Original Article

Evaluation of *Punarnavadi Mandura* for haematinic activity against mercuric chloride-induced anemia in albino rats

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Abstract

Background: *Punarnavadi Mandura,* a compound Ayurvedic formulation, is one of the most commonly used medicine in the treatment of anemia (*Pandu*) in Ayurveda. The safety profile of this formulation is well established; however, no pharmacological study has been reported to provide the scientific basis for its use in the treatment of anemia. **Aim:** To evaluate the hematinic effect of *Punarnavadi Mandura* against mercuric chloride-induced anemia in albino rats. **Materials and methods:** The test drug (*Punarnavadi Mandura*) was suspended in distilled water and administered orally in a dose of 450 mg/kg for 30 days in rats. Anemia was induced by simultaneous administration of mercuric chloride (9 mg/kg) for 30 consecutive days. Body weight was noted for each animals. At the end, haematological parameters, anaemia markers like serum iron, ferritin, and total iron binding capacity (TIBC), as well as relative weight of organs and histopathology investigation, were examined. **Results:** Exposure of mercuric chloride to rats for 30 days resulted in a significant decrease of body weight, an increase in the weight of the liver and kidney and a decrease in hemoglobin content. It also decreased serum ferritin to a significant extent and increased serum TIBC. Histopathology of the liver shows macro fatty changes, vacuolization, marked necrosis, and severe degenerative changes, while the kidney shows cell infiltration. All these changes were significantly attenuated by the administration of *Punarnavadi Mandura*. **Conclusion:** The present data indicate that *Punarnavadi Mandura* has possessing marked cytoprotective activity, significantly attenuated the $HgCl_2$-induced adverse changes on red blood cell related parameters, and showing hematinic activity in albino rats.

Keywords: Anemia, ferritin, mercuric chloride, *Pandu*, *Punarnavadi Mandura*

Introduction

Iron deficiency anemia is one of the most common nutritional disorders worldwide, especially in India and other developing countries. Young children and women in the reproductive age group are the most vulnerable to iron deficiency anemia. Children are at increased risk because their requirement for iron is high.[1,2] With a global population of 6700 million, at least 3600 million people have iron deficiency, and 2000 million out of these suffer from iron deficiency anemia. South East Asia contributes to 1/5th of the population living with Iron deficiency anemia.[3] The prevalence of anemia in India is higher than in other South Asian Countries.[4] National Family Health Survey estimates reveal the prevalence of anemia to be 70%–80% in children.[5] WHO has clearly emphasized that the prevention of anemia in early childhood must be the goal of intervention programmes in reproductive and child health.[6]

Iron deficiency is harmful to all ages. In young children, it impairs physical growth, cognitive development, and immunity at school age. It also affects school performance in adulthood; it causes fatigue and reduces work capacity. Among pregnant women, anemia may cause fetal growth retardation or low birth weight, and is responsible for a large proportion of maternal deaths.[7]

On screening the books of *Ayurveda*, the indigenous system of medicine in India, for a comparable disease, the disease entity described as *Pandu* (disease of pallor) has striking resemblance

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to the descriptions of anemia.[8] There are several formulations described for the management of *Pandu*, among them *Punarnavadi Mandura*[9] is one that is most commonly used clinically. Although the safety profile of this formulation was well established by detailed acute and chronic toxicological studies,[10] no pharmacological study has been reported to provide a scientific basis for clinical findings; hence, this study was aimed to evaluate the effect of *Punarnavadi Mandura* against mercuric chloride-induced anemia in rats.

Materials and methods

Test formulation

The authenticated raw materials [Table 1] of the test formulation *Punarnavadi Mandura* were procured from the attached pharmacy of the institute. The individual ingredients were converted into coarse powder (sieve 40#) after proper shade drying. All the powders were mixed together thoroughly in specified proportions to prepare a homogenous blend, which was shifted to a stainless steel container, added with specified amounts (160 Part) of *Gomutra* and the contents were subjected to mild heat, maintaining a temperature in between 95°C and 100°C. The contents were stirred constantly, to avoid the possibility of settling down of the contents and their charring. When the mixture attained paste-like consistency, 5% gum acacia was mixed in this combination, and the mixture was converted into granules with the help of the granulation equipment and 500 mg tablets were made in tablet making machine and used in the experimental study.

Animals selection

Charles foster strain albino rats of either sex weighing 160 ± 20 g were procured from the animal house attached to the Pharmacology laboratory. They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water was given *ad libitum*. The animals were acclimatized for at least 1 week in lab conditions before the commencement of the experiment in standard laboratory conditions 12 h dark and light cycles, maintained at a temperature of 23°C ± 3°C and 50%–60% humidity. The institutional animal ethics committee had approved the experimental protocol (Approval number; IAEC03/08-11/PhD/03) as per the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

Dose fixation

The therapeutic dose of *Punarnavadi Mandura* mentioned in the classical text is one *Kola* (Approx. 5 g). The same dose was converted to an animal dose based on the body surface area ratio by referring to the standard table of Paget and Barnes.[11] On this basis, the rat dose was found as 450 mg/kg body weight. The test formulation was suspended in distilled water by making uniform suspension with 0.5% sodium carboxy methyl cellulose (Na-CMC) with suitable concentration depending

Table 1: Ingredients of *Punarnavadi Mandura*

Ingredients	Botanical name	Family	Part used	Ratio
Punarnava	*B. diffusa* Linn.	Nyctaginaceae	Dry root	1 part
Sunthi	*Z. officinale* Roxb.	Zingiberaceace	Dry rhizome	1 part
Trivrita	*O. turpethum* Linn.	Convolvulaceae	Dry root	1 part
Maricha	*P. nigrum* Linn.	Piperaceace	Dry fruit	1 part
Pippali	*P. longum* Linn.	Piperaceace	Dry fruit	1 part
Vidanga	*E. ribes* Burm	Myrsinaceae	Dry fruit	1 part
Devadaru	*C. deodara* Roxb.	Pinaceae	Dry hardwood	1 part
Chitraka	*P. zeylanica* Linn.	Plumaginaceace	Dry root	1 part
Kushtha	*S. lappa* C.B. Clarke	Compositae	Dry root	1 part
Daruharidra	*B. aristata* D.C.	Berberidaceae	Dry hardwood	1 part
Amalaki	*E. officinalis* Gaertn.	Euphorbiaceace	Dry fruit	1 part
Haritaki	*T. chebula* Retz.	combrefaceae	Dry fruit	1 part
Bibhitaki	*T. bellirica* Roxb.	Combretaceace	Dry fruit	1 part
Danti	*B. montanum* Muell.	Euphorbiaceae	Dry root	1 part
Chavya	*P. chaba* Hunter	Piperaceace	Dry root	1 part
Indrayava	*H. antidysenterica* Wall.	Apocynaceae	Dry seed	1 part
Pippali	*P. longum* Linn.	Piperaceae	Dry fruit	1 part
Pippalimula	*P. longum* Linn.	Piperaceae	Dry root	1 part
Musta	*C. rotundus*	Cyperaceae	Dry tuber	1 part
Haridra	*C. longa* Linn.	Zingiberaceae	Dry rhizome	1 part
Mandura Bhasma	Purified Red oxide of Iron Fe_2O_3, H_2O	-	-	40 parts
Gomutra	Cow's urine	-	-	160 parts

B. diffusa: Boerhavia diffusa, Z. officinale: Zingiber officinale, O. turpethum: Operculina turpethum, P. nigrum: Piper nigrum, P. longum: Piper longum, E. ribes: Embelia ribes, C. deodara: Cedrus deodara, P. zeylanica: Plumbago zeylanica, S. lappa: Saussurea lappa, B. aristata: Berberis aristata, E. officinalis: Embelica officinalis, T. chebula: Terminalia chebula, T. bellirica: Terminalia bellirica, B. montanum: Baliospermum montanum, P. chaba: Piper chaba, H. antidysenterica: Holarrhena antidysenterica, C. rotundus: Cyperus rotundus, C. longa: Curcuma longa

upon the body weight of animals and administered orally with the help of gastric oral catheter.

Experimental protocol

The selected animals were randomly divided into three groups; each group consisted of six animals of either sex. Group (I), kept as the control group, received the vehicle as 0.5% CMC in distilled water in a volume equal to the volume of the test drug. Group (II) kept as the $HgCl_2$ control group, received vehicle plus mercuric chloride solution (9 mg/kg, po), and served as the negative control group. Group (III) kept as drug-treated, received *Punarnavadi Mandura* (450 mg/kg, po) plus mercuric chloride solution (9 mg/kg, po). *Punarnavadi Mandura* was administered to respective groups for 30 consecutive days. To induce anemia, the solution of mercuric chloride (9 mg/kg, po) was administered to the second and third groups after 1 h of the vehicle and test drug administration, respectively, for 30 consecutive days by referring to the previous study.[12]

On the 31st day, blood was collected from overnight fasted rats by puncturing the supra-orbital plexus by capillary tubes under ether anesthesia for estimation of hematological and biochemical markers. Blood in anti-coagulant, ethylenediamine tetraacetic acid tubes fed to the auto cell analyzer (Sismes KX-21, Trans Asia), which was automatically drawn into the instrument for estimating different parameters like hemoglobin content, total red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, and platelet count. For estimation of serum biochemical markers, serum was separated from collected blood, and parameters such as serum ferritin (ELISA method, ORGENTEC Diagnostika Gmbh-Germany),[13] serum iron, and serum total iron binding capacity (TIBC) (Fe-TR direct method of Wako Junyaku (Nitroso-PSAP, autoanalyzer, Magnetic TIBC kit)[14] were carried out.

The animals were weighed and sacrificed on the 31st day. Important organs like the liver, spleen, and kidney were dissected carefully and cleaned for extraneous tissue, and weighed. The liver and kidney were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. Paraffin sections (4 μm) were cut on glass slides and stained with hematoxylin and eosin by referring to standard procedure.[15] The slides were viewed under binocular research Carl-Zeiss's microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Statistical analysis

Data are expressed as mean ± standard error of mean. Statistical evaluation was carried out with the help of unpaired Student's *t*-test as well as one-way analysis of variance, followed by Dunnett's multiple *t*-test as post-hoc test. A level of $P < 0.05$ was considered statistically significant.

Results

In the course of 30 days, a normal progressive gain in body weight occurred in the control group. Administration of $HgCl_2$ leads to a significant decrease in body weight in comparison to both initial as well as the control group. Concomitant treatment with *Punarnavadi Mandura* significantly mitigated $HgCl_2$-induced body weight changes. [Table 2]

Administration of $HgCl_2$ leads to nonsignificant increase in the relative weight of the spleen and a significant increase in the relative weight of the liver and kidney of albino rats in comparison to the control group. Concomitant treatment with *Punarnavadi Mandura* significantly attenuated the toxicant-induced changes in the weight of the liver and spleen in comparison to the $HgCl_2$ control group. [Table 3]

$HgCl_2$ administration leads to a significant decrease in hemoglobin percentage in comparison to the control group. Concurrent treatment with *Punarnavadi Mandura* significantly attenuated the $HgCl_2$-induced anemia. Total WBC count was nonsignificantly increased in the $HgCl_2$ control group. Treatment with *Punarnavadi Mandura* significantly decreased it in comparison to the $HgCl_2$ control group. Further neutrophil percentage and total platelet counts were nonsignificantly increased in the $HgCl_2$ control group, and treatment with *Punarnavadi Mandura* significantly reversed it. [Table 4]

An apparent and statistically nonsignificant decrease in serum iron was observed in the $HgCl_2$ control group in comparison to the control group. Treatment with *Punarnavadi Mandura* elevated the serum iron content in comparison to the $HgCl_2$ control group. Further, administration of $HgCl_2$ significantly decreased the serum ferritin and increased the serum TIBC levels in comparison to the control group. Treatment with

Table 2: Effect on test drugs on body weight of albino rats

Body weight (g)	Treatments		
	Control	$HgCl_2$ control	*Punarnavadi Mandura*
Initial	156.33±10.65	174.86±07.68	175.67±06.31
Final	190.00±08.31	165.14±09.52	193.33±06.67
Actual change	33.67±04.36	−11.43±02.30*	21.20±03.61@
Percentage change	21.53↑	05.63↓	10.05↑

*P<0.001, when compared with control group, @P<0.001, when compared with $HgCl_2$ control group (unpaired *t*-test). Mean±SEM. ↑: Increase, ↓: Decrease, SEM: Standard error of mean

Table 3: Effect of test drugs on relative organ weight

Organs (g/100 g BW)	Treatments		
	Control	$HgCl_2$ control	*Punarnavadi Mandura*
Spleen	0.25±0.03	0.32±0.03	0.26±0.01@
Liver	3.51±0.13	4.23±0.24*	2.69±0.12#
Kidney	0.61±0.02	0.97±0.09**	1.01±0.08

*P<0.05, **P<0.01, when compared with control group; @P<0.01, when compared with the $HgCl_2$ control group (unpaired *t*-test); #P<0.05, when compared with the $HgCl_2$ control group (one-way ANOVA followed by Dunnett's multiple *t*-test). Mean±SEM. SEM: Standard error of the mean

Table 4: Effect of test drugs on hematological parameters in albino rats

Parameters	Control	$HgCl_2$ control	*Punarnavadi Mandura*
Hemoglobin (g/dL)	14.60±0.25	12.95±0.25**	14.78±0.23$^{\#}$
WBC (10^3/μL)	8450.00±262.10	9914.29±865.59	6950.00±689.81$^{@}$
Neutrophil (%)	24.17±2.39	31.43±2.61	23.67±0.80$^{\#}$
Lymphocyte (%)	67.00±4.39	72.29±2.55	69.50±0.99
PCV (%)	42.60±1.05	42.54±0.60	42.77±0.87
RBC (10^6/μL)	7.55±0.20	7.57±0.14	7.29±0.20
Platelet (10^3/μL)	407.83±21.94	483.14±32.87	336.33±23.90$^{\#}$

**P<0.01, when compared with the control group, $^{@}P$<0.05, when compared with the $HgCl_2$ control group (unpaired t-test), $^{\#}P$<0.05, when compared with $HgCl_2$ control group (one-way ANOVA followed by Dunnett's multiple t-test). Mean±SEM. WBC: White blood cell, RBC: Red blood cell, SEM: Standard error of the mean, PCV: Packed cell volume

Punarnavadi Mandura non-significantly reversed serum ferritin levels not affected the TIBC level in treated albino rats. [Table 5]

Sections of the liver and kidney from the control group have shown normal cyto architecture. [Figure 1a and d] Exposure to mercuric chloride led to marked fatty changes and vacuolization, cell infiltration in the liver [Figure 1b] and marked necrosis, severe degenerative changes, and cell infiltration in the kidney.[Figure 1e] Sections of these two organs from the animals treated with *Punarnavadi Mandura* shows almost normal cytoarchitecture [Figure 1c and f] similar to that of sections of control group.

Table 5: Effect on of test drugs specific markers of anemia in albino rats

Parameters	Control	$HgCl_2$ control	*Punarnavadi Mandura*
Serum iron (IU/L)	96.96±11.70	86.13±10.45	124.17±15.55
Serum ferritin (IU/L)	5.30±1.22	1.81±0.59*	3.27±0.56
Serum TIBC (IU/L)	332.00±20.76	418.00±26.55*	422.00±30.16

*P<0.05, when compared with the control group (unpaired t-test). Mean±SEM. SEM: Standard error of the mean, TIBC: Total iron binding capacity

Discussion

Anemia is one of the important public health problems not only in India but also in most of the South East Asian countries.[16] Despite the interventions at various levels to treat and prevent anemia, its prevalence remains alarming. There is a need of developing a new strategy in the management of anemia with long-term effectiveness. The textbooks of *Ayurveda*, described a disease *Pandu* (disease of pallor), and various metallic and nonmetallic preparations have been described for the management of *Pandu*. The textbooks of *Ayurveda* have given due consideration for various factors that hamper *Agni* and adversely affect digestion and metabolism.[17] The formulations listed in the treatment of *Pandu* include those which can correct the metabolism by which absorption and bioavailability of nutrients like iron can be enhanced as well as hematinic agents. *Punarnavadi Mandura* is such a combination as it contains *Loharaja* (incinerated iron), which is considered the best drug in the management of *Pandu* in the classics of *Ayurveda* which shows that the ancient scholars were well aware of the role of iron deficiency in the pathogenesis of the disease.

Body weight is a useful indicator of growth, and loss of weight is a common in the hemoglobin depletion stage. The present results clearly showed that the body weight of $HgCl_2$ alone treated rats lagged behind in comparison to control rats ($P < 0.001$), while the body weight gain of *Punarnavadi Mandura* treated animals was significant ($P < 0.05$). The loss in the mean weight of the rats treated with mercuric chloride alone shows that toxicity must have been induced in the rats, which led to the loss of appetite, and loss in food intake due to degenerative changes in the liver, kidney, and gut. This confirms the results obtained in previous reports.[18-21] Further, the weight of the liver and kidney was significantly increased by mercuric chloride. It has been reported that these organs are the major site for the accumulation of mercury.[22] Pretreatment with the trial drug significantly arrested and reversed the toxicant-induced change in the weight of the liver. The weight gain observed in the kidney was not reversed to a significant extent.

Exposure of mercuric chloride leads to a significant decrease in hemoglobin content in $HgCl_2$ control group, indicating the occurrence of anemia due to mercuric chloride administration. This may be attributed to a decreased rate of production of RBC or increased loss of these cells. Previous studies also showed impaired erythropoiesis due to the direct effect of metal on hemopoetic centres like the kidney and spleen,[23,24] leading to anemia. Accelerated erythroclasia due to the altered membrane and/or defective iron metabolism, impaired intestinal uptake of iron due to mucosal lesions,[25] and destruction of too much RBC[26] also cause of anemia due to $HgCl_2$ exposure. Further, it has been reported that mercuric chloride induces severe oxidative stress in the RBC.[27] Increase in lipid peroxidation (LPO) in RBC might probably be due to the peroxidation of unsaturated fatty acids in plasma membrane phospholipids of RBC.[28] This increased LPO in RBC may cause a progressive increase in cellular deformity, increase in membrane permeability and rigidity, and disruption of the structural and functional integrity of cell organelles. This may ultimately lead to lysis of the RBC and consequent anemia. Contemporaneous treatment with *Punarnavadi Mandura* not

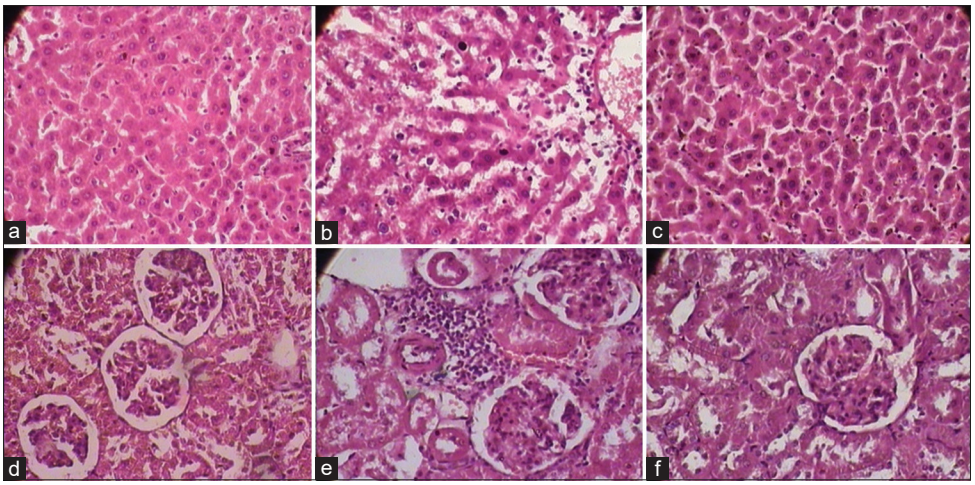

Figure 1: Photomicrographs of sections of the liver and kidney of albino rats (×400 magnification). Liver sections from (a) control group; (b) mercuric chloride control group and (c) PunarnavadiMandura treated group. Kidney sections from (d) control group; (e) mercuric chloride control group and (f) Punarnavadi Mandura treated group

only attenuated the hemoglobin level but also significantly enhanced it. The observed effect may be attributed to interference with one or more of the above-said mechanisms.

Enhancement of the total WBC count following mercuric chloride intoxication could be possible due to leucocytosis, as leukocytosis is an outcome of the proliferation of hemopoietic system leading to progressive infiltration in peripheral blood and stimulation of the immune system in response to tissue damage caused by mercuric chloride.[29,30] *Punarnavadi Mandura* significantly reduced the elevated total WBC count.

Ferritin is an indicator of stored iron in the body. Ferritin is the main protein that stores iron for areas that need it, especially the liver and the bone marrow, where RBCs are made. The iron ferritin level is the first in line to drop if the individual suffers from any iron insufficiency.[31] Low serum ferritin in a patient with low hemoglobin is diagnostic of iron deficiency anemia.[32] Serum iron is a measure of circulating iron bound to transferrin and reflects total body iron.[33] Decreased levels of serum iron and increased levels of TIBC are suggestive of iron depletion and hence used as indicators of iron deficiency state as well as anemia.[34] Exposure to mercuric chloride leads to a significant decrease in the serum ferritin, while it significantly increases the TIBC. Pretreatment with *Punarnavadi Mandura* elevated the serum iron content but did not produce any significant effects on TIBC level in comparison to the $HgCl_2$ control group.

Microscopic examination of the liver obtained from rats treated with mercuric chloride showed destruction of the normal hepatic architecture and a severe pathological alteration which was reported earlier also.[35] The histopathological sections of the kidney also show severe pathological changes such as marked necrosis, cellular infiltration, and severe degenerative changes. *Punarnavadi Mandura* treated group shows almost normal cytoarchitecture in comparison to the mercuric chloride control group. Mercuric ions (Hg^{2+}) have a great affinity to bond with thiol or sulfhydryl (-SH) groups, especially those present on the endogenous thiol-containing molecules such as glutathione (GSH), cysteine, metallothionein, homocysteine, N-acetylcysteine, S-adenosyl-methionine, and albumin.[36,37] GSH is the most important antioxidant and protector of cells such as human erythrocytes, renal cells, and hepatic cells.[38] Hg^{2+}-mediated GSH depletion creates an oxidative stress condition characterized by increased susceptibility of the mitochondrial membrane to iron-dependent LPO. Epithelial cell damage is believed to occur as the result of the enhanced free radical formation and LPO.[39,40] A pivotal role for extracellular GSH has also been identified in the renal and hepatic disposition, toxicity, and excretion of inorganic mercury (Hg^{2+}) in rats.[41,42]

Punarnavadi Mandura contains drugs with antioxidant, cytoprotactive, immunomodulatory, and hepatoprotactive activity like *Boerhaavia diffusa,*[43-45] *Emblica officinalis,*[46,47] *Terminalia belerica,*[48-50] *Terminalia chebula,*[51,52] *Zingiber officinale,*[53,54] *Piper longum,*[55] *Piper nigrum,*[56,57] *Embelia ribes,*[58] *Picrorhiza kurroa.*[59-61] *Mandura bhasma* (incinerated red oxide of iron) which forms the major part of formulation, is a rich source of iron and it has a powerful hematinic and cytoprotective, hepatoprotective activities.[62] *Emblica officinalis* is a rich source of iron and Vitamin C.[63] Their combination as *Triphala* (*Emblica officinalis, T. belerica*, *T. chebula*) is more efficient due to the combined activity of the individual component.[64] Another combination *Trikatu* (*Z. officinale*, *P. longum, P. nigrum)* also has antioxidant and cytoprotective effects as well as they serve as enhancer of the bioavailability of iron. Cow's urine is an important ingredient of the drug and has been proved for its antimicrobial, antioxidant,[65] immunomodulator,[66] and anti-anemic effects due to its erythropoietin stimulating properties.[67] The combination is expected to break the pathogenesis, correct the metabolism and increase the absorption and bioavailability of iron. The observed results are in favor of these hypotheses. However, further studies are required to ascertain the exact mechanism involved in the observed activity profile.

Conclusion

The results obtained in the study clearly show that *Punarnavadi Mandura* has possessing marke cytoprotective activity, significantly attenuated the $HgCl_2$-induced adverse changes on RBC-related parameters and shoshowedematinic activity in albino rats.

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Conflicts of interest

There are no conflicts of interest.

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