>
</table>

Values are means of triplicate determination ± Standard deviation; where AE is atropine equivalent, GAE is gallic acid equivalent, QE is quercetin equivalent and AE is atropine equivalent respectively.

3.2. Result of antihypoxic effect of EME on sodium nitrite haemic induced hypoxia

EME at 500 and 1000 mg/kg significantly (p<0.05) prolonged the death latencies following sodium nitrite induced respiratory arrest, however, EME at 250 mg/kg was found to be insignificant to prolong latencies for death compared to control. The result is presented in Table 2.

3.3. Results of antihypoxic effect of EME on sodium fluoride in circulatory induced hypoxia

EME at all the doses used (250, 500 and 1000 mg/kg) significantly (p<0.05) prolonged the death latencies following sodium fluoride induced hypoxia. The result is presented in Table 2.

Table 2 Anti-hypoxic activity of EME in the different hypoxia models

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses (mg/kg)</th>
<th>Sodium nitrite test (mins)</th>
<th>Sodium fluoride test (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10mL/kg</td>
<td>10.50 ± 0.45</td>
<td>8.74 ± 0.55</td>
</tr>
<tr>
<td>EME</td>
<td>250</td>
<td>11.12 ± 0.41</td>
<td>12.50 ± 0.74*</td>
</tr>
<tr>
<td>EME</td>
<td>500</td>
<td>14.40 ± 1.37*</td>
<td>13.12 ± 0.76*</td>
</tr>
<tr>
<td>EME</td>
<td>1000</td>
<td>14.52 ± 1.30*</td>
<td>12.50 ± 0.45*</td>
</tr>
</tbody>
</table>

Control is 2% Tween 20. Each value represents mean ± SEM of the reaction time. *p<0.05 compared to control (ANOVA, Dunnett's post hoc test). n=6

4. Discussion

This study investigated the antihypoxic effect as well as the phytochemical estimations of ethanol leaf extract of Milicia excelsa (EME) in mice. The finding of this study showed that EME possesses antihypoxic and showed abundance of total alkaloids in the phytochemicals assayed.

An earlier investigation has reported the LD$_{50}$ of EME to be greater than or equal to 5000 mg/kg and doses of 250, 500 and 1000 mg/kg were used in an earlier report [15], hence, this study employed the earlier used doses for the evaluation of the antihypoxic potentials of EME.

The prolongation of the death latency in sodium nitrite-induced chemical hypoxia by EME suggests that EME may possess an antihypoxic effect. The extract may enhance the oxygen-carrying capacity in this hypoxic model since sodium nitrite caused hypoxia by the reduction in oxygen-carrying capacity resulting in respiratory arrest and death [30]. This finding is in agreement with earlier reports of medicinal plants that prolonged death latencies in sodium nitrite-induced chemical hypoxia model and were suggested to exhibit antihypoxic effects [31] [32].
Several research findings have shown that alkaloids isolated from medicinal plants protected against sodium nitrite-induced hypoxia in mice [33-35] and possessed analgesic activities [36]. For example, the protective effects of berberine (an isoquinoline alkaloid found in Berberis, Hydrastis Canadensis and Coptidis rhizoma plants) on acute hypoxia-induced by sodium nitrite in mice have been reported [33]. Likewise, the cerebroprotective effect of isolated harmine alkaloids extracts of seeds of Peganum harmala L. on sodium nitrite-induced hypoxia in mice has also been reported [34]. Since alkaloid is the most abundant phytochemical assayed in EME, it could therefore, be suggested to be responsible for the antihypoxic effect of EME in sodium nitrite-induced hypoxic model as observed in this study.

The prolongation of the death latency in sodium fluoride-induced hypoxia may suggest antihypoxic effects. Literature data affirm that the administration of sodium fluoride (a substance that induces circulatory hypoxia) increases the blood histamine content and decreases the oxygen-carrying capacity [37] thereby resulting in death.

Alkaloids from medicinal plants have been shown to improve cerebral blood flow [38-40]. For example, vinpocetine, a vinca alkaloid, obtained from the leaves of the Lesser Periwinkle (Vinca minor) plant has been demonstrated to improve cerebral blood flow [38-40] and improve resistance to hypoxia [41]. Interestingly, ursolic acid, a triterpenoid is one of the isolated compounds from the leaf of Milicia excelsa [19] and its neuroprotective role in cerebral ischemic stroke has been documented [42-44]. Therefore, ursolic acid, either in synergy or additive with alkaloids in EME may be responsible at least in part for the observed antihypoxic effects.

5. Conclusion

This study concludes that EME has an antihypoxic effect. The antihypoxic effect may at least be due to the abundance of total alkaloids which may either in synergy or additive with other phytoconstituents in the extract be responsible for the observed antihypoxic effect. This may therefore, make the extract a suitable candidate for the prevention and/or management of stroke as well as acute mountain sickness (AMS).

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This experiments were carried out in strict adherence to the internationally accepted principles for Laboratory Animal Use and Care (EEC Directive of 1986; 86/609/EEC) as being strictly ensured by the Postgraduate College, Obafemi Awolowo University vide approval with the registration number PHP 11/12/H/2766.

References


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