Acute Toxicity of *Aeollanthus pubescens* Essential Oil with High Antimicrobial Potential against Multidrug Resistant Bacteria Isolated in Poultry Farms in Benin

Philippe Sessou¹,²*¹, Mahudro Yovo², Rodrigue Towanou¹, Yannick Ayihou¹, Gwladys S. Komagbe¹, Justin Adinci¹, Bruno Ayaoui Yaovi¹, Oscar Nestor Aguidissou¹, Maximin Senou³, Guy Alain Alitonou², Félicien Avlessi², Souaïbou Farougou¹, Dominique Sohounhloue²

¹Research Unit on Communicable Diseases, Laboratory of Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O.Box 2009, Cotonou, Benin.
²Laboratory of Study and Research in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 P.O.Box 2009, Cotonou, Benin.
³Experimental and Clinical Biology Laboratory, National School of Applied Biosciences and Biotechnology, National University of Sciences, Technology, Engineering and Mathematics, P.O Box 14 Dassa-Zoume, Benin.

Abstract

**Background and objectives:** The present work aimed to evaluate the acute toxicity of *Aeollanthus pubescens* essential oil, whose antimicrobial activity has been proven in vitro by previous studies and to examine the effects of this essential oil on the biochemical parameters (transaminases ALT and AST, urea, creatinine and cholesterol) and hematological and histological factors in Wistar rats subjected to this volatile oil. **Methods:** Nine male Wistar rats, 12 weeks old weighing more than 140 g were divided into three groups of three rats each; group one normal control rats; group two normal rats which received the extract of *A. pubescens* by gavage at a dosage of 2000 mg/kg of body weight and finally group three consisting of normal rats receiving the extract of *A. pubescens* by intramuscular injection at a dosage of 2000mg/kg body weight. The duration of the experiment was 14 days. **Results:** At the end of the study, the essential oil of *A. pubescens* did not cause any mortality in the experimental rats, which indicated that the extract did not exhibit acute toxicity at this dose. Biochemical and hematological analyses revealed no adverse effects (p>0.05) on the hepatic, renal, lipid and hematological parameters measured in these animals. Histological examination showed no alteration of the hepatic and renal structures. **Conclusion:** This oil can be considered generally recognized as safe at the experimental dosage and can be used in the fight against pathologies of bacterial origin.

**Keywords:** acute toxicity; *Aeollanthus pubescens*; essential oil; Wistar rats


**Introduction**

Livestock is a very important pillar for food and the economy of Third World countries and is a growing sector in West Africa. Current production methods, allowing greater productivity, have considerably introduced risk factors and the performance of this sector is...
hampered by several health obstacles including pathologies [1,2]. Faced with these pathologies, the means of struggle of breeders rely mainly on the use of synthetic molecules including antibiotics. The misuse of these synthetic molecules has led to a worrying increase in the number of multi-resistant pathogenic strains [3,4]. Antibiotic resistance has become a major concern around the world and is severely impacting the global economy due to economic losses related to the decline in productivity caused by diseases and the rising cost of treatment [5]. As a result, traditional medicine and African pharmacopoeia medications are increasingly emerging as therapeutic alternatives in health systems [6]. In Africa, the use of medicine and traditional pharmacopoeia is a very common practice in the countryside and even in cities, and over 90% of the population in the countries use them for their primary health care and livelihood [7]. The rational exploitation of traditional African medicine can help to solve the problems of geographic and economic accessibility of the majority of populations for effective drugs against multidrug-resistant strains [8,9]. Despite this great appeal, the products of traditional medicine including plant extracts are questionable because of the lack of scientific evidence for their pharmacological properties and their safety [10]. In recent studies, extracts of several plants have been investigated and have shown antiviral, antibacterial, anti-inflammatory, antimalarial, and antifungal properties of interest in vitro. Among these plants, *Aeollanthus pubesens* was mentioned to have strong anti-free radical and antimicrobial potential. The essential oil of this plant has exhibited strong antimicrobial activity against *Escherichia coli* and *Salmonella* isolated from chickens [3,4] and against *Staphylococcus aureus*, *Escherichia coli* ATCC 25922, coagulase-negative *Staphylococcus* and *Klebsiella pneumoniae* ATCC 818 E isolated from humans [11]. Since the effectiveness of a therapeutic molecule depends on several parameters including its biological properties and safety, it is important to evaluate the toxicity of this essential oil in order to better promote this resource. In the present study, the acute toxicity of *Aeollanthus pubesens* essential oil in Wistar rats has been evaluated; also, the effects of this essential oil on certain serum biochemical parameters of the liver, renal and lipid function as well as hematological parameters and histological examination of the liver and kidneys of the animals were investigated.

**Material and Methods**

**Ethical considerations**

The experimental procedures performed were in agreement with the Ethical Directors in Animal Research and approved by Ethical Committee of Research Unit in Applied Microbiology of Natural Substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi (EPAC) under the number 167-07, 2018.

**Plant material**

The leaves of *Aeollanthus pubesens* were collected in June 2017 from the mountainous areas of Dassa-Zoume in Benin at Awaya and authenticated at National Herbarium of University of Abomey-Calavi where it was deposited under voucher specimen AA 6377/HNB.

**Essential oil extraction**

Essential oil used in this study was obtained by hydrodistillation using a Clevenger type apparatus and analysed using GC and GC-MS [12].

**Animals**

Our experiment was performed on 9 male Wistar rats aged 12 weeks and weighing more than 140 g. Upon receipt, they were housed in 50×30×20 cm$^3$ wire mesh cages and maintained under experimental conditions described by the OECD (Organization for Economic Cooperation and Development, Guideline-423, adopted in December 23, 2001). The temperature of the room was 25 degrees and artificial lighting had been provided alternating sequences of 12 hours of light and 12 hours of darkness. The rats were fed with standard food in granulated form, supplied by the Véto Service Group SA (GVS) in Benin and fed ad'libitum in drilling water.

**Acute toxicity studies**

The acute toxicity studies were conducted in accordance with the OECD Guidelines (Organization for Economic Cooperation and Development, Guideline-423, adopted on 17 December 2001). The essential oil of *A. pubesens* was administered once during the experiment as a single dose at 2000 mg/kg body weight following the acute oral toxicity
protocol of the OECD Guidelines for the Testing of Chemicals, Guideline 423, adopted on December 17, 2001 and following good laboratory practice. The rats were divided into three groups of three rats each; group one control rats; group two of normal rats which received the extract of *A. pubescens* by gavage at a dosage of 2000 mg/kg of body weight and finally group three consisting of normal rats receiving the extract of *A. pubescens* by intramuscular injection at a dose of 2000 mg/kg body weight. Twelve hours before administration of the extracts, the animals were deprived of food. After this period of fasting, they were weighed and then the essential oil was administered. The dosage was calculated according to the fasting body weight of each animal. The behaviour of rats were examined during the 14 days of experiment. At the end of the study, the essential oil presented acute toxicity if 50% of experimental rats died.

**Blood sampling**

Blood collection was performed according to the method described by Costa-Silva et al., [13] using hematocrit tubes (capillaries) through the retro-orbital sinus in the eye, before the assay and 14 days after administration of the extract. The rats were anesthetized with ether by inhalation for 2 to 3 minutes under an experimental sealed laboratory rodent jar. The blood was collected in two different tubes, a dry tube for the determination of hematological parameters (NFS) and a second EDTA tube for the determination of biochemical parameters. Each tube was numbered to facilitate identification throughout the analysis process. The biochemical parameters including cholesterol, transaminases (ALT and AST), urea and creatinine were evaluated. These parameters were determined using a spectrophotometer (BioLabo Diagnostics, Kenza Max, and Biochemistry, France) at 505 nm [14]. The study of hematological parameters was based on the Blood Form Count (NFS). The measured hematological parameters were as follows: mean blood contents in number of white blood cells (GB), lymphocytes (Lymph), granulocytes (Gran), hemoglobin (HGB), red blood cells (RBC), hematocrit (HCT), blood volume mean (MCV), mean hemoglobin (TMH), mean hemoglobin concentration (MCHC), platelets (PLT). They were evaluated using Haematologic Automata (Auto Hematology Analyzer, RT-7600, Rayto, China) [14].

**Histological study**

This study consisted of observing any lesions that may be induced by the extract on the liver and kidneys of the animals.

**Rats analyses and organ study**

At the end of the experiment, the surviving rats were euthanized, dissected and then the liver and kidneys were removed for histological examination.

**Histological examination**

Histological examinations were performed according to the method described by Ahossi [15]. The sections previously made with the microtome were stained with hematoxyline eosin, fixed between slide and coverslip before being observed using a photonic microscope equipped with a camera. The observations were carried out at 400X magnification, then the images were captured and transferred in JPEG format.

**Statistical analysis**

The data was processed using SAS (Statistical Analysis System, 2013). One-way analysis of variance was performed. The lot was the variation factor considered. The procedure of generalized linear models (Proc GLM) was used for analysis of variance. The F test was used to determine the significance of the group factor and the averages were compared two by two by the student's t test.

**Results and Discussion**

The survey of the behavioral parameters in the rats reveals that, after oral administration and intramuscular injection of *Aeollanthus pubescens* essential oil at 2000 mg/kg to the experimental rats, no mortality was recorded. In addition, no sign of morbidity or moribund animals was also observed throughout the 14 days of the experimentation. Similarly, no change in behavior and changes of the skin, hair, eyes, mucous membranes and the respiratory system and no physical or behavioral signs of toxicity such as sleep, hyperactivity, agitation, respiratory or convulsions issues were observed. However, inflammation at injection site in group three subjects who received an intramuscular injection at 2000 mg/kg body weight were noted; also, lameness was observed in these same subjects. These results show that the essential oil of *A. pubescens* has no toxic effect (no mortality) after administration at 2000 mg/kg body weight.
which implies that the LD$_{50}$ lethal dose is greater than 2000 mg/kg body weight. With respect to the changes in weight gain (table 1), oral and intramuscular administration of *Aeollanthus pubescens* essential oil at 2000 mg/kg did not influence normal weight growth in the treated rats. There is a gradual change in weight in the experimental rats (186.66 ± 11.54 to 233.33 ± 14.43 for the control, 180.00 ± 30.41 to 230.00 ± 36.05 in the gaved rats and 191.66 ± 30.13 to 231.66 ± 31.75 in the rats injected). The statistical analysis of the weight average values reveals that there is no significant difference between the animal weights of the three groups at days 0 and 14. Our results about the influence of the extract on weight growth are similar to those reported by Jothy et al. [16].

Table 1. Body weight survey results

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g) ± SD</th>
<th>Day 0 (N=3)</th>
<th>Day 14 (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>186.66±11.54</td>
<td>233.33±14.43</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>180.00±30.41</td>
<td>230.00±36.05</td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>191.66±30.13</td>
<td>231.66±31.75</td>
<td></td>
</tr>
</tbody>
</table>

Group 1: Normal control rats; Group 2: Normal rats with *Aeollanthus pubescens* by gavage; Group 3: Normal rats with *A. pubescens* by intramuscular injection; values of same surveys column having the same letters are not different in significance level of 0.05.

Several biochemical parameters such as transaminases (AST and ALT), urea, creatinine and cholesterol have been measured in order to evaluate the effect of *A. pubescens* essential oil on the functioning of vital organs in animals. Table 2 shows the average levels of the above-indicated parameters at the level of the experimental groups as a function of time. The analysis of this table showed that no significant difference (p>0.05) was observed between the levels of these parameters at day 0 and day 14 in the three groups. However, ALT levels at day 14 of the rats in the three groups were slightly lower than those at Day 0. On the other hand, a much more marked increase (p>0.05) in AST levels on day D14 compared to day 0 in groups 1 and 3 was noted. Administration of the essential oil by gavage seemed to be the best way to avoid unusual fluctuation in their AST levels. With regard to cholesterol level, no significant increase (p>0.05) was noticed on day D14 compared to day 0 in the three groups, but the increase was much more marked in group one (table 3). The administration of the essential oil to animals would regulate their cholesterol levels. As for the urea and creatinine levels, the results revealed no significant variations (p>0.05) between day 14 and day 0. However, there was a slight decreased creatinine on day 14 in group one and a slight increase in urea level on day 14 in group 2 (table 2).

The results on the biochemical parameters of the rats showed that there were no significant variations between the levels of ALT, AST, cholesterol, urea and creatinine of the rats subjected to the extract compared to the controls. ALT and AST are two liver enzymes whose role is to transfer an amine group during the chemical processes that take place in the liver [17]. Their activity is proportional to the degree of liver damage [18]. They are therefore two good indicators of hepatotoxicity [19-22]. The double or even triple increase in ALT level indicates hepatic cytolysis [23]. A serum increase in ASAT activity reflects an inflammatory, traumatic or degenerative state of the tissues as a result of cell death and plasma membrane damage [24,25]. The nonsignificant variation in ALT levels observed in both control and experimental rats indicated the absence of hepatic cytolysis, inflammation and tissue degeneration of rats investigated. Therefore, it could be concluded that *A. pubescens* did not have a toxic effect on rat liver at 2000 mg/kg body weight.

The cholesterolemia gives information on the mobilization of body fat reserves by the animal. Cholesterol is present in the diet and can be synthesized by the liver in a mechanism with very fine metabolic regulation [26]. The decrease in cholesterol may be related to environmental conditions, dietary deficit or pathologies [27]. The increase could be due to food origin. Thus, the insignificant increase noted in the three batches would not be related to the *A. pubescens* essential oil, but rather to the food given to the experimental rats because throughout the experimental period, the food was served at will to animals.

Renal function is appreciated by serum creatinine and urea. Urea and creatinine are significant markers of kidney function [28]. These metabolites, finished products from protein metabolism, have a generally constant concentration under normal conditions [29]. Their increase or decrease reflects renal dysfunction [30]. Pritchard et al. [31] have shown that a decrease in serum creatinine may be a sign of cachexia. With regard to the serum concentration of urea, its increase may be a sign
of nephropathy, dehydration, electrolyte imbalance, hypo-albuminemia, tissue catabolism (fever, trauma muscle, myositis) [32]. These levels which did not vary in treated rats compared to controls, show normal renal function. Overall, since there have been no significant variations in the levels of AST, ALT, urea, creatinine and cholesterol, it could be concluded that *A. pubescens*’ essential oil was not toxic to the liver, kidneys and had not disrupted lipid metabolism in rats at 2000 mg/kg body weight. The essential oil of *A. pubescens* administered to rats at 2000 mg/kg body weight did not cause significant changes in white blood cell and red blood cell counts and platelets. White blood cells, being a family of cells composed of granulocytes, lymphocytes and monocytes, play an important role in the fight against infections and in the development of resistance to infection in response to natural exposure or vaccination [33]. Indeed, their rate is increased in case of infections (viral or severe), inflammation, cancer or leukemia and can be decreased by certain drugs, during certain autoimmune diseases, bone marrow failure, splenomegaly, liver disease [32]. Their level did not vary in treated rats compared to controls showing that the essential oil of *A. pubescens* had no toxic effect on the white blood cells of rats at 2000 mg/kg body weight.

Several haematological parameters were evaluated in order to assess the effect of *A. pubescens* essential oil on the functioning of vital organs in animals. Mean blood levels in white blood cells (GB), lymphocytes (Lymph), granulocytes (Gran), hemoglobin (HGB), red blood cells (RBC), hematocrit (HCT), average blood volume (MCV), mean hemoglobin concentration (TMH), mean corpuscular volume (MCV), mean hemoglobin (TMH), mean hemoglobin concentration (MCHC) and red blood cell distribution index (IDR-DS), there was a slight insignificant increase (p>0.05) in all groups on day D14. Platelets (PLT) and plateletocreat (PCT) decreased in group one (p>0.05) but increased in other groups on day 14. Platelet distribution (IDP) showed a non-significant decrease in groups one and three, but a slight increase in group two. For mean platelet volume (MPV), a slight increase was noted in group one but a slight insignificant decrease in other groups. Nevertheless, there were no significant differences between the three groups (p>0.05). The platelet assay can detect a hemorrhagic risk or infectious or inflammatory syndromes after a major haemorrhage [34]. This parameter did not vary in our case showing that the studied oil had no toxic effect on the platelets of rats at 2000 mg/kg body weight. Despite the few variations noted in each lot, the statistical analyzes revealed that there were no significant differences (p>0.05) between the haematological parameters of the rats which had received the *A. pubescens* essential oil and the control rats and there were also no significant variations in these parameters between day 0 and day 14 at each group.

An increase in VGM, TMH and CCMH indicates the presence of macrocytic normochromic red blood cells, whereas a decrease in their levels reflects the presence of microcytic hypochromic red blood cells [35]. In our case, there were no significant variations in these constants, which showed that red blood cells of rats were normochromic normocytic, and therefore, *A. pubescens* essential oil had no toxic effect on red blood cells of rats at 2000 mg/kg body weight. In sum, essential oil of *A. pubescens* did not have a toxic effect on the evaluated haematological parameters. Nevertheless, the insignificant fluctuations recorded in this study could be related to the factors of variation related to the individual concerned and his environment (stress induces a margin of leucocytes, for example), and to all factors related to cell variability, especially their life span [36].

Microscopic liver examination of Wistar rats treated with 2000mg/kg with *A. pubescens* essential oil resulted in no significant changes and showed normal liver parenchyma between normal, gavaged and having received the intramuscular injection extract groups.
Table 2. Effects of Aeollanthus pubescens essential oil on rats biochemical parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>RMSE</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>61.08±0.96</td>
<td>61.02±0.84</td>
<td>60.99±0.97</td>
<td>3.86</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>46.85±0.25</td>
<td>46.62±0.35</td>
<td>46.59±0.34</td>
<td>3.28</td>
<td>NS</td>
</tr>
<tr>
<td>AST</td>
<td>188.32±2.80</td>
<td>187.66±2.34</td>
<td>187.51±2.66</td>
<td>2.97</td>
<td>NS</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.35±0.20</td>
<td>2.34±0.22</td>
<td>2.33±0.22</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinin</td>
<td>1.14±0.23</td>
<td>1.14±0.23</td>
<td>1.14±0.23</td>
<td>0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Chol</td>
<td>1.14±0.23</td>
<td>1.14±0.23</td>
<td>1.14±0.23</td>
<td>0.17</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group 1: normal control rats; Group 2: normal rats with A. pubescens by gavage; Group 3: normal rats with A. pubescens by injection intramuscular; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Chol: cholestérol total; Creat: creatinine; NS: not significant; Values of same surveys column having the same letters are not different at significance level of 0.05; RMSE: Root Mean Square Error; N=3

Table 3. Effects of Aeollanthus pubescens essential oil on rats haematological parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>RMSE</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>11.07±0.39</td>
<td>11.06±0.38</td>
<td>11.05±0.38</td>
<td>0.39</td>
<td>NS</td>
</tr>
<tr>
<td>Hb</td>
<td>4.35±0.35</td>
<td>4.34±0.35</td>
<td>4.34±0.35</td>
<td>0.34</td>
<td>NS</td>
</tr>
<tr>
<td>Hct</td>
<td>54.60±0.41</td>
<td>54.59±0.42</td>
<td>54.58±0.42</td>
<td>0.42</td>
<td>NS</td>
</tr>
<tr>
<td>MCV</td>
<td>18.40±0.48</td>
<td>18.39±0.49</td>
<td>18.38±0.49</td>
<td>0.49</td>
<td>NS</td>
</tr>
<tr>
<td>MCHC</td>
<td>55.60±0.50</td>
<td>55.59±0.50</td>
<td>55.58±0.50</td>
<td>0.50</td>
<td>NS</td>
</tr>
<tr>
<td>MCH</td>
<td>37.17±0.51</td>
<td>37.16±0.52</td>
<td>37.15±0.52</td>
<td>0.52</td>
<td>NS</td>
</tr>
<tr>
<td>MPV</td>
<td>18.90±0.57</td>
<td>18.89±0.58</td>
<td>18.88±0.58</td>
<td>0.59</td>
<td>NS</td>
</tr>
<tr>
<td>platelets</td>
<td>35.39±0.59</td>
<td>35.38±0.59</td>
<td>35.37±0.59</td>
<td>0.60</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group 1: normal control rats; Group 2: normal rats with A. pubescens by gavage; Group 3: normal rats with A. pubescens by injection intramuscular; GB: white blood cells, Lymph: lymphocytes, Gran: granulocytes, HGB: hemoglobin, RBC: red blood cells, HCT: hematocrit, MCV: average blood volum, TMH= Mean Corpuscular hemoglobin Content, MCHC: concentration Mean hemoglobin corpuscularity, PLT: platelets; Values of same surveys column having the same letters are not different at significance level of 0.05; RMSE: Root Mean Square Error; N=3

Figure 1. Lever histology (magnification 400X); VC: centrilobular veins; S: sinuosids; A: liver histology of normal rats; B: liver histology of rats which received Aeollanthus pubescens by gavage; C: liver histology of rats which received A. pubescens by intra muscular injection.
In the gavaged rats (figure 1B) and injected with the extract (figure 1C), the hepatic parenchyma showed the typical appearance observed in the control rats (figure 1A). Hepatocytes (arrows) had no visible atypia and were well arranged in radii around the centrilobular veins (VC). Between these hepatocytes, the sinusoids (S) were clearly visible. The histopathological study of the liver revealed no cases of inflammation or hepatic cell necrosis. These results confirmed that the rats were healthy and that these treatments did not cause hepatic injury.

The kidneys of control rats did not present any renal parenchyma alteration. The same remark was made in rats that were administrated by gavage and intra muscular injection. In fact, the renal parenchyma of the gavaged rats (figure 2B) and the rats injected with the extract (figure 2C) had the typical architecture observed in the control rats (figure 2A). Glomeruli (G), proximal tubes (TP), the distal tubes (TD) and the collecting ducts (CC) showed normal appearance. The histological study confirmed that the mice were healthy and had no nephrotic abnormalities. These histological results were in correlation with the biochemical and haematological analyses which showed that the essential oil of *Aeollanthus pubescens* shows no toxic effects on the liver and kidneys; therefore, this oil can be, through its antimicrobial capacity and its absence of acute toxicity at 2000mg/kg, an suitable remedy for the treatment of microbial infections with *Escherichia coli* and multidrug-resistant *Salmonella* occurring on farms and can be used in the endogenous fight against the pathologies associated with these microorganisms.

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**Author contributions**
Philippe Sessou, Yannick Ayihou and Gwladys S Komagbe conceived the trial design, conducted the trial and drafted the manuscript with input from all authors. Rodrigue Towanou, Yannick Ayihou and Bruno Ayaovi Yaovi performed the biochemical analysis and aided in interpreting the results. Philippe Sessou, Justin Adinci, Gwladys S Komagbe and Oscar Nestor Aguidissou performed the histological analysis under supervision of Maximin Senou who aided in interpreting the results. Mahudro Yovo, Guy Alain Alitonou and Felicien Avlessi contributed in the collection of plant material, its identification, essential oil’s extraction and chemical interpretation of the results. Souaibou Farougou supervise the data analysis. Félicien Avlessi, Souaibou Farougou and Dominique
Sessou P. et al.

Sohounhloue supervised the project. All authors provided critical feedback and helped shaping the research, analyses, and manuscript. They have critically revised and approved the final manuscript.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References


**Abbreviations**