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Interventions of the Hydroethanolic Fruit Extract of *Solanum aethiopicum* on Lead-Induced Testicular Toxicity in Adult Male Wistar Rats

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Abstract— Background*: Solanum aethiopicum (SA), commonly known as garden egg or eggplant can be eaten either fresh or cooked and has a wide acceptance and large history of consumption in West Africa. This study focused on interventions of the hydroethanolic fruit extract of S. aethiopicum (SA) on lead-induced testicular toxicity in adult male Wistar rats.* ***Methods****: Twenty-five (25) adult male Wistar rats were randomly divided into five groups (1-5) of five (5) rats per group. Group 1 served as the control and was given 10 ml/kg/day of distilled water only, Group 2 received 10mg/kg body weight (B.W) of lead acetate only, Group 3 received 100 mg/kg B.W SA and 10mg/kg B.W of lead acetate (Pb), Group 4 received 200 mg/kg B.W of SA and 10mg/kg B.W of lead acetate while Group 5 received 300 mg/kg B.W of SA and 10mg/kg B.W of lead acetate. The administration of the extract was done using gastric gavage once a day, for thirty (30) consecutive days. Semen analysis (to assess sperm parameters such as sperm count, sperm motility, sperm viability and normal morphology), Testicular weight and testicular histology were assessed. Testicular oxidative stress markers, such as Malondialdehyde, Superoxide dismutase, Catalase and reduced Glutathione were also assessed.* ***Results****: Lead acetate caused a significant decrease in the sperm parameters and testicular antioxidant markers. It also caused a significant distortion of the histology of the testes and an insignificant decrease in testicular weight. The hydroethanolic fruit extract of SA however was found to cause an increase in testicular weight, enhanced sperm parameters, increase in the antioxidant parameters and a decrease in Malondialdehyde in all the lead-treated groups. There was also a marked improvement in the testicular histology after SA administration.* ***Conclusion****: It was concluded that S. aethiopicum fruit offers protection against free radical mediated oxidative stress of rats with lead induced testicular toxicity.*

Keywords— *Solanum aethiopicum, Lead, Toxicity, Malondialdehyde and Testicular weight.*

I. INTRODUCTION

Lead (Pb) is a heavy metal that has been abundantly used by humans for centuries. Humans are constantly being exposed to lead through the environment due to its wide range of applications in industries, cosmetics, and medicine. [1] Lead exposure causes numerous adverse effects on health including developmental neurotoxicity and toxicity to the blood, kidneys and endocrine systems. [2] Lead has also been reported in various studies to cause deleterious effects on the entire reproductive function. For instance, lead was found to cause a decline in the activities of testicular key steroidogenic enzymes with a significant depletion in cholesterol, ascorbic acid and reduced glutathione contents in lead-treated animals. [3] It has also been found to cause an undesirable alteration in sperm motility and viability, acrosome reaction, chemiotaxis [4] semen quality [5] and structural abnormality in rats. [6] Lead has also been found to cause a significant decline in the testicular level of Glutathione peroxidase (GPx), Catalase (CAT), and Superoxide dismutase (SOD) and a corresponding elevation of the level of malondialdehyde (MDA) in both the plasma and tissue in lead treated animals. [7, 8] The administration of lead to experimental animals has been found to result in the lumens devoid of sperm in testicular architecture [9], conspicuous degenerative changes in the testis [10], seminiferous epithelium degeneration, a significant reduction in the number of epithelium spermatozoa [11], sharp depressions, membrane folding and granularity at sperm head surfaces. [12] Several studies indicate that one possible mechanism of action of lead toxicity is the production of reactive oxygen species (ROS). Accumulation of lead in the cells has been found to severely affect the mitochondria and alter its normal function by inducing oxidative stress, a predominant reason for the reduction of cellular anti-defense mechanism. However, the complete mechanism of lead toxicity has remained complex. [13-15]

Solanum aethiopicum (egg plant), is a popular traditional vegetable in tropical Africa, which is grown principally for its leaves and fruits. [16] The fruits of *S. aethiopicum* can either be eaten fresh or steamed, pickled, boiled and used in preparing stews with other vegetables or meat. [16] It has been documented that almost all its parts are useful for nutritional, medicinal, cosmetics and industrial uses. [17] It is variously named in different tribes in Nigeria. For instance, the Igbos call the fruit "Aṅara" "Afufa" or "Mkpuruofe", the Yorubas call it "Igbagba" while the Hausas call it "Dauta". [18] This study was therefore aimed at establishing the potential of the hydroethanolic fruit extract of *S. aethiopicum* (SA) to ameliorate lead induced testicular toxicity in adult male Wistar rats.

II. MATERIALS AND METHODS

Plant collection and Preparation of plant extracts

Fresh and healthy fruits of *Solanum aethiopicum* were collected from a farm in Omuokiri in Aluu, Port Harcourt, Rivers State, Nigeria. The plant was identified and

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authenticated by Dr Suleiman, a plant taxonomist from the Department of Pharmacognosy and Phytotherapy in the faculty of Pharmaceutical Sciences, University of Port Harcourt. The sample was given the voucher number: UPHS0474.

The identified fruits were washed and then ground fresh to form a paste. The maceration method was used for the extraction. About 1000 grams of the fruit of the plant after weighing were dissolved in 80% ethanol and 20% water, making up to 2000ml of Hydroethanol, for 72 hours in an extraction vessel well-kept in an air light cupboard. The filtration was done using a glass funnel, 1000ml beaker and Whitman filter paper. The funnel was placed in the beaker. The filtrate was carefully poured into the funnel which filters through the funnel into the beaker. The extraction after filtration was dried using a rotary evaporator which separates the solvent from extract leaving it in a liquid form, which was then completely dried on a steam bath at a temperature of 45^{o}c.

The twenty-five (25) male Wistar rats used for this study were divided into five (5) groups with each group containing five (5) rats as shown in the table below.

At the expiration of 30 days of oral administration, three (3) rats from each group were sacrificed using chloroform anesthesia. The testes were harvested and weighed while semen was obtained from the testes for the analysis of sperm parameters (Sperm count, sperm motility, sperm viability and Sperm morphology) and testicular antioxidant markers. The testes harvested were also stained with haematoxylin and eosin for histopathological examination through light microscope by the usual method described by other researchers. [19]

TABLE 1: Experimental Design of the Study.

S/N	Groups	Substance Administered
1	Group 1	10ml/kg distilled water only
2	Group 2	10mg/kg BW lead acetate only
3	Group 3	100mg/kg BW hydro-ethanol extract of fruit of SA + 10mg/kg BW lead acetate
4	Group 4	200mg/kg BW hydro-ethanol extract of fruit of SA + 10mg/kg BW lead acetate
5	Group 5	300mg/kg BW hydro-ethanol extract of fruit of SA + 10mg/kg BW lead acetate

Ethical approval was obtained from the research Ethics committee unit of University of Port Harcourt (UPH/CEREMAD/REC/MM81/009). The statistical analysis was done using the SPSS version 20.0. The results were analysed using ANOVA with a significant difference at $p<0.05$. LSD comparison was used for the post hoc test, to test for significant differences between the lead group and the control group and between the other groups and the lead group. The results were presented as mean ± SEM.

III. RESULTS

TABLE 2: Effects of Lead Acetate and SA treatment on Sperm parameters for 30 days.

Groups	Sperm viability (%)	Normal morphology (%)	Sperm motility (%)	Sperm count (Million/ml)
1 (control)	88.33±4.41	83.88±4..32	88.33±4.41	61.00±4.93
2 (10mg/kg of lead acetate only)	56.67±4.41	54.76±4.10	70.00±2.89	34.67±2.91
Group 1- group 2	31.66*	29.12*	18.33*	26.33*
3 (100mg/kg SA +10mg/kg of lead acetate)	66.67±1.67	57.78±2.83	78.33±4.41	42.00±3.06
Group 3- group 2	10.00	3.02	8.33	7.33
4 (200mg/kg SA +10mg/kg of lead acetate)	78.33±4.41	60.02±3.43	83.33±4.41	43.33±5.36
Group 4- group 2	21.66**	5.26**	13.33**	8.66
5(300mg/kg SA +10mg/kg of lead acetate)	79.65±4.41	64.21±3.95	91.67±3.33	47.67±2.96
Group 5- group 2	22.98**	9.45**	21.67**	13.00**

The data are presented as the mean ± SEM with * denoting significant differences with respect to the control while '**' denote significant differences with respect to the lead group, at $p<0.05$.

TABLE 3: Effects of Lead Acetate and SA treatment on Testicular Antioxidant markers after 30 days.

Groups	MDA (mg/ml)	SOD (mg/ml)	GSH(mg/ml)	CAT (mg/ml)
1 (control)	78.26±0.80	123.33±6.01	53.67±3.18	133.15±0.52
2 (10mg/kg of lead acetate only)	79.56±0.47	85.67±4.98	36.00±2.31	121.01±5.17
Group 1- group 2	**- 1.30**	**37.66***	**17.67***	**12.14***
3 (100mg/kg SA +10mg/kg of lead acetate)	73.03±1.94	100.00±1.16	38.67±2.33	124.29±5.68
Group 3- group 2	**- 6.53****	**14.33****	**2.67**	**3.28**
4 (200mg/kg SA +10mg/kg of lead acetate)	70.59±3.37	109.00±7.37	41.33±1.86	133.47±2.47
Group 4- group 2	**- 8.97****	**23.33****	**5.33**	**12.46****
5(300mg/kg SA +10mg/kg of lead acetate)	67.17±1.53	111.00±4.36	47.67±1.45	139.48±2.50
Group 5- group 2	**-12.39****	**25.33****	**11.67****	**18.47****

The data are presented as the mean ± SEM with * denoting significant differences with respect to the control while '**' denote significant differences with respect to the lead group, at $p<0.05$.

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TABLE 4: Effects of Lead Acetate and SA treatment on Testicular weight after 30 days.

Groups	Testicular weight (g)
1 (control)	1.56±0.07
2 (10mg/kg of lead acetate only)	1.46±0.08
Group 1- group 2	**0.10**
3 (100mg/kg SA +10mg/kg of lead acetate)	1.56±0.06
Group 3- group 2	**0.10**
4 (200mg/kg SA +10mg/kg of lead acetate)	1.59±0.0.08
Group 4- group 2	**0.13**
5(300mg/kg SA +10mg/kg of lead acetate)	1.63±0.12
Group 5- group 2	**0.17****

The data are presented as the mean ± SEM with ** denoting significant differences with respect to the lead group, at p<0.05.

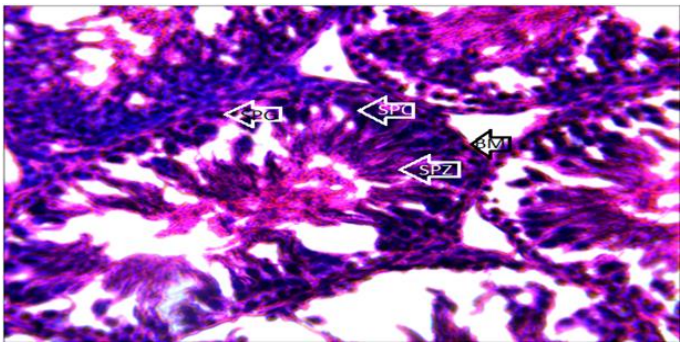

Plate 1: Photomicrograph of a section of the Testes of rat of Group 1 after 30 days. It reveals histologically normal testis with intact seminiferous tubules containing Spermatogonia (SPG), spermatocytes (SPC) and spermatozoa (SPZ), surrounded with a basement membrane (BM) and interstitial spaces (ISS) containing Leydig cells
Stain: H & E. Magnification: X 400.

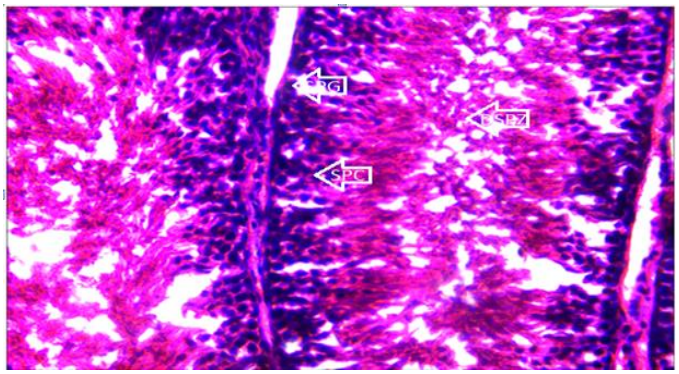

Plate 2: Photomicrograph of a section of the Testes of rat of Group 2 after 30 days of lead administration. It reveals histologically distorted testis with; denatured spermatozoa, spermatocytes (SPC) and spermatogonia (SPG).
Stain: H & E. Magnification: X 400.

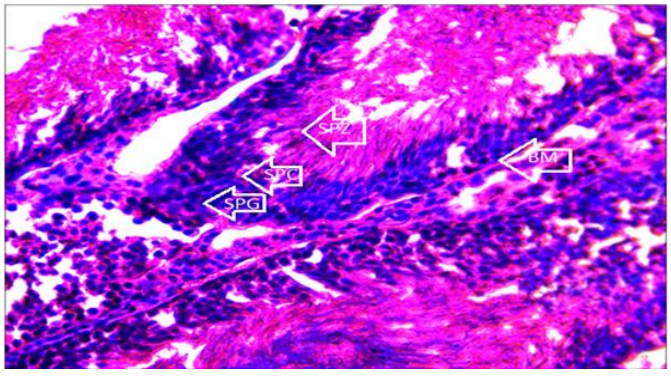

Plate 3: Photomicrograph of a section of the Testes of rat of Group 3 after 30 days of lead administration. It reveals a histologically normal testis with intact seminiferous tubules containing SPG, SPC and SPZ, surrounded with BM and interstitial spaces (ISS) containing Leydig cells.
Stain: H & E. Magnification: X 400.

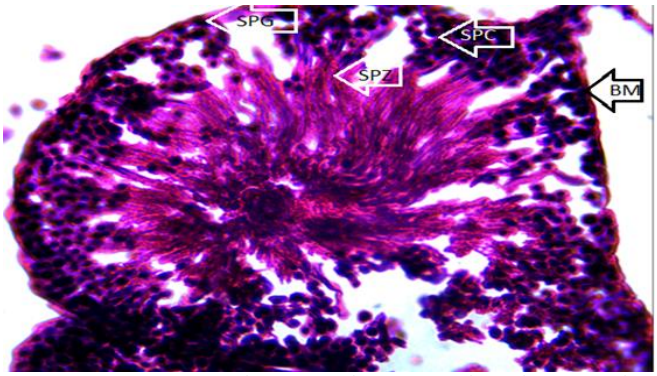

Plate 4: Photomicrograph of a section of the Testes of rat of Group 4 after 30 days of lead administration. It reveals histologically normal testis with intact seminiferous tubules containing SPG, SPC and SPZ, surrounded with BM and interstitial spaces (ISS) containing Leydig cells
Stain: H & E. Magnification: X 400.

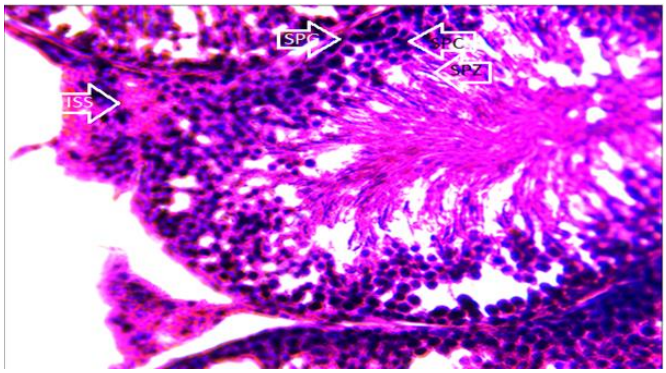

Plate 5: Photomicrograph of a section of the Testes of rat of Group 5 after 30 days of lead administration. It reveals histologically normal testis with intact seminiferous tubules (containing SPG, SPC and SPZ, surrounded with BM) and interstitial spaces (ISS), containing Leydig cells
Stain: H & E. Magnification: X 400.

IV. Discussion of Results

Effects of lead acetate and SA treatment on Sperm parameters after 30 days.

The effects of the hydroethanolic fruit extract of *Solanum aethiopicum* on sperm parameters is shown in table 2. It revealed a statistically significant decrease (P<0.05) in sperm viability, sperm motility, normal morphology and sperm count in the lead group when compared with the control. There was however a significant increase in sperm viability (in groups 4 and 5), normal morphology (in groups 4 and 5), sperm motility (in groups 4 and 5) and sperm count (in group 5), all with respect to the lead treated group (group 2). This means that to a large extent the extract in a dose-dependent manner demonstrated the ability to ameliorate or reverse the damage done on the sperm parameters by lead.

In the present study, the significant decrease in sperm viability, sperm motility, percentage of sperm with normal morphology and sperm count in the rats following exposure to lead acetate, implies that lead may interfere with

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spermatogenesis by crossing the blood-testis barrier and gaining access to the germinal cells. [20] The spermatozoa membranes are reportedly rich in polyunsaturated fatty acids, making them susceptible to reactive oxygen species (ROS) attack and lipid peroxidation as a result of exposure lead. [21] It has been established that lipid peroxidation reaction causes membrane damage which ultimately leads to a decrease in sperm motility, presumably by a rapid loss of intracellular ATP and an elevation in various degrees of defect in sperm morphology. [22, 23]

Several active components in SA account for its ability to scavenge ROS generated by lead, reduce lipid peroxidation and enhance the activity of testicular antioxidant enzymes resulting in protection against lead-induced testicular toxicity, which manifests mostly in an increase in sperm abnormalities. [24, 25, 26] Apart from the ability to scavenge ROS generated by lead, the results of this study indicate that the fruit extract of *Solanum aethiopicum* has an effect on the mitochondria found in the body of the spermatozoon where energy is being synthesized in the form of adenosine triphosphate (ATP), which increases the sperm motility. [27] The antioxidative properties of SA may therefore play a positive role in the defense against oxidative stress induced by lead acetate.

Effects of Lead Acetate and SA treatment on Testicular Antioxidant markers after 30 days.

The effects of the hydroethanolic fruit extract of *Solanum aethiopicum* on testicular oxidative stress markers is shown in table 3. It revealed a statistically significant decrease ($P<0.05$) in the concentrations of superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) in the lead group when compared with the control while there was an insignificant elevation of the level of malondialdehyde (MDA) in the lead group when compared with the control group. There was also a significant decrease in the concentration of MDA (in groups 3, 4 and 5), significant increase in SOD (in groups 3, 4 and 5), GSH (in group 5) and CAT (in groups 4 and 5), all with respect to the lead treated group (group 2). This means that the extract in a dose-dependent manner was able to reverse the damage done on the oxidative stress markers by lead.

In this study, the administration of lead acetate is associated with increased levels of oxidative stress and decreased levels of testicular antioxidants (GSH, SOD and CAT). The decrease in SOD, CAT and GSH levels in the testis suggests the toxic effects of lead in the experimental rats (group 2). The administration of *Solanum aethiopicum* (SA) fruit countered the action of lead on testicular cells thereby mitigating the decrease in the level of the testicular antioxidant markers. Since it is well established that GSH mediates in the detoxification of chemically reactive metabolite in drug-induced toxicity, it then follows that an increase in lipid peroxidation and decreased synthesis of GSH caused by lead, would cause a decrease in GSH levels. [28-29] This therefore implies that an increase in the antioxidant enzyme activities (SOD, CAT and GSH) after administration of SA might contribute to ameliorating oxidative stress.

MDA is a known biomarker of lipid peroxidation and oxidative stress. The elevated level of MDA in the testicular tissue associated with the administration of lead acetate in this study is an important indication of lipid peroxidation as reported by other researchers. [30] The administration of a combination of SA and lead acetate in groups 3 to 5 was found to attenuate the lead-induced increase in MDA, indicating that SA is rich in antioxidant constituents such as phenol, ascorbic acid and flavonoids. [31-33] It can be inferred that these rich antioxidant constituents of SA boosted the testicular enzymatic antioxidants to effectively scavenge the free radicals preventing lipid peroxidation, therefore reducing the lead-induced toxicity.

Effects of lead acetate and SA treatment on Testicular weight after 30 days.

The effects of the hydroethanolic fruit extract of *Solanum aethiopicum* on testicular weight is shown in table 4. It revealed an insignificant decrease ($P>0.05$) in the testicular weight in the lead group when compared with the control and a statistically significant increase ($P>0.05$) in the testicular weight in group 5 with respect to the lead treated group. A higher dose of the extract was therefore able to ameliorate the damage done on the weight of the testes by lead.

The testis and other reproductive organs are structurally and physiologically dependent upon the concentration of testosterone and other androgens. The observable increase in testicular weight in group 5 can therefore be attributable to increase in the levels of testosterone and other androgens by the extract. This is true because studies have shown that the level of androgen is positively correlated with the weight of the testis and epididymis. [34] This therefore implies that any significant reduction in testicular weight (as seen in the lead only treated group), can be attributable to a change in the androgen content. This is because the testosterone among other roles stimulates the growth and secretory activity of the reproductive organs.[35-36] The increase in testicular weight observed in the study may also be due to the ability of the extract to cause an increase in the secretion of seminiferous fluid, which has been found to contribute substantially to the testicular weight. [37] Again, it has been reported that alterations in spermatogenesis is usually accompanied by alterations in testicular weight and that a reduction in the testicular weight is related to a decrease in the number of spermatids and spermatozoa present in the tissues. [38] This is true because a greater part of the testicular weight is contributed by the number of spermatids and spermatozoa present. [39-40] The observed significant increase in testicular weight found in this study could therefore be due to increased spermatogenic activities.

Effects of the extract on the histology of the testis after 30 days of administration following lead-induced toxicity.

The effects of the hydroethanolic fruit extract of *Solanum aethiopicum* on testicular histology is presented in plates 1 to 5. It revealed a histologically distorted testis with denatured spermatozoa, spermatocytes (SPC) and spermatogonia (SPG) in the lead treated group with respect to the control group and a histologically normal testis with intact seminiferous tubules (containing SPG, SPC and SPZ, surrounded with BM), in

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groups 3 to 5, with respect to the lead only treated group (group 2). This implies that the extract was able to reverse the histological distortion caused by lead.

In this study, lead acetate treatment caused the degeneration of the spermatogenic cells of the germinal epithelium, denatured spermatozoa, spermatocytes (SPC) and spermatogonia (SPG). It has been established that testosterone (produced by the interstitial cells of Leydig) is a necessary prerequisite for the maintenance of the process of spermatogenesis. [41] Reduced cellularity of the testicular interstitium has been associated with a decrease in serum level of testosterone resulting in poor spermatogenesis. [20]

The outcome of this research corroborates that of other researchers, which reported that the aqueous extract of *Solanum melongena* (another species of garden egg) fruit did not induce any

lesion in the histology of the testes after twenty-eight days of administration. [42] This can be attributable to the antioxidant property of the fruit since it has been reported that Plants which contain flavonoids are effective in the prevention of lesion, mainly because of their antioxidant properties. [43]

It was observed from this study that in the groups that SA was administered with lead acetate (groups 3 to 5), the testicular histology was protected from the harmful effects of the lead. This protective nature of SA is facilitated by the presence of the antioxidant constituents such as ascorbic acid and flavonoids, which are known for their protection of cell membranes and scavenging effects on free radicals and increased testosterone synthesis by the interstitial cells of Leydig. [44-45]

V. CONCLUSION

We therefore conclude from this study that lead acetate caused a decrease in sperm parameters, testicular antioxidant markers, testicular weight and a distortion or lesion in the testicular histological architecture. However *Solanum aethiopicum fruit when co-administered with lead* caused an increase in sperm parameters, testicular antioxidant markers and the testicular weight and protected the cytoarchitecture of the testis from distortion due to the harmful effect of lead acetate. *Solanum aethiopicum* attenuates lead-induced testicular toxicity through an antioxidant system of activities. We can therefore infer from our findings that *Solanum aethiopicum* fruit has pro-fertility properties which may be beneficial to those who consume it, especially those who are constantly exposed to lead pollution.

VI. ACKNOWLEDGEMENTS

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Competing Interests

No competing interests exist.

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