ANTIOXIDATIVE BURST, CYTOTOXIC AND ANTILEISHMANIAL ACTIVITIES OF ELEUSCINE INDICA

*Jude E. Okokon¹, Anwanga E. Udoh¹, Ette O. Etebong¹, Azare B.A²

¹Department of Pharmacology and Toxicology Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
²Department of Biological Sciences, University of Abuja, Nigeria.

ABSTRACT

The ethanol leaf extract of Eleusine indica were investigated for cytotoxicity activity against HeLa cells using SRB method. Antioxidative burst activity of the extract in whole blood, neutrophils and macrophages was also investigated using luminol/lucigenin-based chemiluminescence assay. The ethanol extract was similarly screened for antileishmanial activity against promastigotes of Leishmania major In Vitro. The ethanol leaf extract was found to exert moderate cytostatic activity. The ethanol crude extract significantly inhibited oxidative burst activity in whole blood (p<0.05) when two different phagocytosis activators (serum opsonizing zymosan-A and PMA) were used. The ethanol crude extract also exhibited moderate antileishmanial activity against promastigotes of Leishmania major In Vitro. These results suggest that the leaf extract of Eleusine indica possess antioxidative burst and moderate cytostatic and antileishmanial activities.

Keywords: Eleusine indica, Cytotoxicity, Antioxidant, Antileishmanial.

INTRODUCTION

Eleusine indica (L.) Gaertn (Poaceae) is an annual or short-lived perennial tufted grass, branching from the base. The ascending culms, 5 to 60 cm high, are sometimes compressed while the leaf blades are flat or sometimes folded, 15 – 30 cm long and 4 -6 cm wide [1]. The grass is used in folklore medicine by the Ibibios of Niger Delta region of Nigeria in the treatment of malaria related fever, diabetes, stomach disorders and infections. Traditionally, the plant is used in the treatment of various ailments and diseases such as amenorrhea, influenza, oliguria, hypertension as well as kidney related disorders in Trinidad and Tobago [2]. The root is used specifically as diuretic, laxative, depurative and febrifuge. Biological activities of the plant include; anti-inflammatory [3], antidiabetic and antiaplasmodial [4, 5] anti-inflammatory and analgesic [6]. Report on antioxidant, antibacterial and non cytotoxic activities have also been published [7]. Two main flavonoids; schaftoside (6-C-β- arabinopyranosylapigenin) and vitexin (8-C-β- arabino pyrano sylapigenin) have been isolated from the plant [3]. In this study, we report the antioxidative burst and antileishmanial activities of this plant.

MATERIALS AND METHODS

Plant materials

Aerial parts of Eleusine indica were collected from University of Uyo premises, Uyo, Akwa Ibom State, Nigeria in May, 2011. The plant was identified and authenticated by Dr Magaret Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen (UUH 1409) was deposited at the University’s Hebarium. The fresh shoots (2 kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100 g was soaked in 95% ethanol (300 ml) for 72 hours. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 1.07% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Corresponding Author: - Jude E. Okokon EMail ID: judeefiom@yahoo.com
Cytotoxic activity

The growth inhibitory and cytotoxic activity of the ethanol crude extract was evaluated against HeLa cells (Cervix cancer cell) by using the sulforhodamine-B assay [8]. The cells (10000 cells/100 µL) in 96-well plate were incubated for 24 h at 37 °C in a humidified 5% CO₂ incubator. The stock solutions of ethanol extract was prepared in DMSO. Various dilutions of the ethanol crude extract (0.1, 1, 10, 100, and 250 µg/mL), were added (100 µL) in each well. After 48 h of incubation, 50 µL of cold TCA (50%) was added gently and left for 30 min at room temperature, followed by washing with distilled water and drying overnight. To each well, 100 µL of SRB solution (0.4% wt/vol in 1% acetic acid) was added and after 10 min, the unbound stain was removed by washing with acetic acid (1%), and air-dried at room temperature. The protein bound stain was solubilized with tris base (pH 10.2), and was shaken for 5 min. Absorbance was measured at 515 nm using a microplate reader. The absorbance of the appropriate blanks, including test substance blank, and control (without drug), was used to calculate the growth inhibition, and cytotoxicity of the test compounds, and represented as GI₅₀, TGI and LC₅₀ (µg/mL) values.

Cellular antioxidant activity of crude extract

The ethanol crude extract was screened for cellular antioxidant activities in whole blood, neutrophils and macrophages using chemiluminescence assay. Briefly, Luminol or lucigenin-enhanced chemiluminescence assays were performed as described by Helfand et al [9] and Haklar et al [10]. Briefly, 25 µL diluted whole blood (1:50 dilution in sterile HBSS**) or 25 µL of PMNCs (1x10⁶) or MNCs (5x10⁶) cells were incubated with 25 µL of serially diluted plant extract/fraction with concentration ranges between 0.5 and 100 µg/mL. Control wells received HBSS** and cells but no extract. Tests were performed in white 96 wells plates, which were incubated at 37°C for 30 min in the thermostated chamber of the luminometer. Opsonized zymosan-A or PMA 25 µL, followed by 25 µL luminol (7x10⁻⁵ M) or lucigenin (0.5 mM) along with HBSS** was added to each well to obtain a 200 µL volume/well. The luminometer results were monitored as chemiluminescence RLU with peak and total integral values set with repeated scans at 30 s intervals and 1 s points measuring time.

Antileishmanial activity

The antileishmanial activity of the extract was evaluated against promastigotes of Leishmania major (DESTO) in culture using microplates. Leishmania major promastigotes were grown in bulk, early in modified NNN biphasic medium, using normal physiological saline. Then the promastigotes were cultured with RPMI 1640 medium supplemented with 10% heat inactivated foetal bovine serum (FBS). The parasites (Leishmania major) were harvested at log phase and centrifuged at 3000 rpm for 10 min. They were washed three times with saline at same speed and time. Finally the parasites were counted with the help of Neubauer chamber under the microscope and diluted with fresh culture medium to give a final density of 10⁶ cells/ml. In a 96-well micro titer plate, 180 µl of the culture medium was added in different wells. The extract was dissolved in PBS (Phosphate buffer saline, pH 7.4 containing 0.5% MeOH, 0, 5% DMSO) to make a stock concentration of 1000 µg/ml. 20 µl of the extract concentration was added to the wells and serially diluted to get working concentrations ranging between 1.0 to 100 µg/ml. 100 µl of parasite culture (final density of 10⁶ cells/ml) was added in all wells. Two rows were left, one for negative and other for positive control. Negative controls received the medium while the positive controls received Pentamidine and amphotericin B as standard antileishmanial compounds. The plate was incubated between 21 - 22°C for 72 h. The culture was examined microscopically for cell viability by counting the number of motile cells on an improved Neubauer counting chamber and IC₅₀ values of compounds possessing antileishmanial activity were calculated [11].

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Student’s t-test and ANOVA (One- or Two-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e. P ≤ 0.01 and 0.05.

RESULTS

Cytotoxic activity against HeLa cells

The results of cytotoxic activity of crude ethanol extract of Eleuscine indica showed a weak cytostatic activity of the crude ethanol extract on HeLa cells. The leaf extract exerted a moderate activity with an average percentage cytostatic activity of 54.03 ± 2.18% and average percentage inhibition of 45.77%. The leaf extract did not possess any cytocidal activity.

Cellular antioxidant activity

Ethanol crude root extract of Eleuscine indica was observed to produce considerable inhibitory effect on the oxidative burst activities of the whole blood, neutrophils and macrophages in a dose-dependent manner. The ethanol extract produced – 17.90 – 36.90% inhibition in whole blood, 0.00 – 46.70% in neutrophils when activated with zymosan-A, 35.60 – 74.50% in neutrophils when activated with PMA and 39.60 – 63.40% in macrophages (Table 1).

Antileishmanial activity

Ethanol crude extract and fractions of root extract of Hippocratea africana exerted prominent antileishmanial activity when tested against promastigotes of Leishmania
major. Ethyl acetate fraction exerted a higher activity than other fractions with EC\textsubscript{50} value of 40.32 ±0.54 µg/ml and ethanol crude extract though uncomparable to the standard drugs, pentamidine and amphotericin B (Table 2).

Table 1. Immunomodulatory activity of Ethanolic leaf extract of *Eleusine indica*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Dose (µG/ML)</th>
<th>% Inhibition (RLU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>1</td>
<td>-17.90 ±1.70</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>21.40±0.23</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>36.90±2.83</td>
</tr>
<tr>
<td>Neutrophils (intracellular)</td>
<td>0.5</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.30±0.98</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>46.70±1.84</td>
</tr>
<tr>
<td>Neutrophils (extracellular)</td>
<td>0.5</td>
<td>35.60±9.01</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>59.00±2.31</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>74.50±3.52</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0.5</td>
<td>39.60±5.27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>42.00 ± 6.39</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>63.40 ± 1.15</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SEM of three independent experiments

Table 2. Antileishmanial activity of *Eleusine indica* (ED\textsubscript{50})

<table>
<thead>
<tr>
<th>Extract/Fraction</th>
<th>ED\textsubscript{50}(µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>58.18±0.14</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>5.09±0.04</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.29±0.05</td>
</tr>
</tbody>
</table>

DISCUSSION

*Eleusine indica* is used in traditional medicine for the treatment of various diseases such as malaria, diabetes and other inflammatory diseases. The leaf extract has been found in this study to demonstrate a moderate cytostatic action which is insignificant to exert cytocidal action against HeLa cells. This finding corroborates an earlier report by Al-Zubairi et al., [6], who reported a non-cytotoxic activity of this plant. The extract was further observed to exert a concentration dependent cellular antioxidant activity. The significant antioxidant activity observed with higher concentrations of the extract could be as a result of the presence of two flavonoids; schaftoside (6-C-β- glucopyranosyl-8-C- α-arabinopyranosylapigenin) and vitexin (8-C- β-arabinopyranosylapigenin), which have been isolated from the plant [3]. Flavonoids are known antioxidant compounds scavenging free radicals and vitexin present in this plant has been reported to possess strong antioxidant activity [12,13]. The leaf extract demonstrated significant antileishmanial activity and antimicrobial activities are known to be promoted by pro-oxidant state. In this study, different concentrations of the extract were observed to exhibit pro-oxidant activity predominantly. This activity has been reported to enhance antimicrobial activity [14] and may have contributed to the antileishmanial activity observed in this study.

CONCLUSION

From the results of this study, leaf extract of *Eleusine indica* possesses cellular antioxidant, moderate cytostatic and antileishmanial activities.

ACKNOWLEDGEMENT

Dr. Jude Okokon is grateful to TWAS for financial support for postdoctoral fellowship and ICCBS for providing research facilities.

REFERENCES