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Acute and sub-acute toxicity evaluation of aqueous decoction extract of *Xylopia aethiopica* fruit pods in post-partum female albino rats

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Abstract

Decoction extracts of Xylopia aethiopica fruit pods are traditionally given to women immediately after childbirth in Eastern Nigeria, because of its widely believed benefits during the puerperium. There is however paucity of reports in available literature on the toxicity and safety of the extracts. The aim of this study was to evaluate the acute and sub-acute toxicity of aqueous decoction extract of Xylopia aethiopica fruit pod in female albino rats during the immediate post-partum period. The new three-stage method of acute toxicity testing with a fourth confirmatory (confidence) stage was used for the acute toxicity evaluation. In sub-acute toxicity evaluation, 16 female rats that just delivered their pups were used. They were randomly assigned to four groups (A, B, C and D), that were orally given 1000, 500, 250 and 0 mg/kg of the extract, respectively, from the day of delivery (day 0 post-partum) to day 7 post-partum. The Group D was the untreated control. Blood samples were collected from the rats on days 1, 3, 5 and 7 post-partum for haematological and serum biochemical assays, following standard procedures. Results showed that the administration of the decoction extract of X. aethiopica fruit pod up to 5000 mg/kg body weight in the acute toxicity test did not lead to any signs of toxicity or mortality. The sub-acute toxicity evaluation showed no obvious signs of toxicity in the females and their neonates and also no clinically significant alterations in erythrocytic and leukocytic parameters and serum biochemical markers of liver and kidney dysfunction. It was concluded that oral administration of Xylopia aethiopica fruit pod decoction extracts as used in this study was acutely and sub-acutely non-toxic, and was well tolerated by the female albino rats, post-partum.

Keywords: *Xylopia aethiopica* fruit pods; Acute toxicity; Sub-acute toxicity; Puerperium; Haematology; Serum biochemistry.

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Introduction

Many people worldwide, especially rural dwellers depend majorly on medicinal plants for their health care needs (Uchendu and Isek, 2008). Almost all cultures have utilized plants as an alternative source of medicine, and plants are thus regarded as the foundation of traditional medicine (Cragg and Newman, 2001). Right from ancient times, people have used parts of medicinal plants to treat various ailments (Cragg and Newman, 2001). In most developing nations, herbal medications are easily obtainable, affordable and appreciated by patients (Sofowora, 1985; George, 2011).

Most medicinal plants are assumed to be safe or non-toxic because they come from "natural" sources, and their traditional use as medication has been based primarily on longterm cultural and clinical experience rather than scientific data on their efficacy and safety (Bent and Ko, 2004; Bhowmik et al., 2009). These herbal products, however, may contain bioactive ingredients that could damage vital organs of the body (Bhowmik et al., 2009). Given the rise in the utilization of medicinal products of plant origin, an in-depth scientific study of these plants is necessary, especially with regards to their safety/toxicity and the validity of their claimed traditional/folkloric uses (Sofowora, 1989).

Xylopia aethiopica belongs to the *Annonaceae* family. It produces odoriferous fruits which are slender, slightly curved pods with about 15 carpels (Fetse *et al.*, 2016). It flourishes in the African Savanna, particularly in Ghana, Nigeria, Cameroon, Ethiopia and Senegal. Its common names include: African pepper, Guinea pepper, spice tree (Tairu *et al.*, 1999). The fruits are used as spices, and the aqueous decoction is commonly given to women in Nigeria after childbirth, as it is believed to boost the immune system and lactation, increase uterine contraction and facilitate placental and lochia expulsion (Imo *et al.*, 2018; Okwunodulu *et al.*, 2023). The fruit is

also used traditionally to treat cough, nausea, dizziness, amenorrhea, bronchitis (when smoked and inhaled), dysentery, boils and skin eruptions (Burkil, 1985). A mixture of the roots and fruits of *X. aethiopica* has been reported to be useful in the treatment of rheumatism (Orwa *et al.*, 2009).

Several of these folkloric and traditional uses been substantiated and validated had scientifically. Woode et al. (2012) reported that the ethanol extract of Xylopia aethiopica fruit extract showed analgesic attributes. Obiri and Osafo (2013) reported that the plant demonstrated extract anti-inflammatory properties by stabilizing cell membranes, thus mast cells from preventing releasing histamine. The anti-diabetic properties of the plant extract were reported by Mohammed et al. (2016). Xylopia aethiopica has also been shown to exhibit anti-microbial activities (Asekun and Adeniyi, 2004; Fleischer et al., 2008). Njoku (2023) showed that oral administration of aqueous decoction extract of the fruit pods of X. aethiopica during the immediate post-partum period enhanced post-partum uterine involution in albino rats. The diverse therapeutic and pharmacological properties of X. aethiopica have been attributed to certain chemical compounds that have been identified and isolated from various parts of the plant. These include sterols, saponins, carbohydrates, glycosides, mucilage, acidic compounds, tannins, balsams, cardiac glycosides, volatile aromatic oils, phenols, rutin, fixed oil, alkaloids and flavonoids (Asekun and Kunle, 2004; Esekhiagbe et al., 2009; Nwaichi and Igbinobaro, 2012; Nworah et al., 2012). The fruit, bark and stem of X. aethiopica have also been shown to contain acidic compounds such as kaurine, kolavane, and trachylobanediterpenes (Adosraku and Oppong-Kyekyeku, 2011).

Though the decoction of the fruit pods of *X*. *aethiopica* is commonly administered to women after childbirth, there had been no reports in available literature on the acute and

sub-acute safety of the decoction in females during puerperium. The present study evaluated the oral acute and sub-acute toxicity of aqueous decoction extract of *Xylopia aethiopica* fruit pod in female albino rats during the immediate post-partum period.

Materials and Methods

Plant Material: The dried *Xylopia aethiopica* fruit pods (Figure 1) were purchased from Ogige Market, Nsukka Local Government Area, Enugu State, Nigeria. The fruit pods were authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria.



Figure 1. A picture of dried *Xylopia aethiopica* fruit pod.

Preparation of the Extract: The dried fruit pods were rinsed under flowing tap water to eliminate contaminants, and air-dried under shade at room temperature. Thereafter, the fruit pods were milled using a laboratory blender. The pulverized fruit pods were placed into a clean pot containing water at the ratio of 1:4, and heat was applied for 15 minutes (Abdullahi and Mainul, 2020). Afterwards the solution was filtered with Whatmann No. 1 filter paper. This decoction extraction procedure was chosen for the preparation of the extract in the present study because it is the traditional method of its preparation and utilization in humans.

Experimental animals: Thirty sexually matured female albino rats (12 weeks of age) were used for the study. They were obtained from the Laboratory Animal Unit of the Department of Veterinary Obstetrics and Reproductive Disease, University of Nigeria, Nsukka. The rats were allowed to acclimatize for four weeks under standard laboratory conditions. Fourteen of the rats were used for the acute toxicity test, while 16 were used for the subacute toxicity evaluation. The ones used for the acute toxicity test were virgin females while the ones used for the sub-acute study were females that just got delivered of their pups: they were batch-bred to become pregnant and deliver almost at the same time. All of the rats were fed standard pelleted rat feed (Vital Grower Feed, Grand Cereals Ltd, Ogbaru, Nigeria) and provided with clean drinking water ad libitum for the entire duration of the study. The rats were handled humanely throughout the study in accordance with the guiding principles of the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The study protocol and design were approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka with the Approval Reference Number: FVM-UNN-IACUC-2023-08/115.

Chemicals and Reagents: The reagents and test kits for evaluation of the serum activities of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST), and levels of serum total proteins, albumins, total cholesterol and creatinine were procured from Quimica Clinica Aplicada (QCA), Spain. That for evaluation of serum bilirubin was sourced from Randox Laboratories Ltd, County Antrim, United Kingdom, while the test reagents for serum urea evaluation were sourced from DIALAB, Neudorf, Austria.

Acute Toxicity Test: The acute toxicity study was done following the new three stage method of acute toxicity testing, with a fourth confirmatory (confidence) stage (Enegide et al., 2013), which is based on an improvement of the two-phase Lorke's method (Lorke, 1983) and the OECD (2001) methods. For the first stage, four rats assigned to four groups were given 50, 200, 400 and 800 mg/kg body weight of the extract and watched for 24 hours. In the second stage, three rats assigned to three groups were given 1000, 1500 and 2000 mg/kg body weight of the extract and watched for 24 hours. For the third stage, three rats assigned to three groups were given 3000, 4000 and 5000 mg/kg body weight of the extract and watched for 24 hours. In the absence of mortality and any obvious signs of toxicity, at the confirmatory stage, the highest dose (5000 mg/kg body weight) was given to three rats and they were observed for 24 hours. Changes in the skin, mobility, behaviour, response to stimuli, respiration patterns and mortality (if any) were looked out for.

Sub-acute Toxicity Test: The 16 pregnant rats that were delivered of pups which were used for the sub-acute toxicity study, were randomly assigned to four groups (A, B, C and D) of four rats each. From the day of delivery (Day 0), each rat in the specific treated groups (A, B, and C) were given 1000, 500 and 250mg/kg of the extract respectively, once daily (every morning) by intra-gastric gavage. The control group (Group D) received distilled water placebo. Treatment started from the day of delivery (Day 0) and went on daily for 7 days. On days 1, 3, 5 and 7 post-initiation of treatment (PIT), the rats were weighed and blood samples collected from them. An aliquot (0.5 ml) of each of the blood samples was dispensed into sample bottles containing ethylene diamine tetra acetic acid (EDTA) for haematology while the remaining (1.5 ml) was dispensed into plain glass test tubes to clot for serum harvest. The clotted blood was centrifuged at 3,000 revolutions per minute

for 20 minutes to separate the serum from clot. The clear serum was dispensed into labeled Eppendorf tubes for clinical biochemistry determinations.

Haematological evaluations: The red blood cell (RBC) counts and total white blood cell (TWBC) counts were done following the manual haemocytometer method, while the differential white blood cell counts were done on air-dried thin blood smears stained by the Leishman technique (Thrall and Weiser, 2002). Packed cell volume (PCV) determination was done by the microhaematocrit method (Thrall and Weiser, 2002), while the haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method (Higgins *et al.*, 2008a).

Biochemical assays: Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed based on the Reitman-Frankel method (Reitman and Frankel, 1957), while the serum alkaline phosphatase (ALP) activity was assayed by the thymolphthalein monophosphate method (Roy, 1970). Serum levels of total protein were determined by the direct Biuret method (Johnson, 2008), while the serum albumin levels were determined by the bromocresol green method (Doumas and Peters, 1997). Serum levels of creatinine were determined by the modified Jaffe method and the serum urea levels were determined by the Berthelot-Searcy enzymatic reaction method (Lamb and Price, 2008). The serum total bilirubin levels were determined by the Jendrassik and Grof method (Higgins et al., 2008b), while the serum cholesterol levels were determined by the enzymatic colorimetric method (Rifai et al., 2008).

Statistical Analysis: Data collected were analyzed using one way analysis of variance (ANOVA), and variant means were separated using the least significant difference (LSD) method. The analysis was done using IBM SPSS software package (version 21.0 for Windows[®], IBM Inc., Chicago, USA). Significant difference was accepted at probability level less than 0.05. Results were presented as mean ± standard deviation.

Results

The percentage yield of the fruit pod extraction was 19.6%, and the decoction was observed to be brownish.

In the acute toxicity testing, administration of the decoction extract of *X. aethiopica* up to 5000 mg/kg body weight, did not lead to any adverse behavioral or physiologic effects on the treated rats. No mortality was recorded at all the doses of the decoction extract administered, up to the maximum of 5,000 mg/kg. The LD_{50} was therefore regarded as being greater than 5,000 mg/kg.

On observations of the rats during the subacute toxicity study, there were no visible signs of toxicity or abnormal behavior by the rat groups given *Xylopia aethiopica* fruit pod decoction extract (*XADE*) at all the doses (250, 500, and 1000 mg/kg). They were able to nurse their neonates effectively. No mortality was recorded in any of the rat groups and their neonates all through the sub-acute toxicity study.

The results of the PCV, RBC and Hb of the rat groups following treatment with graded doses of *XADE* are presented in Figures 2, 3 and 4, respectively. There were no significant (p > 0.05) differences in the PCV, RBC count and Hb of the treatment groups (A, B and C) when compared to Group D (untreated control) all through the treatment period, except on day 1 post-initiation of treatment (PIT), where the means of these parameters for Group C was significantly (p < 0.05) lower than that of Group D (Figure 2, 3 and 4).



Figure 2. Packed cell volume (PCV) of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.







Figure 4. Haemoglobin concentration (Hb) of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, postpartum.

The mean TWBC counts of the Group B and D rats was significantly lower (p < 0.05) than that of Group A rats on day 1 PIT, while on day 3 PIT the mean TWBC counts of Group A was significantly lower than that of Group B (Figure 5). Further on day 5 of treatment, the mean TWBC count of Group C rats was significantly lower than those of all other groups (Figure 5). The mean lymphocyte counts of the Group A rats was significantly (p < 0.05) higher than those of all the other groups on day 1 PIT, but on day 5 PIT, only the mean lymphocyte counts of Group C rats was significantly lower (p < 0.05) than that of all other groups (Figure 6). There were no significant differences (p >0.05) in the mean neutrophil counts of the rats groups except on day 7 PIT when the mean neutrophil count of Group B rats was significantly (p < 0.05) higher than that of Group D rats (Figure 7). The mean eosinophil counts of the Group A rats was significantly (p < 0.05) higher than those of all other groups on day 1 PIT, but on day 3 PIT, that of group C was significantly (p < 0.05) higher than those of groups B and D (Figure 8). Further on day 5 PIT, the mean eosinophil counts of the Group C rats was significantly (p < 0.05) higher than those of all other groups, while on day 7 PIT, those of Groups B and C were significantly (p < 0.05) higher than those of Groups A and D (Figure 8). There were no significant (p > 0.05)variations in the mean monocyte counts of the rat groups on days 1, 3 and 5 PIT, but on day 7 PIT, the mean monocyte counts of Group A rats was significantly (p < 0.05) higher those of all other groups (Figure 9).

There were no significant variations (p > 0.05) in the mean serum level of urea across the various groups (Figures 10) but the mean serum creatinine of Group D rats was significantly (p < 0.05) lower than those of Groups A and B on day 5 PIT (Figure 11). The mean total protein of Group A rats was significantly (p < 0.05) higher than that of Group D rats on day 3 PIT (Figure 12), whereas there were no significant variations (p > 0.05) in the mean serum levels of albumin across the various groups (Figures 13) all through the study. The mean serum ALT activity of Group C rats was significantly (p < 0.05) lower than those of Groups A and D on day 5 PIT (Figure 14), while those of Group B was significantly (p < 0.05) higher than that of Group D on day 7 post-partum (Figure 14). The mean serum AST activity of Group C rats was significantly (p <0.05) higher than those of all other groups on day 5 PIT (Figure 15). The mean serum ALP activity of Group A, B and C rats was significantly (p < 0.05) lower than that of Group D rats on day 3 PIT (Figure 16), while that of Group C was significantly (p < 0.05)higher than those of Groups A and D on day 7 PIT (Figure 16). Mean total bilirubin levels of Group C rats was significantly (p < 0.05) lower than that of Group D rats on day 5 PIT (Figure 17), but was significantly (p < 0.05) higher than those of Groups A and D rats on day 7 PIT (Figure 17). The mean total cholesterol level of Group C rats was significantly (p < 0.05) lower than that of the Group B rats on day 3 PIT and that of Group D rats on day 5 PIT, but on day 7 PIT, the serum total cholesterol levels of Group C rats was significantly (p < 0.05) lower than that of Group D (Figure 18). Most of the significant differences in serum biochemical parameters were considered incidental, because the values that were statistically significant were within the reference/normal range for rats.



Figure 5. Total white blood cell (TWBC) counts of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.

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Figure 6. Absolute lymphocyte counts of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 7. Absolute neutrophil counts of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 8. Absolute eosinophil counts of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 9. Absolute monocyte counts of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 10. Serum urea levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 11. Serum creatinine levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.

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Figure 12. Serum Total Protein levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 13. Serum Albumin levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 14. Serum Alanine amino transferase (ALT) activity of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.



Figure 15. Serum Aspartate amino transferase (AST) activity of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.







Figure 17. Serum Total Bilirubin levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, postpartum.



Figure 18. Serum Total Cholesterol levels of female rat groups given varied doses of *Xylopia aethiopica* decoction extract, post-partum.

Discussion and Conclusion

The 19.6% yield of a brown colored extract following the decoction procedure of extraction used in this study is comparable to and slightly higher than the 17.8% yield of dark brown extract reported for methanol extract of fruits of *X. aethiopica* by Okagu *et al.* (2018). The 13.6% yield reported by Keren *et al.* (2022) using aqueous extraction was however relatively lower than the 19.6% recorded in this present study.

In the acute toxicity test, administration of the decoction extract of X. aethiopica as used in this study, up to the maximum dose of 5,000 mg/kg did not lead to any signs of toxicity; the LD₅₀ of the extract was therefore regarded as being greater than 5,000 mg/kg body weight, which implies that the extract is acutely safe for use, and therefore falls within Class 5 Drugs/Medicines, designated as 'not acutely toxic', according to the Global Harmonized Classification System for Chemical Substances and Mixtures (OECD, 2001). The LD₅₀ greater than 5,000 mg/kg recorded for the decoction aqueous extract in the present study differs from the LD₅₀ of 3,464 mg/kg reported by Akinloye et al. (2019) for ethanol extract of the X. aethiopica fruits. The LD₅₀ recorded in this present study (> 5,000 mg/kg) also differs from the LD₅₀ of 1,296.15 mg/kg reported by Ogbuagu et al. (2022) on ethanol Soxhlet extract of *X. aethiopica* fruits and the LD_{50} of 2,154 mg/kg reported by Keren *et al.* (2022) on aqueous ethanol extract of *X. aethiopica* fruits. It is thought that the differences in LD_{50} reported in the different studies and the present study reflect differences in the solvent used and the method of extraction. Earlier reports had substantiated the effects of extraction using varied solvents and extraction methods on activity and toxicity of extracts from the same plant parts (Dirar *et al.*, 2019; Mohammed *et al.*, 2022).

In the sub-acute toxicity study, treatment with the aqueous decoction extract of *Xylopia aethiopica* at the doses of 250, 500 and 1000 mg/kg body weight for seven days postpartum did not lead to any signs of toxicity or death in the female rats (does) and their pups during and after the treatment period of the experiment. This is considered significant because the safety of both the mothers to which such extracts are administered and the neonates is very important: an efficacious therapeutic agent that is toxic to the mother, neonates or both should be considered useless!

Haematological evaluations are critical because blood is the body's primary transport medium. Almost all metabolic processes, tissue and cellular requirements and wastes are transported and detectable in the blood; this makes it easy to detect any deviations from normal in the blood profile (Ihedioha et al., 2004; Reagan et al., 2010). The lack of variations between the PCV, RBC counts and Hb of the treated groups when compared to the untreated control suggests that the treatment did not lead to any significant alterations in these erythrocytic parameters; the significantly lower erythrocytic profile of the Group C rats on Day 1 is considered incidental and may be as a result of individual difference. The variations in total white blood cells count and the differential white blood cells count values across groups were not considered to be of clinical value, though

there were incidental points of statistically significant differences, which did not deviate from the reference/normal values.

It was noted that not all the haematological values obtained in this study (both in the treatment and the control groups) were within the reference intervals reported by Ihedioha et al. (2004). It is thought that the differences may probably be due to the physiological changes that occur during pregnancy in the haematological parameters; the reports of Ihedioha et al. (2004) which was being compared with were on non-pregnant females. The mean RBC counts of the postpartum rats were higher when compared to the reference intervals reported by Ihedioha et al (2004), and this higher RBC count may be attributed to the reported increase in maternal erythropoietin production in pregnant humans, which results in an increase in red cell mass during pregnancy and the immediate post-partum period (Chandra et al., 2012). The mean TWBC and mean lymphocyte counts of the postpartum rats were higher compared to the reference values reported for non-pregnant females by Ihedioha et al. (2004). Earlier reports had shown that total white blood cell counts are usually increased during pregnancy due to physiologic stress caused by the pregnancy (Fleming, 1975). It has also been reported that during pregnancy, the number of lymphocytes in blood decrease in the first and second trimester, but increases during the third trimester (Chandra at el., 2012). The findings of this study differ from the reports by Ogbuagu et al. (2022), which showed a decrease in white blood cell parameters following treatment with the ethanol Soxhlet extract of the fruit of X. aethiopica in albino rats. However, Nnodim et al. (2011) reported that administration of the aqueous fruit extract of X. aethiopica to young rats led to dose dependent higher PCV, RBC and Haemoglobin concentration after 14 days treatment, when compared to untreated controls.

Some parenchymatous organs whose early pathologies and/or defects may not be quickly evident, frequently require evaluation of their functional status using biochemical assays. Such assays/analyses are crucial, particularly for conditions that might not show up early as clinical illnesses or even in tissue biopsies or histopathological analyses. Some of these organs (liver, kidney, pancreas, gonads, adrenal glands, etc.) whose abnormalities could lead to altered biosynthesis/biotransformation of their secretory/breakdown products or metabolites are evaluated for their functional status using serum biochemistry assays (Coles, 1986). Serum biochemistry evaluations may be used to assess the degree of organ damage, the neoplastic growth, existence of the effectiveness of the treatment being used, and the interpretation of toxicological and safety studies (Stockham and Scott, 2008). Two frequently used serum biochemical evaluations are the liver and kidney function tests (Giknis and Clifford, 2008). The result of the serum biochemical profiling in this present study showed that administration of the extract as used in the present study did not lead to any clinically significant alterations in liver and kidney function markers in serum. The finding in this present study that administration of aqueous decoction extract of X. aethiopica did not lead to liver or kidney injury/damage concurs with the reports of Abaidoo et al. (2011) that Xylopia aethiopica ethanol extract did not incite hepatotoxicity. Findings in the present study however differs from that reported by Ogbuagu et al. (2021), who showed that administration of high doses (518 mg/kg) of ethanol extract of X. aethiopica led to significantly higher serum ALT, AST and ALP activity in rats treated for 28 days with the extract when compared to untreated controls.

Based on the results obtained in this study, it was concluded that oral administration of *Xylopia aethiopica* fruit pods aqueous decoction extract, both acutely and sub-

acutely post-partum, at doses used in this experiment, did not result in any clinically obvious signs of toxicity or any clinically significant alterations in the haematological and serum biochemistry markers of liver and kidney dysfunction. This suggests that the use of *Xylopia aethiopica* decoction extract postpartum is acutely and sub-acutely non-toxic.

Conflict of interest

The authors declared no conflict of interest.

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