Biochemical Studies on hydroethanolic extracts of *Eremomastax polysperma* (Benth.) Dandy used in the management of sickle cell disease

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In the West African tropics of Nigeria, various plant extracts are used in the management of sickle cell disease (SCD) by indigenous herbalists. Preliminary studies using one such hydroethanolic extract of *Eremomastax polysperma*, on some prominent symptoms of SCD were investigated. Standard methods were used to assess the effect of the extract on anti-anaemic parameters in albino wistar rats. The extract was also studied for its anti-inflammatory and analgesic effects, which are involved in the pathophysiology of SCD. The extract was found effective in improving anaemic indices of the experimental animals as follows: haemoglobin (14.54 ± 0.20 g/dL), white blood cell count (11.04 x 10³ µL), platelet count (1015.40 ± 22.67µL), lymphocyte (9.22 ± 1.38µL) and hematocrit (44.36 ± 1.70%); these were significantly higher (p < 0.05) than for the negative control groups. The extract affected two of the major enzymes responsible for liver function significantly, viz. decreased (p < 0.05) levels of Aspartate Amino Transaminase (AST) and Alkaline Amino Transaminase (ALT) while Alkaline Phosphatase (ALP) was not significantly affected. Pain was relieved significantly by the extract and the effect was dose dependent as shown by the percentage inhibition of 18.61, 48.62 and 51.03%, respectively, for 100, 200, 300 mg/kg body weight, although it is significantly lower than 83.10% recorded for the standard drug assayed (aspirin at 30mg/kg body weight). The mechanism of sickle cell inhibition though not clear, is thought to possibly arise from possible nutrigenomic and epigenetic influences of the extract.

**Key words:** Sickle cell disease, anti-anaemic, analgesic, *E. polysperma* leaf extract, liver function.

INTRODUCTION

In many countries of the West African tropics, the potential benefits of using medicinal plants as herbal remedies in the management of sickle cell disease (SCD) has a distinct niche in the rural and even sub-urban populations. The holistic use of plants in healthcare affords the benefit of maximizing all the essential ingredients that nature has endowed to humanity. Many of these plants have antioxidant, anti-inflammatory, anti-microbial, anti-sickling and nutritive supplemental properties. Traditional herbalists without formal science backgrounds have used nature’s abundant resources, to manage SCD with a great degree of success, through trial and error methods.

The use of phytomaterials from *Piper guineense*, *Pterocarpus osun*, *Eugenia caryophyllata* and *Sorghum bicolor* extracts for the treatment of SCD has been reported by Wambebe, *et al.*, (2001). The extracts of *Pterocarpus santalinoides* and *Aloe vera* were reported by Ugbor (2006) to increase gelling time of sickle cell blood. It was further shown to inhibit sickling in vitro. *Terminalia catappa* could be an effective antisickling agent inhibiting osmotically induced haemolysis of human erythrocytes in a dose dependent manner (Mybemene et al., 1999). *Scoparia dulcis* is also reported to exhibit reasonable potential in this direction (Orhme et al., 2005; Orhue and Nwanze, 2006). The authors used *Trypanosoma brucei* to investigate the effect of the plant extract on haematological parameters and biochemical indices due to lack of animal models. The role of crude extracts of *Macraphylla* roots as an anti-drepanocytany
agent has also been highlighted (Elekwa et al., 2005). Ekeke and Shode (1985) reported sickling reversal by Cajanus cajan extracts.

Eremomastax polysperma belongs to the family Acanthaceae; it is a stout, erect, much branched herb of the forest zone; forms weeds on cocoa farms, dispersed from Guinea to Western Cameroons and widespread in tropical Africa. The leaves are eaten in Congo DR, (Burkil, 1985). The plants found in Ivory Coast and Nigeria also contain saponins. It is used by indigenous people in Nigeria for the management of symptoms of SCD including pains and anaemic conditions. The aim of this study was to validate scientifically the analgesic, anti-inflammatory, anti-anaemic and liver function effects of E. Polysperma leaf extracts on biochemical parameters using experimental Wistar albino rats.

MATERIALS AND METHODS

Plant material

The leaves of E. polysperma (Benth.) Dandy was collected from the wild with the help of a local herbalist in Uyo Local Government Area of Akwa Ibom State, Nigeria. The plant sample was authenticated at the University of Uyo Herbarium 50 g of air dried mixture of mature and young leaves were pulverised and extracted using 30:70% aqueous ethanol, filtered and concentrated at 45°C in a water bath.

Animals

Studies were carried out using six month old Wistar albino mice and rats weighing 28 – 33 g and 130 – 154 g, respectively. The animals reared in the animal house of the faculty of Basic Medical Sciences were maintained under standard laboratory conditions with 12/12 h dark-light cycle for two weeks before commencing the experiments. They were allowed free access to standard rat feed (Pfizer, Nig.) and given water ad libitum.

Analgesic activity

Acetic acid induced writhing test as outlined by Saadogo et al. (2006) was used. Nociception was induced by an intraperitoneal injection of 0.6% acetic acid (10 ml/kg) body weight. The animals which were divided into five groups of six mice each, were administered E. Polysperma (EP: 100, 200 and 300 mg/kg), Acetyl Salicylic Acid (ASA: 30 mg/kg) and distilled water, 1 h orally before acetic acid injection. The number of writhes occurring between 5 and 20 min after the injection was recorded. The analgesic was calculated as the difference in number of writhes in treated mice compared to those in the control group.

Anti-Inflammatory activity

The method of Winter et al. (1962) was used with slight modification using egg albumin as inflammatory agent in place of carragenan. Acute inflammation was induced by injection of 0.2 ml of egg albumin into the sub plantar region of the right hind paw. The mice were divided into four groups of five mice each while the extract, EP (250, 500 mg/kg), distilled water and aspirin (ASA) were orally administered 1 h prior to the injection of egg albumin. The paw size was measured using vernier calipers and anti-inflammatory activity was calculated as:

\[
\% \text{ inhibition} = \frac{(A - B) \text{ control} - (A-B) \text{ treated}}{(A-B) \text{ control}} \times 100
\]

Where:

\( A = \text{paw size at time}, \ t, \ B = \text{paw size before albumin injection}, \ A - B \text{ is the edema.} \)

Haematological and biochemical studies

Wistar albino rats (5 per group of three) were administered with the EP leaf extracts (250 mg/kg wt.; 500 mg/kg wt.) for 28 days. At the end of the 28 days, the animals were fasted for 12 h, anaesthetized and blood samples obtained by cardiac puncture were placed in and EDTA bottles for haematology and biochemistry analyses, respectively.

RESULTS

Results of analgesic activity are presented in Table 1.

Table 1. Analgesic activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Writhes</th>
<th>% Inhibition</th>
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<tbody>
<tr>
<td>I</td>
<td>47.00 ± 8.13</td>
<td>18.67</td>
</tr>
<tr>
<td>II</td>
<td>29.80 ± 7.74</td>
<td>48.64</td>
</tr>
<tr>
<td>III</td>
<td>28.40 ± 7.34</td>
<td>51.03</td>
</tr>
<tr>
<td>IV</td>
<td>9.80 ± 5.44</td>
<td>83.10</td>
</tr>
<tr>
<td>V</td>
<td>58.00 ± 9.92</td>
<td>-</td>
</tr>
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</table>

EP = E. polysperma values, expressed as mean ± SEM. I = 100 mg of EP, II = 200 mg/kg of EP, III = 300 mg/kg of EP, IV = Vehicle (control)
dard drug administration. Inactivation of mast cells, macrophages, platelet and the Ep extracts may one of the body defenses and therefore relieved incidence against ages, alliative for anaemia. Imme useful to sickle cell disease patients who portray oxidative stress. This showed that extract of the plant extract relieved pain by 51%, though less than that produced by the standard drug (aspirins). During inflammation, activation of mast cells, macrophages, eosinophils and neutrophils are known to produce reactive oxygen species (ROS) such as superoxide radicals with NADPH oxidase playing an important role (Eduarda et al., 2004). These radicals can act as secondary messengers thereby provoking the production of other mediators involved in the inflammatory response (Ouedrago et al., 2011). It appears the Ep extracts may have prevented inflammation through its inert antioxidant revealed by the initial phytochemical analysis of the plant material. The extract possessed anti-inflammatory properties that peaked at the third hour of administration.

It can be deduced from the results presented in Table 3 that the extracts improved both haematological and biochemical parameters significantly (P < 0.05). Even though, the haemoglobin of the treated groups was not significantly higher than the control group receiving only the vehicle, it showed improvements in this parameter consequently, it can serve as a palliative for anaemia which is a common issue in sickle cell disease. Moreso, the significant differences in the WBC, platelet and lymphocyte counts portrays the fact that the extract has immune boosting effects on the treated animals. This potential is very useful to sickle cell disease patients who suffer frequent attack of diseases due to lowered immunity prevalent in the situation. All the indices were higher in treated groups than those for control groups. Liver function enzymes were higher in control serum than those in the treated groups, demonstrating that the extract was safer to the liver than the latter.

**Discussion**

Vaso-occlusive pain is a common symptom of SCD; the plant extract relieved pain by 51%, though less than that produced by the standard drug (aspirins). During inflammation, activation of mast cells, macrophages, eosinophils and neutrophils are known to produce reactive oxygen species (ROS) such as superoxide radicals with NADPH oxidase playing an important role (Eduarda et al., 2004). These radicals can act as secondary messengers thereby provoking the production of other mediators involved in the inflammatory response (Ouedrago et al., 2011). It appears the Ep extracts may have prevented inflammation through its inert antioxidant revealed by the initial phytochemical analysis of the plant material. The extract possessed anti-inflammatory properties that peaked at the third hour of administration. This showed that extract can mob up excess radicals that is generated in sickle cell disease and therefore relieved the condition of oxidative stress. Mukhtar et al. (1994) noted that antioxidants present in consumables like fruits, herbs and vegetables have received attention considerably as chemo protective agents. The lowered serum levels of AST, ALT and ALP (significant at 0.05 confidence level) compared with control showed *E. polysperma* to be hepatoprotective. The incidences of anaemia in SCD due to increased loss of RBC, resulting from haemolysis is of great concern. The WBC is the first line of the body defence against invading organisms. This suggests that the extract of the plant contains agent that stimulate the production of these haematological indices. The presence of such agents had been reported for *Viscum album* (Mittletoe) and other commonly prescribed medicinal plants. (Sonkar et al., 2012). The increased levels Hb implies that there may be a positive change in oxygen carrying capacity of the blood and transferring of respiratory gases (Ashafa et al., 2009). Though the mode of action is not known, it

**Table 2. Anti-inflammatory study.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in paw size (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1h</td>
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<tr>
<td>I</td>
<td>2.93 ± 0.17</td>
</tr>
<tr>
<td>II</td>
<td>2.18 ± 0.32</td>
</tr>
<tr>
<td>III</td>
<td>1.67 ± 0.11</td>
</tr>
<tr>
<td>IV</td>
<td>1.07 ± 0.03</td>
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</tbody>
</table>

* = significant difference from 1, P < 0.05, I = Control, II = 250 mg/kg of EP, III = 500 mg/kg of EP, IV = Aspirin (30mg/kg wt).

**Table 3. Haematological and biochemical results.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Hb (g/dL)</th>
<th>Wbc x 10⁹ (µL)</th>
<th>Plt x 10⁹ (µL)</th>
<th>Lym x 10⁹ (µL)</th>
<th>Hct (%)</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>14.08 ± 0.20</td>
<td>8.20 ± 0.75</td>
<td>731.50 ± 46.24</td>
<td>600 ± 0.54</td>
<td>42.80 ± 6.60</td>
<td>98.00 ± 6.99</td>
<td>25.40 ± 4.89</td>
<td>78.68 ± 4.13</td>
</tr>
<tr>
<td>II</td>
<td>14.54 ± 0.38</td>
<td>11.04 ± 1.51*</td>
<td>1015.40 ± 22.67*</td>
<td>9.22 ± 1.38*</td>
<td>44.36 ± 1.70</td>
<td>80.80 ± 9.47*</td>
<td>15.00 ± 1.10*</td>
<td>77.56 ± 1.12</td>
</tr>
<tr>
<td>III</td>
<td>14.72 ± 0.22</td>
<td>12.68 ± 2.40*</td>
<td>1023 ± 33.01*</td>
<td>16.10 ± 1.87*</td>
<td>44.00 ± 1.06</td>
<td>69.60 ± 7.32*</td>
<td>12.00 ± 1.41*</td>
<td>80.44 ± 1.42</td>
</tr>
</tbody>
</table>

Keys: Hb = haemoglobin; Wbc = white blood cell count; Plt = Platelet count; Lym = Lymphocyte concentration; Hct = Haematocrit. Values are expressed as mean ± S.E.M; n = 5, I = Control, II = 250 mg/kg of Ep, III = 500 mg/kg of Ep.
may be attributed to the ability of the extracts to lower lipid peroxidation level that causes haemolysis of erythrocytes (Devaki et al., 2012; Oyedemi et al., 2011). It is reported that the plant extracts possessed compounds such as phenols, flavonoids, and tannins which are strong antioxidants, therefore could inhibit peroxidation of polyunsaturated fatty acids in the cell membrane hence haemolysis of RBC. Devaki et al. (2012) reported altered neutrophils. Lymphocyte and platelet counts of wistar albino rats fed with aqueous extract of Passiflora edulis. This is also supported by the findings that Hermania incana also produced mild changes in haematological indices of rats through sensitization of bone marrow (Devaki et al., 2012).

REFERENCES