Haematological and Kidney Function Indices of
Piliostigma thonningii Leaf Extract Administration
Following Pefloxacin Induced Toxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author KD designed the study and
performed the statistical analysis. Authors KD and AAA wrote the protocol and wrote the first draft of
the manuscript. All authors managed the analyses of the study. Authors AAA, OOO and MO managed
the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: The study investigated haematological and kidney function indices of Wistar rats
administered Piliostigma thonningii ethanol leaf extract after being induced with therapeutic doses
of pefloxacin (400 mg/5 mL).

Place and Duration of Study: Department of Medical Biochemistry, Cross River University of
Technology, Okuku Campus, between August 2013 and June 2014.

Methodology: Twenty four male Wistar rats weighing between 185-200 g were assigned into four
groups. Administration was orally, with groups A (control), B and C receiving 0.5 mL each of
distilled water, P. thonningii extract and pefloxacin respectively, while group D was co-administered
P. thonningii extract and pefloxacin (1:1). The rats were sacrificed after 21 days and blood
collected for study.

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Results: *P. thonningii* extract co-administration with pefloxacin produced a significant (*P*<0.05) increase in hematocrit (HCT), lymphocytes and neutrophils and a significant (*P*<0.05) decrease in platelets count compared with the rest of the groups. The effects of pefloxacin co-administration with *P. thonningii* extract on serum electrolyte and kidney function indices were not clear-cut, however creatinine levels were normal.

Conclusion: Synergistic and antagonistic effects of pefloxacin co-administration with *P. thonningii* leaf extract were demonstrated. These effects when properly harnessed could be useful in the management of anaemic conditions, immune responses as well as bone demineralization relating to drug toxicity.

Keywords: Drug toxicity; serum electrolytes; kidney integrity.

1. INTRODUCTION

Pefloxacin is a third generation fluoroquinolone drug with broad spectrum antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria. Pefloxacin a preferred option to chloramphenicol in the treatment of typhoid and other bacterial infections has proven to be quite effective. However the drug has been implicated in several toxic actions on the muscle, tendon, synovial membrane, blood etc [1].

The use of plants, plant extracts and essential oils, isolated and purified compounds have been a source of medicinal agents for thousands of years and have become the major active component of a number of modern drugs. Many of the isolations were based on the use of the medicinal agents in traditional medicine [2]. Their safe use and reduced side effects of many herbal extracts have also projected them as good sources of new pharmaceutical formulations [3].

*Piliostigma thonningii* is a leguminous plant belonging to the family of Fabaceae-caesalpinioideae [4]. It is known across Africa and the Sub-Sahara as camel’s foot and recognized in various local languages in Nigeria ranging from abefe in Yoruba, kalgo in Hausa and okpoatu in Igbo among others [5,6]. *Piliostigma thonningii* has been reported in literatures to have age-long folkloric use in traditional medicine. The leaves and bark have been associated with the treatment of malaria, wounds, ulcers, gastric/heart pain, gingivitis, fever, haemorrhoids and backache, leprosy, digestive disorders and cough. Analgesic properties are also ascribed to the bark as preparations are used for the treatment of sore throat, tooth-ache, stomach-ache and ear-ache [5,7,8]. Its root and twig have been used for the treatment of dysentery, fever, infections, respiratory ailments, snake bites, hookworm and skin diseases [9].

The plant has been reported in a study to demonstrate aphrodisiac potentials [10]. It is therefore imperative to know if co-administration of pefloxacin with *P. thonningii* extract may ameliorate or reduce the adverse effects caused by pefloxacin on haematological and kidney function indices in Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh *P. thonningii* leaves was obtained from Igoli-Okuku road, Cross River State, Nigeria in January, 2013. Identification and authentication was carried out by Mr. E.S Ogunlusi at the Federal College of Forestry, Jos, Plateau State, Nigeria, with the voucher number #25.

2.2 Preparation of *Piliostigma thonningii* Ethanol Leaf Extract

The leaves of *P. thonningii* were collected and air dried for 14 days until constant weight was obtained. The dried leaves were then pulverized after which 300 g was extracted in 1000 mL of ethanol for 72 h with constant shaking using the electric shaker. This was later filtered using Whatman No. 1 filter paper. The filtrates were then concentrated in a water bath at 40°C. The resulting slurry was weighed and reconstituted in distilled water to obtain the required dose for administration.

2.3 Experimental Animals

Twenty four male Wistar rats were obtained from the animal holding unit, Department of Medical Biochemistry. The rats were housed in wooden cages and allowed to acclimatize for 7 days before start of experiment. The animal room was well ventilated and kept at room temperature and relative humidity of 29±2°C and 70% respectively.
with 12 h natural light/dark cycle and were allowed free access to standard feed and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

2.4 Animal Grouping and Administration of Extract

Twenty four male Wistar rats were picked according to body weight and placed into wooden cages labelled A to D. Six male Wistar rats each were placed per cage, with the group labelled A serving as the control group, while B, C and D were the test groups. The animals in the control group (A) were administered daily with standard feed and clean tap water orally, using cannula and syringe. The animals in group B were administered orally with same volume (0.5 mL) corresponding to 200 mg/kg body weight of the ethanol leaf extract, group C was administered pefloxacin (400 mg/5 mL) alone, while group D was co-administered pefloxacin and the extract (1:1) for 21 days. The animals in each group were sacrificed 24 h after the completion of their respective doses for blood samples collection by cardiac puncture and organs harvested for future evaluation [11]. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and use for scientific purposes- ETS-123 [12].

2.5 Blood Sample Collection

Blood was collected from all the rats, by cardiac puncture under chloroform anaesthesia into 2 different sample test tubes for each rat. Heparinised test tubes were used to collect blood samples for haematological indices assay, while plane and sterile test tubes were used to collect blood samples for serum electrolytes, preceded by centrifuging and subsequent separation of the blood plasma with a standard pipette.

2.6 Assay Kits

The assay for serum electrolytes and haematological parameters such as white blood cells count (WBC), red blood cells count (RBC), hematocrit (HCT), neutrophils, lymphocytes, monocytes, platelets, haemoglobin (Hb), were carried out by automated techniques using Microlab 300 from ELITech Group, Germany and Sysmex KX-21N from Sysmex corporation, Japan.

Data obtained were expressed as mean ± SD. SPSS 16.0 was used for one way analysis of variance (ANOVA) and then post hoc (LSD) for the determination of statistical significance, which was accepted at P< 0.05.

3. RESULTS AND DISCUSSION

3.1 Results of Haematological Indices

The ethanol leaf extract of Piliostigma thonningii and pefloxacin administration on haemoglobin, hematocrit, red blood cell count and absolute red blood cell indices of Wistar rats in Table 1 showed significant (P< 0.05) increase in red blood cell (RBC) count, haemoglobin (Hb.) and hematocrit on administration of P. thonningii, pefloxacin and their co-administration compared with the control. In Fig. 1, the administration of P. thonningii extract, pefloxacin, and their co-administration also produced a significant (P<0.05) increase in white blood cell (WBC) count, lymphocytes, neutrophils and MxD when compared with the control. Pefloxacin and P. thonningii leaf extract each produced a significant (P<0.05) increase in platelets counts while a significant (P<0.05) decrease was observed on co-administration of pefloxacin with P. thonningii extract (Fig. 2).

3.2 Results of Kidney Function and Serum Electrolytes Indices

The results of kidney function indices and serum electrolytes of rats administered P. thonningii extract, pefloxacin and their co-administration are given in Fig. 3 and Table 2 respectively. There was a significant (P<0.05) decrease in serum creatinine on administration of P. thonningii extract, while no significant (P>0.05) difference was observed in groups administered only pefloxacin and pefloxacin co-administered with P. thonningii extract compared with the control (Fig. 3). The test groups showed a significant (P<0.05) increase in serum urea and uric acid concentrations when compared with the control (Fig. 3). The effect of administration of P. thonningii extract, pefloxacin or their co-administration on serum electrolyte profile of Wistar albino rats resulted in significantly (P<0.05) raised serum K⁺, Na⁺ and PO₄²⁻ except Ca²⁺ which was significantly (P<0.05) decreased in all the test groups when compared with the
A further significant \((P<0.05)\) decrease in serum \(\text{Ca}^{2+}\) was observed in the group administered only \(P.\ thonningii\) leaf extract compared to the rest of the groups (Table 2).

**Fig. 1. Effect of ethanolic leaf extract of *Piliostigma thonningii* and pefloxacin on white blood cells, lymphocytes, neutrophils and MXD**

*Values are mean ± SD (n=6); Significance accepted at \(P<0.05\)*

*Groups A, B, C and D represent the control, \(P.\ thonningii\) extract only, Pefloxacin only and Pefloxacin + \(P.\ thonningii\) extract respectively*

**Fig. 2. Effect of ethanolic leaf extract of *Piliostigma thonningii* and pefloxacin on platelets**

*Values are mean ± SD (n=6); Significance accepted at \(P<0.05\)*

*Groups A, B, C and D represent the control, \(P.\ thonningii\) extract only, Pefloxacin only and Pefloxacin + \(P.\ thonningii\) extract respectively*
Table 1. *Piliostigma thonningii* ethanol leaf extract and pefloxacin administration on haemoglobin, hematocrit, red blood cell count and absolute red blood cell indices of wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Hb (g/dL)</th>
<th>HCT (%)</th>
<th>RBC (10⁶/µL)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>10.62±0.22b</td>
<td>33.61±0.00d</td>
<td>5.50±0.02²</td>
<td>61.16±0.05c</td>
<td>19.33±0.08bc</td>
<td>31.60±0.00a</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>12.08±0.28c</td>
<td>38.65±0.22b</td>
<td>6.39±0.02c</td>
<td>60.48±0.11d</td>
<td>18.90±0.36bc</td>
<td>31.25±0.88bc</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>13.49±0.16a</td>
<td>42.92±0.05c</td>
<td>6.85±0.09a</td>
<td>62.66±0.05b</td>
<td>19.69±0.28bc</td>
<td>31.43±0.11b</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>13.63±0.15a</td>
<td>43.52±0.02a</td>
<td>6.85±0.09a</td>
<td>63.54±0.22a</td>
<td>19.90±0.38a</td>
<td>31.32±0.33a</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SD (n = 6).
Values with different letters (a, b, c, d) are statistically different (P<0.05).
Groups A, B, C and D represent the control, *P. thonningii* extract only, Pefloxacin only and Pefloxacin + *P. thonningii* extract respectively.

Fig. 3. Effect of ethanolic leaf extract of *Piliostigma thonningii* and pefloxacin on serum creatinine, urea and uric acid
Values are mean ± SD (n=6); Significance accepted at P<0.05.
Groups A, B, C and D represent the control, *P. thonningii* extract only, Pefloxacin only and Pefloxacin + *P. thonningii* extract respectively.

Table 2. *Piliostigma thonningii* ethanol leaf extract and pefloxacin administration on serum electrolytes of wistar rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>K⁺(mmol/L)</th>
<th>Na⁺(mmol/L)</th>
<th>Ca²⁺(mg/dL)</th>
<th>P0₄²⁻(mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>4.720±0.164b</td>
<td>174.40±16.802a</td>
<td>9.19±0.147a</td>
<td>8.52±0.000c</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>5.420±0.10a</td>
<td>198.8±6.573a</td>
<td>6.67±0.186c</td>
<td>11.11±0.00b</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>5.5±0.00a</td>
<td>196.0±0.00a</td>
<td>7.86±0.054b</td>
<td>12.60±0.613a</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>6.2±0.219b</td>
<td>199.2±1.095a</td>
<td>7.73±0.377b</td>
<td>12.87±0.695b</td>
</tr>
</tbody>
</table>

All values are presented as mean ± SD (n = 6).
Values with different letters (a, b, c) are statistically different (P<0.05).
Groups A, B, C and D represent the control, *P. thonningii* extract only, Pefloxacin only and Pefloxacin + *P. thonningii* extract respectively.
3.3 Discussion

The effect of *P. thonningii* ethanol leaf extract and pefloxacin on haematological parameters, serum electrolytes and kidney function indices were evaluated. The assessment of haematological parameters serves as a biomarker for evaluating the hematotoxic potential of an extract/drug in the area of pharmacognosy [13]. Therefore, the increase recorded for WBC following the administration of *P. thonningii* extract and pefloxacin suggests they contain some bioactive constituents which may have boosted the production of WBCs and hence the immune system [14]. It has also been reported that granulocytes, macrophage colony stimulating factor interleukens IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC [15,16].

In differential WBC count, *P. thonningii* extract and pefloxacin each produced increased levels of lymphocytes and neutrophils with their co-administration producing a further increase in both lymphocytes and neutrophils. This shows the potential of *P. thonningii* extract to work synergistically with pefloxacin in the fight against infections or foreign bodies. Similar trend observed for Hb, RBC and HCT indicated that the bioactive agents in the *P. thonningii* extract might have hematopoietic and erythropoietic properties, which was further expressed on co-administration with pefloxacin.

The platelets count was increased on administration of *P. thonningii* extract and pefloxacin separately; suggesting thrombocytosis. But this effect was reversed on co-administration of pefloxacin with *P. thonningii* extract suggesting thrombocytopenia. The assumption drawn from this case is that thrombocytosis induced by pefloxacin can be reversed by using one half the amount of *P. thonningii* leaf extract to the amount of pefloxacin instead of 1:1 ratio used in this study.

Other biochemical indices evaluated in this study were used to assess the functional state of the kidney. This included the biomarkers such as urea, uric acid, creatinine and the serum electrolytes Ca$^{2+}$, PO4$^{2-}$, Cl$^{-}$, Na$^{+}$, K$^{+}$ and HCO3$^{-}$. Electrolytes occur in large quantities, both in extracellular and intracellular fluids due to their ability to dissociate readily into their constituents ions or radicals. They are the single most important factor in the transfer and movement of water between three divisions of extracellular and intracellular regions [17]. Serum phosphate released during cell break down can be used in building nucleic acid of cell. It can be inferred that the increase in serum phosphate might suggest alterations in tubular dysfunction of the nephrons.

The increased level of serum K$^{+}$ following the administration of *P. thonningii* ethanol leaf extract and pefloxacin, suggest that the extract and the drug induced hypernatremia, due to alteration in the physiological and metabolic roles of the kidney. However, there was a concomitant increase in K$^{+}$ which when compared with that of Na$^{+}$ indicates a rather increased level of K$^{+}$ as one moves down the group.

Urea is the major nitrogen-containing product of protein catabolism while uric acid is the major product of the catabolism of purine nucleotides, however, the bulk of purine are ultimately excreted as uric acid from degradation of endogenous nucleic acids. The increased level in serum urea concentration following the administration of pefloxacin shows that the drug may have caused increased protein catabolism [19], which could be counteracted when co-administered with *P. thonningii* extract. Elevation of serum uric acid levels have been implicated in various disorders including gout, increased nuclear breakdown and renal diseases. Ciprofloxacin has been reported to inhibit pyrimidine nucleotide formation and cellular growth [20]. The plant extract, pefloxacin or its combination with the plant extract increased serum uric acid levels. However urea and uric acid levels alone cannot be used as determinants of kidney function as they are affected by quite a number of factors. But creatinine concentration, considered a significant marker in renal dysfunction was not altered hence the overall effect produced by co-administration of pefloxacin with *P. thonningii* extract on the kidney function was minimal or none [21,22].
4. CONCLUSION

This study showed synergistic effects with increased level of lymphocytes and neutrophils and HCT on co-administration of pefloxacin with P. thonningii leaf extract, demonstrating potentials towards fight against infection or foreign bodies as well as hematopoietic and erythropoietic properties. Antagonistic effect against thrombocytosis was also demonstrated. These effects when properly harnessed could be useful in the management of anaemic conditions, immune responses as well as bone demineralization relating to drug toxicity. The co-administration of pefloxacin with P. thonningii leaf extract normalized the serum creatinine levels and this may provide helpful knowledge in addressing the adverse effects of pefloxacin or other drugs on the kidney function.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Faculty of Basic Medical Sciences, Cross River University of Technology (CRUTECH) Research and Ethics Committee (CR/BCM/13/034).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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