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Original Research Article

Acute and Sub-Chronic Toxicity Studies of the Aqueous Ethanol Leaf Extract of *Pavonia senegalensis* (Cav.) Liestner in Wistar Rats

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ABSTRACT

The leaf of *Pavonia senegalensis* is used traditionally for the treatment of bone and soft tissue infections. In this study, the aqueous ethanol leaf extract was investigated for its acute and sub-chronic toxicological effects. Acute toxicity study was done using Lorke method while the 28-day sub-chronic toxicity study was done using OECD guideline in Wistar rats via the oral route. The LD_{50} from the acute toxicity study was > 5000 mg/kg indicating that the extract is non-toxic. In the sub-chronic toxicity study, there was no significant changes in body weight and relative organ weights of organ assessed in both the extract treated and control groups. There were no significant variations in the haematological indices in the extract treated groups compared to control. There was a significant ($p \leq 0.05$) increase in creatinine levels at 600 mg/kg dose of the extract compared to control and there were a dose-dependent glomerular and tubular necrosis on sections of the kidney in the extract treated groups. There were no significant changes in liver enzymes markers in the extract treated groups compared to control. The section of the liver showed a slight to moderate necrosis, vacoulation and vascular congestion that is dose-dependent in the extract treated groups. The section of the spleen showed a slight lesion that is dose-dependent. In conclusion, the aqueous ethanol leaf extract of *P. senegalensis* is non-toxic when given orally over a short period but the 28 days administration showed that the extract is nephrotoxic and slightly hepatotoxic in rats.

Keywords: LD_{50}, Liver, Creatinine, Kidney, Necrosis, *P. senegalensis*

Introduction

Herbal preparations currently serve the health needs of many populations especially those in the underdeveloped countries and there is clear evidence of their pharmacological effects.[1] It is a widely held belief that herbal preparations are safe. However, despite the belief and claim of being natural and safe, herbal remedies have been associated with adverse effects, which have been attributed to several factors. These factors include hepatic toxicity of the main constituents and contamination of preparations by heavy metals or microorganisms.[2] The goals of toxicity testing are to identify possible adverse effects of exposure to environmental agents, to develop dose-response relationships that can elucidate the severity of effects associated with known exposures, and ultimately to predict the effects of exposure of human populations.[3]

Pavonia senegalensis (Cav.) Liestner synonyms *Pavonia hirsuta* Gull. & Perr., *Pavonia arabica* Hoschst ex Steud and *Pavonia argentina* Gurke. It is called *Tsu* in Hausa. It is found in drier parts of tropical Africa on sandy-clayey soils, humid sands and sometimes on rocky screen in savannah areas; often near villages; in woodland with Grewia, Terminalia, etc.; along rivers and in seasonally dry riverbeds; usually in light shade.[4] *Pavonia senegalensis* is usually an annual plant, but occasionally lives longer. A spreading, short-lived perennial with semi-prostrate to ascending branches, up to 1.25 m. Stems somewhat angular with harsh stellate hairs. Leaves suborbicular in outline, angular to shallowly lobed; lower surface densely stellate-hairy. Stipules up to 10 mm, filiform. Flowers solitary in the leaf axils, up to 8 cm in diameter sulphur-yellow with a maroon centre. Epicalyx bracts 12-16, narrowly linear, usually shorter than the calyx lobes.[5]

The roots are put into cold water to draw and the infusion is taken as a remedy for diarrhoea in South and East Africa [6]. The powdered seed is taken with milk and used as a contraceptive in Sokoto North-west Nigeria.[7] The infusion of the roots is used in antenatal care for general wellbeing in Katsina North-west Nigeria.[8] The water-extract of the leaf is used in Zaria North-west Nigeria to treat wounds and bone infections.[9] In Botswana cold-water infusion of the dry roots is taken to induce labour, particularly if the onset has been delayed.[4]

Due to the importance of toxicity studies of medicines and standardized herbal remedy discovery and development, the aqueous ethanol leaf extract of *P. senegalensis* was investigated in this study for its acute and sub-chronic toxicological effects.

Materials and Methods

Plant Collection, Identification and Extraction

Leaves of *P. senegalensis* were collected in November, 2018 from Rafin Yashi, Giwa Local Government Area of Kaduna State. The plant was identified and authenticated by Taxonomist U.S Gallah at National Research Institute for Chemical Technology, Zaria, Kaduna State Nigeria and assigned a voucher number 24011.

The leaves collected were air dried at room temperature for seven days after which the dried leaves were pulverized using pestle and mortar. Two hundred (200) grams of the powdered leaves was weighed and this was macerated in 70% aqueous ethanol for 5 days with occasional

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shaking. On the fifth day, the macerate was filtered through a No. 6 Whatsman filter paper and the filterate was evaporated under reduced pressure using rotary evaporator. The dried extract was kept in a desiccator until use.

Experimental Animals

Mature Wistar rats, were bred in the laboratory animal unit of the Department of Pharmacology and Therapeutics Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. They were housed in an environment of normal ambient temperature and the lighting period was 12 h daily and relative humidity of 40–60%. The weight of the rats varied between 170 and 190 g. The rats were kept in stainless steel cages, supplied with clean drinking water and fed ad libitum with standard rat commercial pelleted feed (Vital feeds, Nigeria). Ethical conditions governing the conduct of experiments with life animals was strictly observed as stipulated by Ward and Elsea [10] and the experiment was conducted in compliance with NIH Guide for Care and Use of Laboratory Animals (pub. No. 85-23, Revised 1985). The experimental protocol was approved by the Ahmadu Bello University ethical committee for the use of laboratory animals with approval number ABUCAUC/2017/001.

Acute Toxicity Study

Lethal median dose (LD_{50}) determination was conducted using Lorke's method[11] using the oral (p.o) route in rats. The method was carried out in two phases. In the initial phase, 3 groups of three rats each were treated with the aqueous ethanol extract of the plant at doses of 10, 100 and 1000 mg/kg body weight p.o. and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one rat were administered the dose of 1200, 1600, 2900 and 5000 mg/kg of the extract. The LD_{50} value was determined by calculating the square root of the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

Sub-Chronic Toxicity Study

Repeated dose oral toxicity study was carried out according to OECD Guideline 407.[12] Rats were randomly divided into 4 groups of 6 animals each (3 females and 3 males). First group received 10 mL/kg distilled water orally to serve as the control. The second, third and fourth received 150, 300 and 600 mg/kg of the extract respectively daily for 28 days orally.

The rats access food and water throughout the duration of the experiment. Observation was done on daily basis for general symptoms of toxicity and mortality. Weights of the animals were taken weekly. Rats were anesthetized using chloroform and sacrificed after the 29th day. Paired blood samples, heparinised and non-heparinised were collected from the heart by direct needle puncture following chloroform anaesthesia for hematological and serum biochemical assays. Relative organ weight of vital organs which included liver, kidneys, heart, lungs and spleen were assessed. Histopathological examination was done on the liver, kidney and spleen.

Statistical Analysis

The results were presented as mean ± SEM and analyzed using one-way analysis of variance (ANOVA). The differences between the means were tested using Dunnet Post-hoc test and values of $p<0.05$ were considered statistically significant. SPSS for windows version 22 was used for the statistical analysis.

Results and Discussion

Acute toxicity tests evaluate the adverse effects of short-term exposure and are considered an integral step in the assessment of the toxic potential of a substance [13]. In the acute oral toxicity study of the aqueous ethanol extract of the leaf of *P. senegalensis*, the LD_{50} was determined to be > 5000 mg/kg as there were no deaths recorded in both phases of the study. According to the Hodge and Sterner scale of oral acute toxicity, 1 mg/kg is considered highly toxic, 10 mg/kg is considered toxic, 100 mg/kg is moderately toxic, 1000 mg/kg is slightly toxic, and > 5000 mg/kg is considered practically non-toxic.[14] From acute toxicity study carried out, the aqueous ethanol extract of the leaf of *P. senegalensis* was found to be non-toxic.

In the 28 days' sub-chronic toxicity study, administration of *P. senegalensis* extract at the doses of 150, 300 and 600 mg/kg did not produce any significant effect on the weight of rats and organ body ratio of vital organs (kidney, liver, heart, lungs and spleen) over the entire duration of administration compared to the control (Figure 1 and Table1). No significant changes in food and water intakes were observed. The normal body weight increment observed was due to the normal physiological changes in rats due to metabolic processes in their systems. Changes in body and internal organ weights are sensitive indices observed after exposure to toxic substances.[15]

The haematological indices are very sensitive to toxins, hence values obtained after exposure of an animal to toxic compounds can be used to evaluate the pathological or physiological status of the test animals.[16] After 28 days' administration of different doses (150, 300 and 600 mg/kg) of *P. senegalensis* extract there were no significant difference in the haematological indices assessed compared to that of the control (Table 2) showing no signs of haematological toxicity.

Urea and creatinine are the major indicators of renal toxicity. Serum urea accumulation is used as the acute marker, while serum creatinine accumulation is used in detecting renal toxicity.[2] In this study, the oral administration of *P. senegalensis* extract showed a significant ($p \leq 0.05$) increase in creatinine levels at the dose of 600 mg/kg of the extract (Table 3) indicating signs of chronic renal toxicity with increase in dose of the extract. The histological section of the rat kidneys showed a dose-dependent glomerular and tubular necrosis (Figure 2). The elevated creatinine levels in the serum and tissue necrosis of the kidney indicates that the aqueous ethanol extract of *P. senegalensis* leaf is toxic to kidney after prolong (28 days) administration.

The degree of liver damage induced by a chemical substance can be evaluated by determining the level of biochemical markers of the liver function such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP). ALP is located in the cytoplasm and is released into circulation after cellular hepatic damage. ALT and AST are also enzymes released as a result of liver injury, especially damage to mitochondria of liver cells. Elevation of level of these enzymes can be an indication of cellular damage, leakage and loss of functional integrity of hepatic cell membrane.[17]

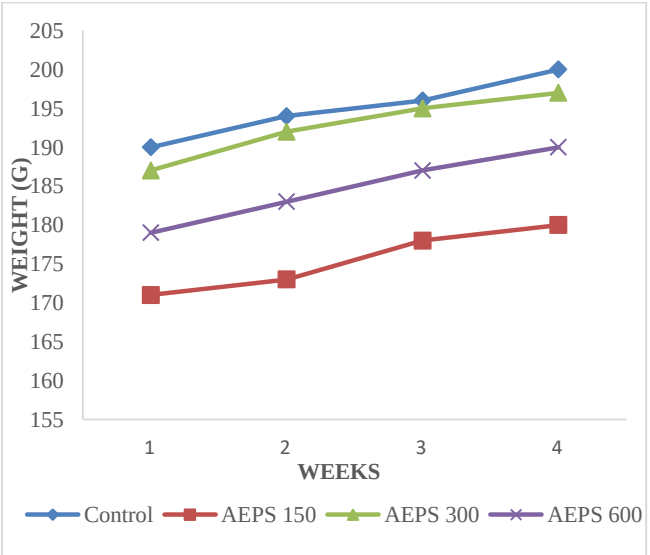

Figure 1: Effect of Aqueous Ethanol Leaf Extract of *P. senegalensis* Compared to Control on Body Weight Changes Following 28 days Sub-Chronic Oral Treatment in Wistar Rats.

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There was slight elevation of these liver enzymes but were not statically significant at $p \leq 0.05$ with the extract treated groups compared to the control group (Table 4). The histological section of the liver reveals slight to moderate necrosis, vacoulation and vascular congestion in the extract treated groups indicating that the extract is slightly hepatotoxic (Figure 3). The histological section of the spleen (Figure 4) showed mild lesions that are dose-dependent in the extract treated groups indicating a mild effect on the spleen that may be exacerbated with higher doses and prolonged usage.

Table 1: Effect of Aqueous Ethanol Leaf Extract of *P. senegalensis* Compared to Control on the Relative Organ weight of Some Vital Organs Following 28 days Sub-Chronic Oral Treatment in Wistar Rats

Treatment (mg/kg)	Relative Organ Weight (Per 100 g Body Weight)				
	Liver	Kidney	Heart	Spleen	Lungs
Control	4.50 ± 0.41	0.65 ± 0.03	0.34 ± 0.03	0.41 ± 0.04	0.91 ± 0.32
AEPS 150	3.77 ± 0.20	0.62 ± 0.06	0.36 ± 0.07	0.38 ± 0.03	0.95 ± 0.13
AEPS 300	3.92 ± 0.31	0.66 ± 0.04	0.33 ± 0.11	0.35 ± 0.03	0.92 ± 0.20
AEPS 600	3.86 ± 0.35	0.73 ± 0.06	0.38 ± 0.04	0.48 ± 0.02	0.89 ± 0.36

Data expressed as Mean ± SEM, SEM = Standard Error of Mean, n = 6.

Table 2: Effect of Aqueous Ethanol Leaf Extract of *P. senegalensis* Compared to Control on Heamatological Indices Following 28 days Sub-Chronic Oral Treatment in Wistar Rats

Treatment (mg/kg)	WBC (x 10^3 µL)	LYMP (%)	MID (%)	GRAN (%)	RBC(x 10^3 µL)	HCG (g/dL)	PLT (x 10^3 µL)
Control	4.18 ± 0.19	63.78 ± 2.09	4.94 ± 0.34	31.02 ± 1.80	5.90 ± 0.04	12.94 ± 0.51	170.22 ± 5.46
AEPS 150	4.87 ± 0.24	59.63 ± 1.74	5.31 ± 0.37	35.80 ± 1.34	6.05 ± 0.06	12.75 ± 0.44	194.83 ± 10.45
AEPS 300	4.85 ± 0.41	57.65 ± 2.15	4.83 ± 0.68	37.18 ± 2.09	6.05 ± 0.09	13.65 ± 0.34	174.00 ± 11.56
AEPS 600	4.80 ± 0.84	60.33 ± 0.68	5.38 ± 0.74	34.15 ± 1.78	5.93 ± 0.15	13.53 ± 0.28	166.25 ± 3.54

Data expressed as Mean ± SEM. SEM= Standard Error of Mean, n = 6, WBC= White Blood Cell Count, LYMP = Lymphocytes, MID = other types of white blood cells not classified as lymphocytes or granulocytes, GRAN = Granulocytes, RBC=Red Blood Cells, HCG = Haemoglobin, PLT = Platelate, AEPS = Aqueous Ethanol Leaf Extract of *Pavonia senegalensis.*

Table 3: Effect of Aqueous Ethanol Leaf Extract of *P. senegalensis* Compared to Control on Kidney Function Following 28 days Sub-Chronic Oral Treatment in Wistar Rats.

Treatment (mg/kg)	Urea (mg/dL)	Creatinine (meq/L)	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mg/dL)	HCO_3 (mg/dL)
Control	33.53 ± 1.61	0.73 ± 0.06	20.17 ± 1.62	139.15 ± 2.45	22.17 ± 1.94	83.67 ± 3.04
AEPS 150	41.90 ± 1.61	0.76 ± 0.04	20.67 ± 0.97	138.38 ± 2.66	23.83 ± 2.63	82.50 ± 4.86
AEPS 300	42.50 ± 3.62	0.78 ± 0.05	21.18 ± 0.46	142.05 ± 3.08	23.75 ± 1.97	82.50 ± 2.06
AEPS 600	48.65 ± 4.73	0.98 ± 0.05*	17.73 ± 1.26	141.38 ± 4.81	18.75 ± 0.95	82.50 ± 4.41

Data expressed as Mean ± SEM, SEM = Standard Error of Mean, n = 6, *statistically significant at $p<0.05$, Na^+ = Sodium ion, K^+ = Potassium ion, Cl^- = Chloride ion, HCO_3 = Bicarbonate, AEPS = Aqueous Ethanol Leaf Extract of *Pavonia Senegalensis*.

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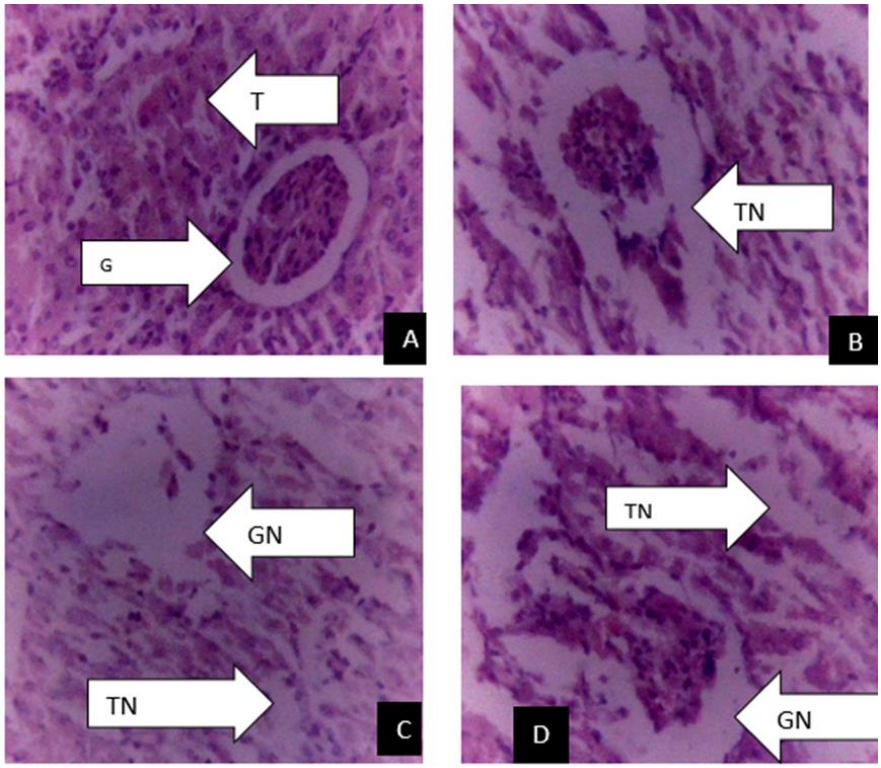

Figure 2: Photomicrograph of the section of rat kidney treated with the AEPS following 28 days' oral administration (x100). (A) is the control group showing normal glomerulus(G) and tubules(T), (B) is the group treated with 150 mg/kg of the extract showing intense tubular necrosis(TN), (C) is the group treated with 300 mg/kg of the extract showing intense glomerular necrosis(GN) and tubular necrosis(TN) While (D) is the group treated with 600 mg/kg of the extract showing glomerular necrosis(GN) and tubular necrosis(TN).

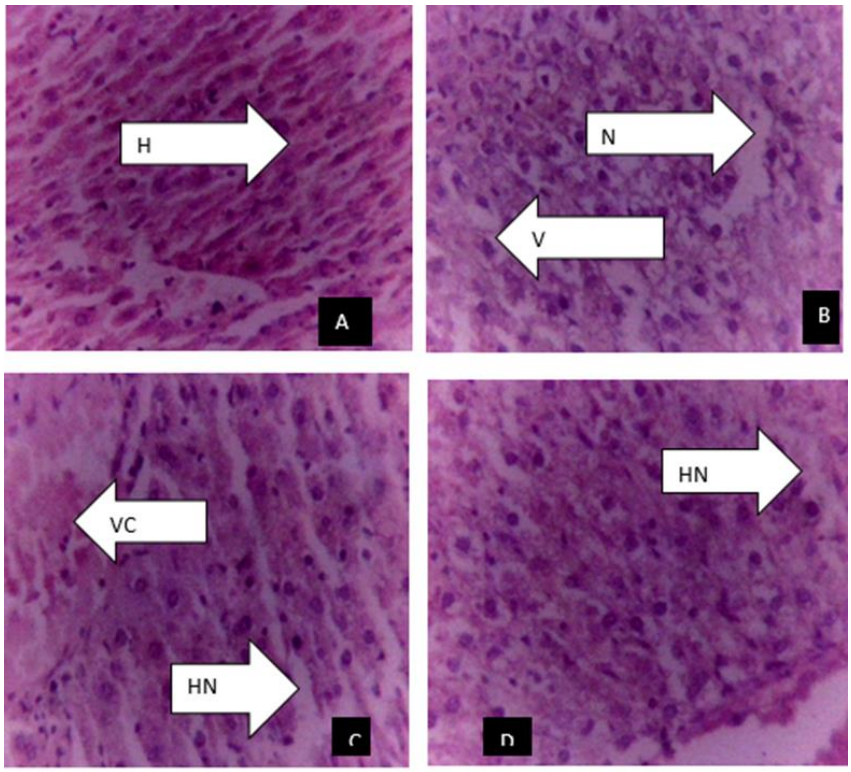

Figure 3: Photomicrograph of the section of rat liver treated with the AEPS following 28 days' oral administration (x100). (A) is the control group showing normal hepatocytes (H), (B) is the group treated with 150 mg/kg of the extract showing moderate necrosis (N) with vacoulation (V), (C) is the group treated with 300 mg/kg of the extract showing vascular congestion (VC) and slight hepatic necrosis (HN), While (D) is the group treated with 600 mg/kg of the extract showing moderate hepatic necrosis (HN).

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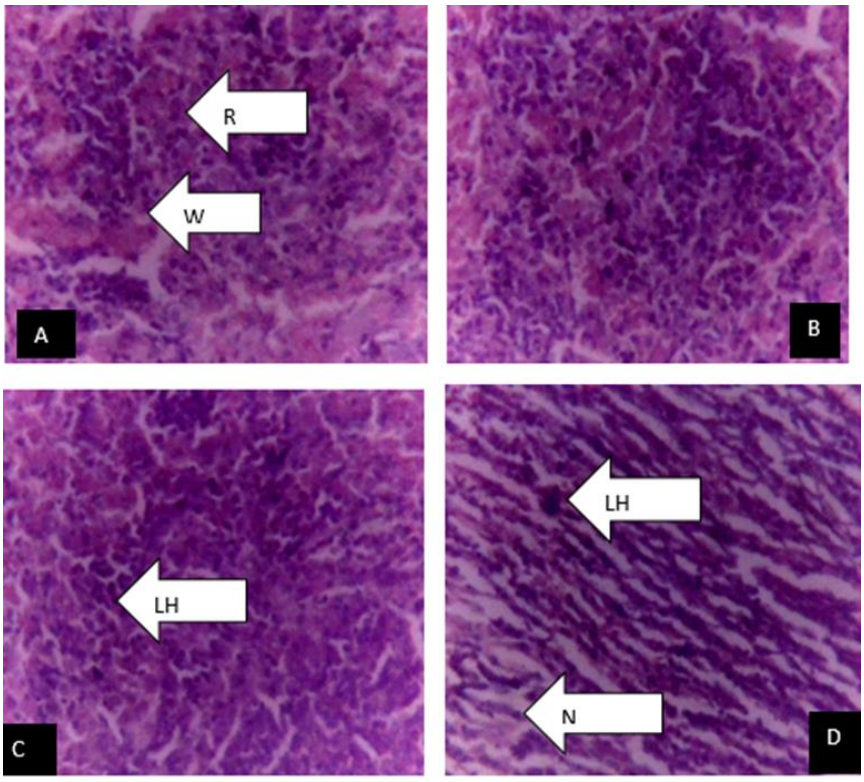

Figure 4: Photomicrograph of the section of rat spleen treated with the AEPS following 28 days' oral administration (x100). (A) is the control group showing normal red pulp (R) and white pulp (W), (B) is the group treated with 150 mg/kg of the extract showing normal features of the spleen, (C) is the group treated with 300 mg/kg of the extract showing slight lymphocyte hyperplasia (LH), While (D) is the group treated with 600 mg/kg of the extract moderate lymphocyte hyperplasia (LH) and necrosis (N).

Conclusion

In conclusion, the acute toxicity study of the aqueous ethanol extract of *Pavonia senegalensis* leaves showed that the extract is non-toxic when given orally over a short period while the sub-chronic (28 days) showed that the extract is nephrotoxic and slightly hepatotoxic in rats. These levels of toxicity may not be significant in healthy individuals, but they may exacerbate pre-existing hepatic and renal disturbances.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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