Effects of aqueous extract of *Nelsonia canescens* leaf on the osmotic fragility of red blood cell and blood parameters of Wistar albino rats.

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**ABSTRACT**

The herbaceous plant *Nelsonia canescens* is a medicinal plant used in Asian and African traditional medicine for various diseases of humans and animals. The plant has been used for the treatment of pains and inflammatory action related diseases, cancer, gout, cough, fever, cardiovascular diseases, chicken pox and even malaria. Decoction of it has also been used as immune booster in patients by the traditionalists. This study was aimed at evaluating the effects of the aqueous leaf extract of the plant on the osmotic fragility of rats’ red blood cells, and also to determine the changes that occur in haematology and serum chemistry of the rats exposed orally to the extract for 28 days. Three groups of rats were administered orally with 200, 400, and 800mg/kg of the plant extract respectively while the fourth group which is the control was administered also orally with distilled water and their blood were then analyzed. The 2, 4 and 6mg/ml concentrations of the extract inhibit hypotonic solution induced rats erythrocytes hemolysis in concentration dependent manner and the inhibition is comparable to that of Indomethacin. The blood analysis showed a significant increase (p<0.05) in total white blood cells and the lymphocytes for the groups administered with 400 and 800 mg/kg of the extract while the neutrophils decreased significantly. It was then concluded that the aqueous extract of the plant inhibits red blood cell hemolysis and hence its anti-inflammatory activities. Also, the significant increase in the total white blood cells and the lymphocytes could be the reason why the plant is useful as immune booster by the traditionalists.

**Keywords**: *Nelsonia canescens*, osmotic fragility, erythrocytes, rats, blood.

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INTRODUCTION

*Nelsonia canescens* (Lam. Spreng) otherwise known as blue pussyleaf is an herbaceous species of Acanthaceae family. It is a small perennial herb with soft decumbent villous branches; creeping, sprawling or prostrate plant that often grows in disturbed and open habitats where it can be weedy [1]. The plant is also known as agricultural weed as it grows in rice and oil palm plantations thereby reducing crop yield by competing with crop plants for common resources [2], it is a very agile dispenser thereby having high dispersal ability and thus distributed across the tropics[3]. *Nelsonia canescens* is a native to Africa, Asia and Australia [4] where it is also reported to be used as cover crop to suppress the growth of weeds in banana plantations as it invades large areas of the plantation with no visible adverse effects on the banana crop but limiting the possibility of other weeds to invade [5]. The plant is a medicinal plant used in African and Asian traditional medicine; for the treatment of fever and as an analgesic in cold, flu, cough and viral infections in Africa, use in Indian traditional medicine to treat pain and inflammation [6], the plant is also used in the treatment of cancer, gout, cardiovascular and inflammatory diseases [7]. The traditional healers use the root, fruit and leaves of the plant for different disease conditions [8]. The root is used by traditional healers of Gandhamardan hills of Orissa in the local name of *Badarasna* (*Rasna*) for the management of pain and inflammatory conditions such as arthritis, cutting wound, and bone fractures [9] while the whole plant is used for its hepatoprotective action [10]. *Nelsonia canescens* grows fast in Western part of Nigeria where it is used for the treatment of various ailments. The traditional practitioners in this part of the country use the plant for the treatment of malaria [11], pain, chickenpox, constipation and gastric ulcer; the patient is required to take a full cup of the plant decoction at least three times daily or take after meal instead of water [12], they also use decoction of the plant to boost the immunity of patients. The phytochemical analysis of *Nelsonia canescens* shows the presence of phenolic compounds such as flavonoids in the polar extract; two flavones (apigenin and luteolin), one flavonol (quercetol), four cinnamic acid derivatives (p-coumaric acid, caffeic acid, chlorogenic acid and ferulic acid) and one benzoic acid (gentisic acid) derivative were identified [13]. Phenolic compounds are important constituents of the human diet and they have been recognized largely as beneficial antioxidants, antibacterial and enzyme inhibitors. This study was to determine the effects of aqueous extract of the leaf of the plant on osmotic fragility of red blood cell and blood parameters including serum chemistry.

MATERIALS AND METHODS

**Plant Materials**

The fresh samples of *Nelsonia canescens* were collected from Ologun eru area of Ibadan in Ido Local Government area of Oyo State, Nigeria where it grows as weed. The confirmatory identification of the plant was done at Forest Reserve Institute of Nigeria (FRIN) Ibadan. The leaves were plucked, washed with water to remove dirt and then shade-dried at room temperature. After drying, the leaves were milled into powder using mortar and pestle and then stored in an air tight container for extraction.

**Extraction of the Leaves**

The cold maceration extraction method was employed in which 250g of the powdered sample of the leaves was added to 1 liter of distilled water and allowed to stay for three days with intermittent shaking. Thereafter, the mixture obtained was filtered using filter paper and the filtrate which is the extract was concentrated using vacuum rotary evaporator (IKA, Germany) at an optimum temperature of 50°C. The percentage yield of the extract was 5.77%. A fresh 10% (w/v) solution of the extract was prepared with normal saline to make appropriate dosage required for the studies.
Animals for the study
Adult Wistar albino rats of both sexes weighing 120–175g, obtained from National Veterinary Research Institute Vom, Plateau State Nigeria, were used for the study. The animals were housed in steel metal cages at the Animal House of the Faculty of Veterinary Medicine, University of Abuja and acclimatized for two weeks before the experiments. The animals were given free access to water and fed with growers mash bought from the local market.

Solutions for osmotic fragility test

Standard Drug
0.10mg/ml of Indomethacin was prepared in isotonic saline (0.85% NaCl) to make the concentration required for the study.

Red Blood Cell Suspension
10% (v/v) of rat red blood cell suspension was prepared with normal saline and kept in refrigerator at 4°C as stock erythrocytes and the osmotic fragility activity of the extract on the red blood cells was assessed using hypotonic solution-induced rat erythrocyte hemolysis.

Test Solution
4.5ml of test solution consists of 2ml of hypotonic saline (0.25%w/v); 1ml of phosphate buffer (pH 7.4); 1ml of test extract (1mg/ml - 8mg/ml) in normal saline and 0.5ml of rat red blood cells in isotonic saline.

Test Control
4.5ml of test control consists of 2ml of hypotonic saline (0.25% w/v); 1ml of phosphate buffer (pH 7.4); 1ml of isotonic saline and 0.5ml of rat red blood cells in isotonic saline

Standard Drug Solution
4.5ml of standard solution consists of 2ml of hypotonic saline (0.25%w/v); 1ml of phosphate buffer (pH 7.4); 1ml of Indomethacin (0.1mg/ml) and 0.5ml rat red blood cells in isotonic saline.

Procedure for osmotic fragility test
The method used was as described by Shinde et al in 1999 [14]. Whole blood was obtained from rats through cardiac puncture into a heparinized tube, centrifuged and supernatant was carefully pipetted. The remaining packed cells was washed four times with equal volume of isotonic buffer solution (154 mM NaCl in 10 mM sodium phosphate buffer; pH 7.4), the packed cells were been centrifuged each time at 1000 rpm for 10 minutes. 10% rat erythrocytes suspension was prepared with normal saline and kept in the refrigerator at 4°C as stock erythrocytes.

1 ml of varying concentrations of the extract (2, 4 or 6mg/ml) or 0.10 mg/ml of indomethacin in the case of standard drug solution, was mixed with 1 ml of phosphate buffer, 0.5 ml of stock erythrocytes and 2 ml of the hypotonic solution was added to make 4.5 ml. The test control consists of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH 7.4), 1ml of isotonic saline and 0.5ml of rat red blood cells in isotonic saline, the reaction mixtures were incubated at 37°C for 30 minutes, centrifuged for 10 minutes at 1000 rpm and the absorbance of the supernatant solution was measured with spectrophotometer at 540 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of hemolysis or membrane stabilization was calculated according to modified method described by Shinde et al in 1999.

% inhibition of hemolysis = 100 x (OD₁ – OD₂)/OD₁
Where OD\textsubscript{1} is the optical density of hypotonic buffer-saline solution alone (control) and OD\textsubscript{2} is the optical density of test sample in hypotonic solution.

**Procedure for evaluation of blood and serum parameters**

Twenty four Wistar albino rats divided into 4 groups I, II, III and IV containing 6 rats per group were used for this study. The first 3 groups were administered orally with 200, 400 and 800 mg/kg body weight of the plant extract, while the fourth group served as control and was administered with 3 ml/kg of distilled water. The plant extract and distilled water were administered to the respective group orally for 28 days and blood samples were thereafter obtained from the anaesthetized rats through cardiac puncture for analyses. Blood samples were collected under light Ether anaesthesia by cardiac puncture into heparinized bottles for haematological studies. Another set of blood samples were collected into a clean bottle (non-heparinized) and allowed to clot. The clotted blood with serum was centrifuged at 3000 rpm for 5 min. Serum samples were collected into sterilized eppendorf tubes for biochemical analysis.

**Determination of haematological parameters:** Blood samples collected from rats into heparinized bottles were analyzed to determine the packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration, platelet count, total and differential white blood cell (WBC) count using standard methods [15].

**Determination of serum biochemical parameters:** the serum collected was analysed for total protein using biuret reaction while albumin was measured by colorimetric estimation using the sigma diagnostics albumin reagent (Sigma Diagnostic, U.K.) which contained BromoCresol Green (BCG). Globulin was measured from the difference between total protein and albumin. Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenyl hydrazine while aspartate aminotransferase (AST) was determined by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenyl hydrazine.

**Statistical Analysis**

The entire data collected was statistically analyzed using one-way ANOVA and Duncan New Multiple Range post hoc test from SPSS 16.0 package to compare the mean values of the test groups with the control.

**RESULTS**

The results on osmotic fragility of the red blood cells shows the extract to inhibit the hypotonic solution induced hemolysis of the red blood cells, the extract at all the concentrations used showed an inhibition which is comparable to that of indomethacin which is the standard drug used in the study (Table I).
Table I: Effect of aqueous leaf extract of *Nelsonia canescens* on hypotonic solution induced hemolysis of rat erythrocytes. The values are expressed as mean ± SEM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration</th>
<th>Optical density (OD)</th>
<th>% Inhibition of hemolysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotonic soln</td>
<td>50mM</td>
<td>0.451 ± 0.007</td>
<td>-</td>
</tr>
<tr>
<td>Extract 2mg/ml</td>
<td></td>
<td>0.308 ± 0.021</td>
<td>31.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract 4mg/ml</td>
<td></td>
<td>0.270 ± 0.101</td>
<td>40.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract 8mg/ml</td>
<td></td>
<td>0.236 ± 0.071</td>
<td>47.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.1mg/ml</td>
<td>0.201 ± 0.028</td>
<td>55.47</td>
</tr>
</tbody>
</table>

Superscripted items indicate significant values compared to indomethacin; a standard drug.

Table II shows the results of blood analysis as it affects erythrocytes parameters. The red blood cell count, packed cell volume and even the hemoglobin concentration does not show any significant difference from the control group.

Table II: Haematological parameters (Mean±SEM) of rats exposed to aqueous leaf extract of *Nelsonia canescens*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>RBCx10&lt;sup&gt;6&lt;/sup&gt;/µl</th>
<th>PCV(%)</th>
<th>HB(g/dl)</th>
<th>MCV(fl)</th>
<th>MCH(pg/cell)</th>
<th>MCHC(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>4.8±1.4</td>
<td>36.5±3.1</td>
<td>8.8±1.3</td>
<td>76.04±7.3</td>
<td>18.33±1.8</td>
<td></td>
</tr>
<tr>
<td>400mg/kg</td>
<td>5.7±2.3</td>
<td>34.7±2.9</td>
<td>8.6±0.9</td>
<td>60.88±3.7</td>
<td>15.09±2.5</td>
<td></td>
</tr>
<tr>
<td>800mg/kg</td>
<td>5.8±2.5</td>
<td>37.3±1.7</td>
<td>8.9±0.6</td>
<td>64.31±4.8</td>
<td>15.34±3.1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>55.7±1.1</td>
<td>37.9±2.5</td>
<td>8.4±0.8</td>
<td>66.49±5.7</td>
<td>14.74±2.6</td>
<td></td>
</tr>
</tbody>
</table>

Control is distilled water at 3ml/kg body weight. Superscripted items indicate significant difference at p<0.05 between the test compared to control group.

The further blood analysis on total white blood cells, neutrophil and the lymphocytes shows a significant difference (p<0.05) in all the three parameters as shown in table III. The groups administered with 400mg/kg and 800mg/kg showed significant increase in total white blood cell and the lymphocytes but a significant decrease in the neutrophil count.
Table III: Total white blood cell and differentials (Mean±SEM) of rats exposed to aqueous leaf extract of *Nelsonia canescens*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TWBC×10 /µl</th>
<th>Lymp(%)</th>
<th>Neut(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>4.8 ± 0.9</td>
<td>54.7 ± 6.7</td>
<td>43.8 ± 5.2</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>5.7 ± 1.7a</td>
<td>68.1 ± 8.9b</td>
<td>31.9 ± 2.9c</td>
</tr>
<tr>
<td>800mg/kg</td>
<td>5.9 ± 0.6a</td>
<td>67.8 ± 3.2b</td>
<td>32.2 ± 4.7c</td>
</tr>
<tr>
<td>Control</td>
<td>4.1 ± 1.2</td>
<td>53.9 ± 4.8</td>
<td>46.1 ± 1.3</td>
</tr>
</tbody>
</table>

Control is distilled water at 3ml/kg body weight. Superscripted items indicate significant difference at p<0.05 between the test compared to control groups.

The result of serum analysis showed a significant (p<0.05) decrease in the level of ALT for the group of rats administered with 400 and 800 mg/kg of the extract while the increase in AST is not significant. The increase in total proteins is not significant (p>0.05) for the groups administered with 200, 400 and 800 mg/kg of the extract, while the albumin:globulin ratio slightly decreased but the decrease was not significant, as shown in table IV.

Table IV: Some serum chemistry parameters (Mean±SEM) of rats exposed to aqueous leaf extract of *Nelsonia canescens*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT(U/L)</th>
<th>AST(U/L)</th>
<th>T. Prot(g/l)</th>
<th>Albu(g/l)</th>
<th>Glob(g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mg/kg</td>
<td>28.1±1.4</td>
<td>41.3±1.7</td>
<td>43.2±5.7</td>
<td>26.1±1.3</td>
<td>17.1±5.3</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>29.1±2.4a</td>
<td>42.9±2.0</td>
<td>39.9±2.1</td>
<td>27.7±2.7</td>
<td>19.5±4.1</td>
</tr>
<tr>
<td>800mg/kg</td>
<td>21.7±2.1a</td>
<td>43.0±1.7</td>
<td>38.2±1.1</td>
<td>24.3±3.1</td>
<td>18.7±3.2</td>
</tr>
<tr>
<td>Control</td>
<td>37.4±0.9</td>
<td>42.6±0.7</td>
<td>37.8±3.2</td>
<td>23.6±1.9</td>
<td>15.2±3.1</td>
</tr>
</tbody>
</table>

Control is distilled water at 3ml/kg body weight. Superscripted items indicate significant difference at p<0.05 between the test compared to control group.

DISCUSSION

*Nelsonia canescens* is a medicinal plant that has been acclaimed to treat many ailments including pains and inflammatory action related diseases. In this study it was discovered that the extract of the plant inhibits the hypotonic solution induced rats erythrocytes hemolysis in concentration dependent manner and the hemolysis is comparable with that of indomethacin which is a known non-steroidal anti-inflammatory drug. It is already a known fact that the vitality of any cell depends on the integrity of its membrane [16] and exposure of such cell to substances such as hypotonic solution results in lysis due to the inflow of fluid from the region of low concentration to high concentration inside the cell causing turbidity of the cell and thus the rupturing of the membrane of such a cell. The cell releases its contents into the surrounding environment.
and these results into different kinds of reactions such as inflammatory reactions. The hemolytic effect of hypotonic solution apart from rupturing of membrane will further render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation [16]. The red blood cell membrane resembles the lysosomal membrane and as such, the effect of drugs on the stabilization of red blood cell membrane could be extrapolated to the stabilization of lysosomal membrane [17]therefore, as the membrane stabilizes, it interfere with the release and or action of mediators like histamine, serotonin, prostaglandins, luekotrienes etc [14] which are very important for early phase of inflammatory reaction. Substances with membrane-stabilizing activities such as this extract are known for their ability to interfere with the early phase of inflammatory reactions, such as the prevention of the release of phospholipases that trigger the formation of inflammatory mediators [18] thereby preventing inflammatory reactions. It could therefore be said that the anti-inflammatory activities of Nelsonia canescens was due partly to the inhibition of the early phase of inflammatory reactions as a result of increase in lysosomal membrane integrity.

This study showed a significant increase in total white blood cells and the lymphocytes and these increase observed could be suggestive of increased immunity due to the extract. The immune system is a complex network of cells such as lymphocytes and organs that work together to defend the body against foreign substances. Lymphocytes are one of the main types of immune cells and are divided mainly into B-lymphocytes and T-lymphocytes, while the B-lymphocytes produces antibodies through plasma cells, the T-lymphocytes are programmed to recognise, respond to and to remember the antigens. It has been observed that some plants contain different compounds and have long been used to modulate the humoral and cell-mediated immune responses in Wistar albino rats [19] and the result of this study corroborates the use of Nelsonia canescens by the traditionalists as immunity booster in the treatment of some diseases. The significant increase in neutrophils could be suggestive of the fight against active bacterial infections but in this case a significant decrease was observed.

The blood serum analysis showed a significant decrease in the activities of alanine aminotransferase; this enzyme is present in the liver and other cells and it is particularly useful in measuring hepatic necrosis especially in small animals [20]. Serum levels of both alanine aminotransferase and aspartate aminotransferase become elevated whenever disease processes affect liver [21] and thus alanine aminotransferase is used to detect liver diseases [22]. Conversely, the activity of alanine aminotransferase in this study was observed to be significantly decreased while that of aspartate aminotransferase remained statistically unchanged. These corroborate the use of this plant by the traditionalists as an
hepatoprotective agent. The total protein, albumin and globulin were slightly increased but the increase was not significant. The slight but not significant increase in the ratio of albumin to globulin suggests a slight increase in the globulin production with an attendant increase in IgM, IgA, and IgG which may in turn suggest a boost in immunity as there is no sign of hyperproteinaemia which may be due to other systemic problems. These results corroborate the use of Nelsonia canescens as medicinal plant by the traditionalists and further studies on the plant could bring out good remedy for different ailments as it affects humans and animals. It has been observed that some of the phytochemical compounds extracted from the plant have promising medicinal potentials. Studies have shown that p-coumaric acid, a Cinnamic acid derivative decreases low density lipoprotein peroxidation and possesses a potential protection on cardiac oxidative damage induced by doxorubicin, an anticancer antibiotic [23]. Cinnamic acid derivative; ferulic acid is another compound present in Nelsonia canescens and it has been reported to possesses a high antioxidant potential due to its resonance-stabilized phenoxy radical structure and this compound has been approved in some countries as food additives to prevent lipid peroxidation as it is an effective scavenger of free radicals [24]. It has also been observed that normal cell damage can be prevented in cancer patients by pretreatment with ferulic acid which protects the cell from γ-radiation-induced damage by inhibiting peroxidation of membrane lipids and free radicals induced DNA strand break formation [25]. Quercetol which is the flavonol extracted from the plant can prevent oxidant injury and cell death by protecting against lipid peroxidation, so also, the antioxidant capacities of its flavones; the apigenin and luteolin in free radical scavenging and against 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radicals respectively have been reported [26]. The Caffeic acid which is a Cinnamic acid derivative with Chlorogenic acid is known for its in vitro antioxidant, anti-inflammatory and antitumorigenic activities while chlorogenic acid with its protective effects on oxidative stress in vivo has also been reported [27]. It can be concluded that the aqueous leaf extract of Nelsonia canescens possesses red blood cell membrane stabilizing effects and this may be part of the mechanism of its anti-inflammatory activities especially by inhibiting the early phase of inflammatory reactions. The extract could also boost the cellular immunity of the animals by increasing the total white blood cell count viz-a-vis the lymphocytes count of the rats.

REFERENCES


