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Research Article

Histomorphological and Therapeutic Evaluation of *Chrysophyllum Albidum* (G. Don) Leaf Extract in Broiler Chickens Infected with *Salmonella Gallinarum*

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Abstract

This study examined the therapeutic and histomorphological efficacy of ethanol leaf extract of *C. albidum* (G. Don) on broiler chickens infected with *Salmonella enterica serovar Gallinarum* (Gr. D1-1, 9, 12). A total of 108-day old broiler chickens were brooded and allotted into a clinical trial at three weeks old involving six treatments (T1 - T6) replicated thrice with six birds per replicate in a completely randomised design (CRD). T1: Infected birds but not treated (negative control). T2: Infected and treated with standard drug (gentamicin®) which served as the positive control; T3: Not infected and not treated. T4 - T6 were infected and given 100, 250 and 625 mg/kg/day of extract respectively. After the clinical trial, antimicrobial efficacy index of bacterial count was established. Analysed results showed that the therapeutic index was high as depicted in observed results of reduced level of bacterial load count in organs and samples in the different experimental set up. Significant difference ($p < 0.05$) occurred among post infection, post therapy and controls. Histomorphological examination of the liver and kidney of the experimental birds did not show any significant abnormal presentation. It was concluded that ethanolic leaf extract of *C. albidum* demonstrated highly significant therapeutic effect without any toxic influence on the vital organs and comparable to conventional antibiotic against *Salmonella gallinarum* which could be further explored as novel herbal antibiotic formulation against fowl typhoid.

Keywords: Antibiotic Resistance; Leaf Extract; *S. Gallinarum*; *C. Albidum*

Introduction

The multiplicity of the current unabated global crisis of antimicrobial resistance necessitates decisive solution [1] for the achievement of Sustainable Development Goals (SDGs). According to reports, about 75% of bacteria on chicken meat in Kenya were resistant to at least one antibiotic and the possibility of these bacteria spreading to humans through food chain, water, air and manure is very high [2]. It was also reported that avalanche of facts abound on the animal-to-man and man-to-man spread of resistant bacteria [3,4] as corroborated by European Medicines Agency [5]. The fact that about 84 countries experiencing drug resistance to many bacterial disease calls for attention [6]. High death rates have been recorded as a result of infections relating to antimicrobial resistance. The report of [7] substantiates this observation that antibiotic resistance is becoming a global killer phenomenon. One important disease of economic importance causing great loss in poultry industry worldwide is Fowl typhoid. The resistance of *Salmonella gallinarum*, the causative agent of this disease to many

commercially available antibiotics calls for sourcing a novel antibiotic from natural source. Bacteria of the genus Salmonella have for several years presented untold challenges to poultry production and concurrently the causative agents of health problems in other avian species and one of the multidrug resistant bacteria [8,9]. Infection due to *Salmonella gallinarum* is a source of highly significant economic losses to poultry farmers and food production industries [10]. To solve the problem of infections and antimicrobial resistance, the usage of medicinal plants has been advocated [11]. Plants have been explored over the years and found to contain biological properties of high pharmaceutical effects for the remedy of various diseases [12-15]. The leaves of plants contain bio active compounds of therapeutic usefulness such as tannins, flavonoids, alkaloids, saponins etc. These compounds could inhibit the growth or annihilate many pathogenic microorganisms [16]. *Chrysophyllum albidum* is a useful medicinal plant that has ethnobotanical history of traditional use against bacterial infections, wherewith its various parts have been experimented to be potential source of antimicrobial compounds [17,18]. This experiment therefore was conceptualized and conducted, hinging on the salient facts above and dearth of relevant information on the therapeutic effect of *C. albidum* extract against *S. gallinarum Gr. D1-1, 9, 12*.

Materials and Methods

Experimental site

This experiment was carried out at the Animal Parasitology and Microbiology Unit of the Department of Animal Production and Health, Federal University of Technology, Akure Ondo State, Nigeria. The birds were reared in a partitioned and well sanitized deep litter pen. The dimension of each of the cubicle that housed 6 birds (a replicate) measured 120 cm x 90 cm. The geographical location of the site revealed coordinates (a) 5° 07′ E, 7° 19′ N, (b) 5° 09′ E, 7° 19′ N (c) 7° 17′ N, 5° 07′E and (d) 7° 17′ N, 5° 09′ E. The region has a tropical climate of double annual maximal of rainfall (2000 mm - 2380 mm) and a temperature range of about 24 - 28°C [19].

Plant collection and authentication

Fresh, matured, and disease-free leaves of *C. albidum* G. Don Sapotaceae were plucked from its natural habitat within the environment of the Federal University of Technology Akure (Plate 1a, 1b, 1c, and 1d). The plant was authenticated by an experienced botanist at Medicinal Plant Herbarium, Department of Pharmacology, Obafemi Awolowo University Ile-Ife Nigeria with voucher specimen number: FPI 2267. The leaves were gently rinsed in distilled water in order to rid off dust particles before air-drying under shade for three weeks (Plate 1f), pulverized into fine powder (Plate 1e) in pulverizing machine (Thomas-Willey machine) and eventually stored in airtight container before further consideration.

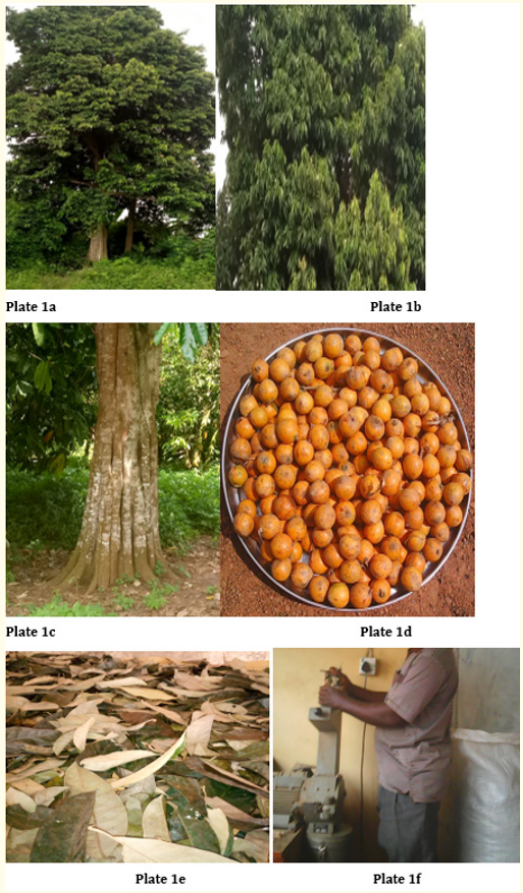

Plate 1: 1a: *C. albidum* tree, 1b: aerial view of the leaves of *C. albidum*, 1c: The bark of *C. albidum,* 1d: The fruits of *C. albidum,* 1e: Air-drying stage of *C. albidum* leaves, 1f: Grinding stage of *C. albidum* leaves.

Preparation of the leaf extract of *C. albidum*

The ground sample measuring 100 g was soaked in 500 mL of ethanol for 72hours and thereafter double filtered using muslin cloth and Whatman No 1 (125 mm) filter paper. The wet extract was concentrated by using rotary evaporator set at 40^0C and finally left to clean open air for the total removal of the solvent to dryness [20]. The extract was preserved in the fridge until bioassayed.

Source of experimental chicks, infectious agent and management

One hundred and thirty-five (135) day old arbor acre broiler chicks were purchased from a reputable hatchery for the experiment. Typed bacterial isolate, *Salmonella gallinarum Gr. D1-1, 9, 12* was sourced from National Veterinary Research Institute (NVRI), Vom Jos, (Nigeria). The organism was kept in slant at 4°C until when needed for the experiment. Commercial feed from a reputable feed company was fed *ad libitum* to the experimental broiler chickens, both at starter and finisher stage. Routine vaccinations were administered accordingly and other management practices duly carried out. The experiment lasted for fourteen (14) weeks, meaning seven weeks of passaging and seven weeks of clinical trial. Some of the experimental birds are shown in-situ in plate 2.

Plate 2: Experimental broiler chickens at the project site.

Experimental design

One hundred and eight (108) broiler chicks were used for the clinical trial while twenty seven (27) were used for the passaging of the infectious agent at the age of three weeks. Six (6) birds were allotted to a treatment (T1 - T6) in a completely randomised design (CRD) and replicated thrice. Treatment one (T1) = Infected but not treated (negative control). Treatment two (T2) = Infected and treated with standard drug gentamicin as positive control (PC). Treatment three (T3) = Not infected and not treated. Treatment four (T4) = Infected and treated with 100 mg/kg/day. Treatment five (T5) = Infected and treated with 250 mg/kg/day and Treatment six (T6) = Infected and treated with 625 mg/kg/day of the ethanolic leaf extract of *C. albidum*.

Passaging of the infectious agent

The bacterial organism was passaged thrice to gain virulence *in-vivo* using a total of 27 broiler chickens (nine birds per phase) following the modified method described by [21]. Bacterial samples were taken from agar slant maintained at 4°C with the aid of sterile inoculating loop into prepared Selenite F broth (HiMedia Laboratories Pvt. Ltd., India). The broth was incubated at 37°C for 24 hours as recommended by the manufacturer. Birds were administered orally with 1mL of the broth culture of the infectious agent serially diluted to the concentration of $x10^6$ Cfu/mL (0.5 McFarland Standard). *S. gallinarum* was recovered from the birds after five days. The process was repeated twice and *S. gallinarum* was recovered eventually for the final inoculation of the experimental broiler chickens.

Inoculum standardization and infection of the experimental broiler chickens

Samples were taken from the liver of the broiler chickens and grown on Selenite F broth at 37^0C for 24 hours to recover and isolate the infectious agent. Thereafter, another broth culture of the test organism was prepared overnight and standardized to 0.5 McFarland scale ($x10^6$ Cfu/mL). Exactly 0.5 mL of the broth culture of *S. gallinarum* was drawn and administered orally to each of concerned experimental broiler chickens [22] when the broilers attained the age of three (3) weeks.

Administration of *C. albidum* leaf extract

The result of the acute toxicity obtained in the preliminary study of *C. albidum* of this experiment showed the LD_{50} to be greater than 5000 mg/kg body weight indicating the safety and non-toxicity of the crude ethanol extract of the plant in oral formulations [23]. Therapeutic doses of the extract were determined and given in milligram per kilogram (mg/kg/day) of body weight for five days [24].

Antibacterial efficacy of ethanolic leaf extract of *C. albidum*

The antibacterial efficacy of the leaf extract was assessed through enumeration of the colonies of *S. gallinarum* in the cloaca swab of the experimental birds through bacterial growth culture on day one (pre-infection), three days post infection, two days post-treatment and from the faeces, spleen, liver, and heart at termination of the experiment [25,26].

Histopathology

Tissue samples were taken from the liver and kidney of the experimental birds immediately after slaughtering. These samples were preserved in 10% neutral buffered formalin in order to preserve their freshness and wholeness prior to processing [27]. The tissues were then passed through tissue processing stages viz; dehydration, clearing, infiltrating, embedding, sectioning, (cutting by using a rotary microtome with 4-5 µm thickness), labelling (using India ink), water bathing, staining (with haematoxylin and eosin), clearing, drying, cover-slipping and drying in hot air for about 5 minutes [28]. The stained specimens were examined under low (40X) power magnification with the aid of light microscope and thereafter photographed.

Data collection and analysis

Sterile swabbing sticks were used to take samples from the cloaca of the experimental broiler chickens a day pre-infection, three days post-infection and two days post-treatment with the extract of *C. albidum*. The samples were cultured in the laboratory at 37°C for 24 hours using Selenite F broth. Grown *S. gallinarum* on Figures were enumerated with the aid of electronic colony counting machine and recorded as Cfu/mL x 10^6. At the end of the experiment, three birds per replicate were sacrificed and samples from the faeces and selected organs (spleen, liver and heart) were also cultured as formerly highlighted and colonies were enumerated as above. Data collected were subjected to analysis of variance using SAS, version 9.2 [29], and means appropriately separated by Duncan's Multiple Range Test [30]. Probability values of less than 0.05 ($p < 0.05$) were considered significant.

Ethical Consideration

This experiment was performed following the ethical code, guidelines and regulations for animal use of National Institute of Health [31] as well as that of the Federal University of Technology Akure.

Results and Discussion

The results of the histopathological examination of the liver and kidney as well as those obtained through the cloacal bacterial count (Cfu/mL x 10^6) faeces, and some selected organs of the experimental broiler chickens are as presented in Plates 3a, 3b, 3c, and 3d for the liver while Plates 4a, 4b, 4c, and 4d showed that of the kidney. Tables 1 and 2 showed the laboratory bacteriological results in each of the treatments. The liver section of the infected but not treated birds (Plate 1b) was highly vacuolated with faded liver cell nuclei. The slight histological change (wider sinusoids) in the liver and the kidney observed in the positive control group was found normalizing. This showed that the standard drug equally had some level of positive effect on the organ. Both the Plates showing the liver and the kidney sections of the infected and untreated birds clearly demonstrate great divergence in structure from that of T1 and the rest groups. T2, T3, and T6 present similar architectural configuration of normal liver and kidney cells but T6 showed a better liver and kidney cells configuration than T2. The histological examination of the birds administered 620 mg/Kg *C. albidum* leaf extract showed that the leaf extract had no deleterious effect and can prevent severe disruptions of liver and kidney structures which actually unveils the ameliorative effect of extract on supposedly damaged liver and kidney cells. This observation agrees with [32]. The positive effect of *C. albidum* leaf extract on the selected organs of the experimental broiler chickens may be attributed to the presence of the various health beneficial phytochemicals (tannins, saponins, terpenoids, steroids, flavonoids and cardiac glycosides) in the extract as reported by [33].The results of the cloacal swab of the experimental broiler chickens at 1 day pre-infection were not significant statistically but significant difference occurred ($p < 0.05$) at 3 days post-infection with the values 176.17 ± 23.47 for zero extract administration, 102.33 ± 23.07 for 100mg/ml, 42.67 ± 14.31 for 250 mg/mL, 42.67 ± 20.43 for 625 mg/mL and 160 ± 9.24 standard drug group as shown in table 1. A sharp increase in bacterial load was noticed in all groups after three days of infection (Table 1). At the end of the experiment, bacterial load count from the cloaca of the experimental broiler chickens was highest in the negative control group (224 ± 19.86), followed by the positive control group (70 ± 16.29), 100mg/mL group having 8.83 ± 5.29, 250mg/mL with 4.83 ± 1.68, and 625mg/mL with 1.67 ± 1.09 as presented in table 2. There were significant differences ($p < 0.05$) in the mean cloacal bacterial load among the groups. The implication of this is that the leaf extract of *C. albidum* administration had probably contributed to the low bacterial population in all the groups that were treated with the extract. The outcome of this cloacal examination agrees with [17] that *C. albidum* leaf extract had antibacterial (broad spectrum) effect on *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Shigella spp*. There were significant differences ($p < 0.05$) in the bacterial count from the faeces, spleen, liver and heart of the experimental broiler

chickens as shown in Table 2. The faecal analysis at termination of the experiment showed average mean values of bacterial load (cfu/mL) to be highest in the negative control group (75.67 ± 12.09[a]) followed by the positive control group (50.67 ± 5.81[ab]). There was a trend in the reduction of bacterial load in the spleen and the heart following the dosage level showing that the lowest dosage (100 mg/mL) had the highest bacterial load among the three administered dosage and this agrees with [16] that the leaves of plants have therapeutic effect on many pathogenic organisms. The lowest bacterial load was recorded in the highest administered dosage (625 mg/mL) and vice versa. This observation supported [34] that antimicrobial activities of extracts increase as the concentration of the extract increase. This trend also was observed in the bacterial load in the heart of the experimental broiler chickens. The significant differences ($p < 0.05$) observed in the bacterial load from the faeces, spleen, liver and heart of the experimental broiler chickens in this study shows that the extract possesses antimicrobial effect on the experimented infectious agent. This observation agrees with the reports of earlier researchers [35,36] that *C. albidum* leaf extract has broad spectrum antibacterial potential.

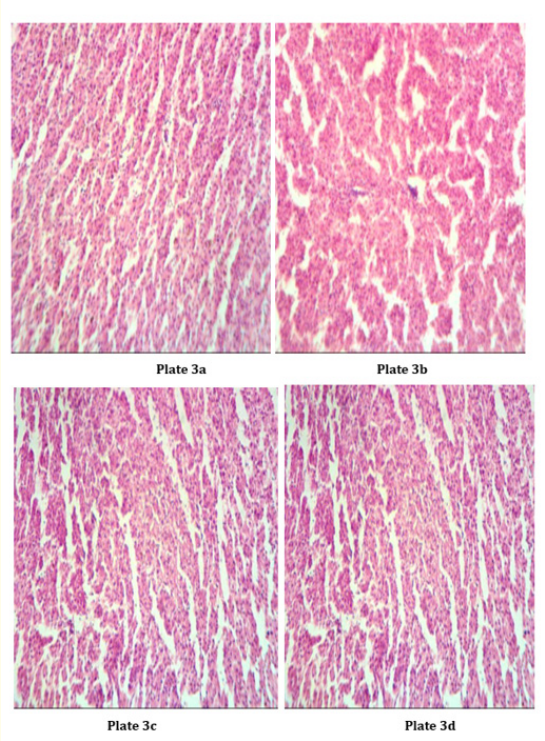

Plate 3: 3a: Photomicrograph of liver section stained with H and E (X400) of the experimental broiler chickens (T3) uninfected and untreated, showing normal architectural morphology of the liver. (3b): infected but not treated (T1) showing karyolitic reaction as more of liver cell nuclei faded off. (3c): treated with standard drug (Gentamicin®) showing prominent hepatic cells. (3d): infected and administered 625mg/kg with no visible lesion.

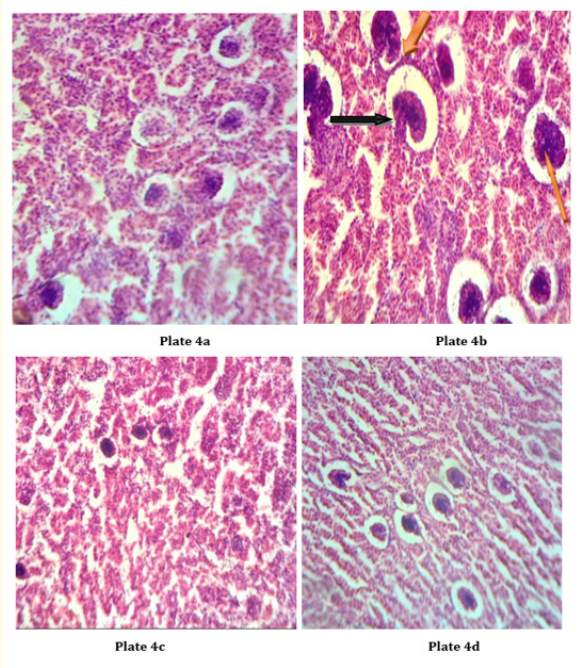

Plate 4: (4a): Photomicrograph of the kidney section stained with H&E (X400) of the experimental broiler chickens (T3) uninfected and untreated, showing normal architectural morphology of the kidney. (4b): infected but not treated (T1) showing prominent and widened bowman capsule (brown arrow) space and highly stained constricted glomerulus (black arrow). (4c): infected and treated (T2) with standard drug (Gentamicin®) showing thickening of glomerular basement membrane and collapse of glomerulus (arrowed brown). (4d): infected and administered 625mg/kg (T6) with no observed visible change.

Infection	Dosage level	1 day pre-infection	3 days post-infection	2 days post-treatment
Not infected	-	6.75 ± 3.48	56.83 ± 18.12^{b}	53.25 ± 28
Infected	-	10.27 ± 3.14	132.07 ± 16.27^{a}	67.13 ± 24.46
(T1)	NC (T1)	10.5 ± 6.17	176.17 ± 23.47^{a}	224 ± 19.86^{a}
(T2)	PC	8.67 ± 8.67	160 ± 9.24^{a}	70 ± 16.29^{b}
(T3)	NCC (T3)	6.75 ± 3.48	56.83 ± 18.12^{b}	53.25 ± 28
(T4)	100 mg/kg/day	10.5 ± 5.34	102.33 ± 23.07^{b}	8.83 ± 5.29^{c}
(T5)	250 mg/kg/day	3.5 ± 3.5	42.67 ± 14.31^{c}	4.83 ± 1.68^{c}
(T6)	625 mg/kg/day	10.33±4.88	42.67 ± 20.43^{c}	1.67 ± 1.09^{c}
Not infected	NCC	15 ± 11.59	56.83 ± 18.12	53.25 ± 28
Infected and not treated	NC	6 ± 6	208.33 ± 16.41	241.33±11.62
Infected	100mg/kg/day	21 ± 5.69	150 ± 15.28	16.67 ± 8.82
Infected	250mg/kg/day	0 ± 0	68.67 ± 18.42	6.33 ± 0.88
Infected	625mg/kg/day	15.67 ± 8.09	73.33 ± 33.83	1.33 ± 1.33
	PC	8.67±8.67	160 ± 9.24	70 ± 16.29
Status		0.4265	0.0002	0.2313
Level		0.8008	< .0001	< .0001
Status*Level		0.1184	0.7329	0.6463

Table 1: Cloacal bacterial count (Cfu/mLx10^{6}) from the experimental broiler chickens.

Where NC: Negative Control and PC: Positive Control. NCC: not infected and not treated. Means with different superscripts within the same column are significantly different from each other ($p < 0.05$).

Infection	Level	Faeces	Spleen	Liver	Heart
Not infected	-	35.5 ± 7.82	26.17 ± 5.46^{b}	34.83 ± 11.21^{b}	23.92 ± 9.65^{b}
Infected	-	44.73 ± 8.34	51.8 ± 8.24^{a}	98.2 ± 23.26^{a}	85.6 ± 26.26^{a}
T1	NC (T1)	75.67± 12.09^{a}	68 ±12.3^{a}	135.17± 46.41^{a}	162.17±38.5^{a}
T2	PC	50.67 ± 5.81^{ab}	65 ± 14.01^{a}	55.33 ± 15.06^{ab}	145.67±28.2^{a}
T3	NCC (T3)	35.5 ± 7.82	26.17 ± 5.46^{b}	34.83 ± 11.21^{b}	23.92 ± 9.65^{b}
T4	100 mg/kg/day	25.17 ± 8.51^{b}	35 ± 8.74^{b}	64 ± 24.97^{ab}	12 ± 2.13^{b}
T5	250 mg/kg/day	20.33 ± 9.93^{b}	28.5 ± 11.56^{b}	36 ± 18.53^{b}	10 ± 2.44^{b}
T6	625 mg/kg/day	36.33 ± 7.86^{b}	17.83 ± 1.74^{c}	52.33 ± 29.9^{ab}	4.83 ± 1.56^{b}
Not infected	NCC	58 ± 15.53	47.33 ± 17.29	51.67 ± 17.27	78.33 ± 7.26
Infected	NC	93.33 ± 13.33	88.67 ± 5.46	218.67 ± 59.15	246 ± 22.48
Infected	100 mg/kg/day	18.33 ± 9.28	45.67 ± 15.34	92.67 ± 37.1	16 ± 1.15
Infected	250 mg/kg/day	24.67 ± 18.12	41.67 ± 22.18	55.67 ± 34.48	13.67 ± 3.28
Infected	625 mg/kg/day	36.67 ± 5.21	18 ± 3.79	68.67 ± 55.81	6.67 ± 2.4
Infected	PC	50.67 ± 5.81	65 ± 14.01	55.33 ± 15.06	145.67±28.2
Status		0.4147	0.0172	0.0098	< .0001
Level		0.0043	0.0057	0.0443	< .0001
Status* Level		0.3281	0.4189	0.2490	< .0001

Table 2: Bacterial count (Cfu/mLx10^{6}) from the faeces and selected organs of experimental broiler chickens.

Where NC: Negative Control and PC: Positive Control. NCC: not infected and not treated. Means with different superscripts within the same column are significantly different from each other at ($p < 0.05$).

Conclusion and Recommendation

The undamaged liver and kidney cells, coupled with low bacterial count (Cfu/mL) observed in all the infected groups of broiler chickens administered with *C. albidum* leaf extract compared with the infected and untreated indicates the worthy to reckon with antimicrobial activity of ethanolic extract of the leaf *C. albidum* on *Salmonella gallinarum Gr. D1-1, 9, 12.* Further study is hereby recommended for the isolation, characterization and elucidation of the bioactive compounds in the leaf extract of *C. albidum* in order to enhance their potentials as phyto-medics especially in poultry production.

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Competing Interests

There are no competing interests to be declared by the authors.

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