Anti-Inflammatory and Anti-Microbial Potential of *Plumbago zeylanica* L.: A Review

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ABSTRACT

*Plumbago zeylanica* L. (Pz) is one of the most important medicinal plant belonging to the family Plumbaginaceae. It is a perennial shrub, growing throughout India and most places of Sri Lanka. It contains various bioactive compounds like alkaloids, flavonoids, naphtoquinones, glycoside, saponins, steroids, tri-terpenoids, coumarins, phenolic compounds etc. Of all the chemical constituents, plumbagin is the principal active compound. Plumbagin ([5-hydroxy-2-methyl-1, 4-naphtoquinone]-C11H8O3) is primarily present in roots in higher amounts with only about 1% in the whole plant. The literature reveals that the root and root bark have a wider claim in traditional medicines against various diseases as a memory enhancer, anti-inflammatory, anti-microbial, wound healing, anti-malarial, anti-inflammation, anticancer, blood coagulation, and anti-oxidant activities. The present study aims to evaluate the anti-inflammatory and antimicrobial properties of this plant.

**Keyword:** Plumbago zeylanica; Sheetraj; Chitramol; Anti-inflammatory; Antimicrobial; Traditional uses

1. INTRODUCTION

Inflammatory processes play a key role in the body's initial protection following infection or tissue injury, thereby minimising more harm to the affected place. While inflammation reaction plays an important role in biological defense mechanisms in the early stages of infection. Chronic inflammation has long been associated with a wide variety of non-infectious diseases, including arthritis. Steroids play an important role in inflammatory disease management, but due to their toxicity, they should only be used for brief periods or in extremely severe situations where the risks are appropriate. Prolonged use of NSAIDs often involves severe side effects, especially gastrointestinal haemorrhage. After the "golden age" revolution, when almost all classes of essential antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the key problems of chemotherapy were solved in the 1960s, history nowadays is repeating itself and these exciting compounds are in danger of losing their effectiveness due to the rise in microbial resistance. Its impact on treatment failures associated with multidrug-resistant bacteria is currently substantial and has become a global concern for public health.

As a result, the use of medicinal plants for the treatment and management of diseases like inflammatory and bacterial infections that have existed since time immemorial is becoming increasingly widespread globally. *Pz* is one of the most important medicinal plant, commonly known as Chitraka/Chitramol in Sanskrit, White Leadwort/Ceylon leadwort/Doctorbush in English, Sheetraj Hindi in Urdu, belonging to the family Plumbaginaceae. It is widely used in traditional medicine for anti-inflammatory and antimicrobial agent. It is a perennial shrub, growing most places of Sri Lanka and throughout India. In India, especially in Uttar Pradesh, Andhra Pradesh, Karnataka, Kerala and Bengal. Moreover, it can be found in subtropical and tropical regions above 2 km from sea level, where soils are rich in trace elements. It grows in lead polluted areas. The Plumbago genus name originated from Latin words plumbum, lead and against various infections that have existed since time immemorial is becoming increasingly widespread globally.

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traditional medicine the root, stem bark and leaves are used as medicinal herbs for a variety of treatments but in Indian traditional medicine dried mature root is mostly used for medicinal purpose. It contains various chemical constituent like alkaloids, flavonoids, naphthoquinones, glycoside, saponins, steroids, tri-terpenoids, coumarins and phenolic compounds. Many studies showed that plumbagin can be used in combination with existing anticancer drugs, which help to treat chemotherapy-resistant patients. The literature reveals that the root and root bark have wider application in the traditional system of medicines against various diseases as an anti-inflammatory, antimicrobial etc.

2. MATERIAL AND METHODS

The literature of Pz was obtained from online databases including Pub Med, Google Scholar, Scopus, Web of Science and Science Direct. A library search was also conducted from classical Textbooks, PhD Theses, and published Books. The keywords used for the search were Plumbago zeylanica, Shetraj, Ceylon Lead Wort, Chitrak, Doctorbush. Scientific name and synonyms were validated through the Plant list (www.theplantlist.org).

3. VERNACULAR NAME

Arabic: Sheetraj, Shitraj; English: Lead Wort, Ceylon Lead Wort, White Flowered Lead Wort, White Lead Wort; Persian: Shitrah, Shitrak; Sanskrit: Agnimata, Chitraka; Ayurveda: Chitraka, Agni, Vahni, Jvalanaakhya, Krshaanu, Hutaasha, Dahana, Sikh; Unani: labediyyoon, Sheetraj Hindi

4. BOTANY

The family Plumbaginaceae consists of 10 genera and 280 species. There are three species in the genus Plumbago, namely Plumbago indica L/ P. Rosea L., P. capensis L., and P. zeylanica L, which are spread across India. In India, Pz grows as a wild species in various parts but is also cultivated because of its large therapeutic applications. There is no consensus in the literature citing Pz as being listed as herb or shrub. Its roots are 30 cm or more in length, 6 mm or more in diameter, slightly branched with very fewer secondary roots, having a smooth and unbroken texture, light yellow colour when fresh and reddish to pale brown when dry. Dried roots are uniform, cylindrical in shape, smooth surface and woody hard with very strong having a bitter taste and a distinct odour with acrid. Stems are somewhat woody, spreading, terete, striate, globous. It is around 0.5–2 m (1.6–6.6 ft) tall. The bark is thin and brown. Leaves are dark green and are simple, elliptical with hairy margins along with alternate positioning on the stem with the gap of up to 3 inches and thickness of 1.5 inches. Petioles are thin and with an approximate length of 0.5 mm and native stipules are present. The inflorescence is about 6–30 cm long and many-flowered terminal raceme-type. Flowers are Bisexual, white in colour, 10–25 cm long, with a diameter of 1/2 to 3/4 inch. Flowers are borne in spikes, whereas the rachis of the spike is pubescent or glandular. Flowers also have a tubular calyx (7–11 mm long and 5-ribbed) with glandular trichomes which secrete a sticky mucilage. Corolla is white, very slender, and tubular and stamens are 5, free. Ovary superior, 5-gonous, one-celled, ovule one basal. The plant flowers round the year and pollination are primarily by insects. The mucilaginous glands aid in trapping insects and fruit dispersal by animals. Fruits are oblong (7.5–8 mm long) five-furrowed capsule containing a single seed. Each seed is oblong in structure, 5–6 mm long and reddish-brown to dark brown and its capsules are enclosed by persistent viscid calyx. Panda et al., 2015 examine the patterns and levels of morphological and genetic variability within/among populations and ascertain whether these variations are dependent on geographical conditions; and to evaluate genetic differentiation and population structure within the species.

Plumbago zeylanica flower

Source: http://ayurveda.alandiashram.org/ayurvedic-herbs/chitrak-plumbago-zeylanica
5. TRADITIONAL USES

In the traditional system of medicine, Pz possesses Abortifacient; Aperient; Antipyretic; Antiseptic; Aphrodisiac; Astringent; Carminative; Choleretic; Diaphoretic; Digestive; Diuretic; Emollient; Expectorant; Laxative; Stimulant and Tonic actions. Its root decoction mixed with roots of Moringa borziana, has been used to treat Gonorrhoea. In Iwo, Ibadan and Southwest Nigeria, infusion of Pz root along with other ingredients are used to treat Lung and breast cancer. In Ethiopia, the root has Crushed, squeezed and applied to skin lesions to treat anthrax while the powdered roots or leaves are used to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, swellings and wounds. In Nigeria, the roots are used to treat rheumatic swelling, scabies and ulcers. In leprosy and other obstinate skin diseases, root paste prepared with milk or vinegar or salt and water is used externally. Its cold infusion is used to treat influenza and black-water fever. Experimentally, plumbagin which is a naphthoquinone derivative, prevent the accumulation of triglycerides in liver and aorta. Intake of 10 gm of roots powder of Pz with honey ensures quick delivery.

6. CHEMICAL CONSTITUENT

Pz comprises a range of essential chemical compounds. The GC–MS analysis of Pz revealed the presence of 40 compounds that could contribute to the medicinal property of the plant. Different parts of the plant possess alkaloids, carbohydrates, triterpenoids, flavonoids, gums, mucilage, protein, fatty acids, saponin, plumbagin, plumbagic acid, chitanone, plumbaginol, dihydroflavinol, campesterol, stigmasterol, sitosterol, glucopyranoside, sitosterol, naphthoquinones, glycosides, steroids, tannins, phenolic compounds and coumarins.

Of all the chemical constituents, plumbagin is the principal active compound. Plumbagin (5-hydroxy-2-methyl-1, 4-naphthoquinone-C11H8O3) is primarily present in roots in higher amounts with only about 1% in the whole plant. All parts of the plant are used but roots are considered as the
most active, prominent and effective herbal drug. Its Roots contains Naphthoquinone like 2-hydroxy-1,4-naphthoquinone (kwson) (5,7-dihydroxy-8-methoxy-2-methyl-1,4-
naphthoquinone (plumbagin), Biplumbagin (Chitranon), Chloroplumbagin, Maritifone, Elliptinone, Lapachol, Plumbic acid, enzymes like invertase and protease along with other compounds such as plumbazeylanone, drosone, isozeylinone, fructose, glucose, zeylinone, Coumarins like 5-

methoxyxoselin, seselin, suberosin, xanthyletin, xanthoxylin, oleic acid (19.95%), b-asarone (14.08%),
naphtho(2,3-β)furano-2(3H)-one (7.68%), ethyl p-

methoxycinnamate (4.58%), and n-hexadecanoic acid (2.18%).

7. PHARMACOLOGICAL ACTIVITY

Pz used for centuries to treat a wide variety of diseases has shown great potential as a safe and effective multifunctional medicinal agent. Many parts of the plant are used but there are considerable pharmacological properties in the roots. The powdered bark, root or leaves are used to treat gonorrhea, syphilis, tuberculosis, rheumatic pain, wound healing, plies, skin diseases and also reported to have antibacterial, antifungal and cantharides properties. Besides its traditional uses, several recent reports have demonstrated hepatoprotective, immunomodulatory, antitumor anti-inflammatory, anti-diabetic memory inducing, lipid metabolism, anti-malarial allergic and modulatory, anti-fertility, anti-bacterial, anti-viral, anti-cancer, antioxidant and larvicidal activity.

7.1. Anti-inflammatory activity

Plumbago species are one of the most important medicinal plants which are used for anti-inflammatory activity. Oyedapo, 1996 investigated the phosphate-buffered saline extract of the roots of Pz for anti-inflammatory activity. The extract stabilized red blood cells subjected to both heat and hypotonic induced lyses. The extract exhibited a biphasic response and the enzymatic activities of both alkaline and acid phosphatases were reduced. Another study showed that Pz reduces oedema thus conforting the body part, and also prevent graft versus host disease. Sheela et al, 2010 carried out the anti-inflammatory activities of various leaf extracts of Pz using in vivo experimental models at two dose levels (200 and 400 mg/kg, p.o.). The acetone extract (p < 0.01) significantly decreased inflammation in rats induced by carrageenan compared to the control group. Reducing the anti-inflammatory process by extracting petroleum ether, acetone extract, and fractions F2 and F3, obtained during 3–4 h, is possibly linked to a reduction in prostaglandin synthesis and release, rather than preformed inflammatory agents. In another study, the methanolic extracts of the root of Pz at 300 and 500 mg/kg produced 31.03 and 60.3% inhibition of acute inflammation, respectively, in Carrageenin induced raw paw oedema. Another study reported that Pz reduces oedema significantly compared to aspirin. V. el al, 2014 investigated the Anti-inflammatory activity of hydroalcoholic extract of Pz root bark through in-vitro Human Red Blood Cell Membrane protective activity, and in-vivo through Carrageenan induced rat paw oedema and Complete Freund's Adjuvant induced chronic inflammatory model in rat. In both acute and chronic model of inflammation hydroalcoholic extract of root bark of Pz showed moderate anti-inflammatory at the dosage of 250 mg/kg bw comparable with standard Indomethacin and diclofenac.

The mechanism of anti-inflammatory activity of hydroalcoholic extract Pz might be due to prostaglandins, lysosomes and proteases. Carrageenan injection in the rat paw induces the release of bradykinin which results in the development of paw oedema in the rat. The first step is due to histamine and serotonin release lasting one hour. The second step is triggered by the release of prostaglandins, lysosomes and proteases. Carrageenan injection in the rat paw induces the release of bradykinin which results in the development of inflammation in adjuvant arthritis rats. The development of inflammation in adjuvant arthritis rats

Another research conducted to determine the anti-inflammatory potential in vivo and in vitro of selected medicinal plants used in traditional Indian medicine. The sequentially extracted plant samples including Pz in water, ethanol and hexane were evaluated in-vitro for COX-1 and 2 inhibitory and antioxidants activities. The in vivo anti-inflammatory activity was assessed using carrageen and Phorbol Myristate Acetate induced mice oedema. The results reveal that Pz inhibits COX-2 (mean activity 42.86%) as compared to COX-1 (mean activity 25.91 %). PZ-E-6, a freeze-dried ethyl acetate fraction, purified from the roots of Pz, was efficacious in preventing joint inflammation when treatment was started before the onset of joint inflammation. PZ-E-6 also substantially suppressed arthritis by reducing paw volume, clinical score and delayed-type hypersensitivity reaction. Moreover, 20 mg/Kg of PZE-6 was found to inhibit the development of inflammation in adjuvant-induced arthritis rats. The dichloromethane extract of Pz showed a significant (p<0.05) dose-dependent protection against carrageen-induced paw oedema. At 500 mg/kg Pz was comparable to diclofenac (25 mg/kg) in the inhibition of paw oedema. The effect of DMEPZ may be attributed to its free radical scavenger activity and protection of apoptosis. Another study, Plumbagin prominently hampered the development of inflammatory cascade, as nuclear factor κB (NF-κB), tumour necrosis factor-alpha (TNF-α) and myeloperoxidase (MPO) activity.

7.2. Anti-microbial activity

The emergence and prevalence of drug-resistant pathogenic microorganisms have led to a decline in the efficacy of traditional antimicrobial therapy. Thus, treatment of infections is increasingly becoming more difficult. To address this emerging issue, novel therapies must be developed to spare existing broad-spectrum antimicrobials including antibiotics. In vivo study was carried out by Abdul and Ramchend, 1995; in this study, the bactericidal capacity of plumbagin-treated mouse macrophages was estimated against Staphylococcus aureus. The result indicates that the macrophage bactericidal activity increases with low concentration plumbagin by potentiating the oxynradical release whereas it has an inhibitory effect at the higher concentration.
In another study, Pz (root) was prepared in extracts of three different forms, namely hexane, alcoholic and aqueous extracts and tested against Staphylococcus aureus, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Salmonella typhimurium and Proteus Vulgaris. The result possesses that Pz produced outstanding antimicrobial effect with inhibition zone greater than 20 mm and alcoholic extracts exhibited the highest degree of antimicrobial activity as compared to aqueous and hexane extracts fractions 46. The maximum activity of leaves of P. zeylanica were almost equally sensitive to these plant extracts. The hexane extract of leaves of P. zeylanica demonstrated relatively high activity as compared to other species.

Lew et al., 2002 47 studied the antibacterial activity of polar and non-polar extracts of roots Pz by using hole plate diffusion method against some pneumonia-causing pathogens. The result showed that the aqueous extract did not exhibit any activity while petroleum ether extract was found to have strong antibacterial effects as compared to the ethanol extract. The synergistic activity of Pz-based antimycobacterial constituents was assessed against four atypical species, namely Mycobacterium intracellulare, M. smegmatis, M. xenopoi, and M chelonai, in combination with isonicotinic acid hydrazide (INH). The findings showed that the plumagbin MIC values were reduced from 1.25-2.5 to 0.15-0.3 μg/ml due to collaboration with INH. When tested against the resistant strain of M. tuberculois H37Rv, plumagbin and 7β-hydroxyabieta-8,13-dien-11,12-dione exhibited inhibitory activity at <12.5 μg/mL, while others were inactive at this concentration 48. By using agar diffusion and lysis plates ethyl the ethanol, ethyl acetate and acetone extracts of Pz showed higher anti-H. pylori activity except for water extract 49. Aqil et al., 2006 50 studied the antimyelin-resistant Staphylococcus aureus (MRSA) activity of ethanolic extracts of four medicinal plants including Pz (root). The result shows the ethyl acetate, acetone and methanol fractions of Pz demonstrated antibacterial activity and inhibition zone size ranged from 11 to 44 mm and minimum inhibitory concentration (MIC) varied from 0.32 to 3.25 mg/ml. The potency of these fractions based on the zone of inhibition and MIC value was relatively higher in ethyl acetate fraction of Pz. 51 screened ethanolic extracts of 66 plants against nine different bacteria. The highest activity against all groups of bacteria was found in Pz. Also, these extracts showed synergistic interaction with tetracycline, chloramphenicol and ciprofloxacin against S. aureus and/or E. coli. Six plants, Pz, H. indicus, A. calamus, P. granatum, H. antidysenterica and D. regia, were further subjected to fractionation-based study. Gram-positive and Gram-negative MDR bacteria are almost equally sensitive to these extracts/fractions, indicating their broad spectrum nature. Ahmad and Aqil, 2007 52 studied in-vitro efficacy of alcoholic crude extracts of 15 medicinal plants against extended-spectrum β-lactamases (ESBL) producing multidrug-resistant enteric bacteria. The result showed that Pz demonstrated relatively high activity as compared to other plant extracts.

In another research, an in vitro antimicrobial activity of plumagbin was done by (Jayachandran et al., 2009. The result indicates Plumagbin (20 μg/disc) showed good activity against Escherichia coli (25.6±0.56 mm), Salmonella typhi (24.3±0.23 mm), Staphylococcus aureus (21.6±0.55 mm), Klebsiella pneumoniae (19.8±0.76 mm), Serratia marcescens (17.6±0.65 mm) and Bacillus subtilis (14.5±0.76 mm). Proteus vulgaris (10.1±0.14 mm) and Pseudomonas aeruginosa (9.6±0.67 mm) displayed mild aggression against the other species. In this study also Methanol, chloroform and aqueous extracts of Pz root were tested against various gram-negative and gram-positive bacteria. The result shows that Pz’s chloroform root extracts displayed strong activity against Escherichia coli (16.7±0.14 mm), Salmonella typhi (14.3±0.04 mm), and Staphylococcus aureus (12.0±0.54 mm). The inhibition of Klebsiella pneumoniae (9.2±0.73 mm), Serratia marcescens (8.6±0.07 mm), and Bacillus subtilis (8.0±0.61 mm) was moderate and poor against Proteus vulgaris (5.9±0.55 mm) and Pseudomonas aeruginosa (4.8±0.87 mm) 52. Another study, the different alcoholic solvents of the root of the Pz were tested against the three human pathogenic bacteria viz., Escherichia coli, Salmonella typhi and Staphylococcus aureus by adapting the disc diffusion method. The result showed that all of the extracts had a varied degree of antimicrobial activity against the pathogens tested. However, the acetone extracts at 100% concentrations exhibited higher inhibition zone (27 mm) against the bacteria, Salmonella typhi 53.

Jetty et al., 2010 54 isolated, separated and evaluated the antimicrobial properties of compounds such as neoisoshinanolone and 1-epineo-isoshinanolone from the roots of Pz. 1-epineo-isoshinanolone is more active with a MIC of 12.5 to 25 μg/ml, whereas neoisoshinanolone has recorded a MIC of 50 to 100 μg/ml. The activities are compared with plumbagin (0.78 to 3.13 μg/ml) and standards streptomycin for bacteria and nystatin for fungi. The root extract of Pz possesses good antimicrobial activity. In another study, methanolic extract of Pz at 50 and 100 mg/ml concentrations showed marked inhibitory effect against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi with MIC values 0.3125, 0.3125, 2.5 and 0.625 μg/ml respectively 55. In an in vitro study, petroleum ether, chloroform and alcoholic extract of leaves of Pz were evaluated on microbial strains like gram-positive species Staphylococcus aureus, and Bacillus subtilis and gram-negative species Escherichia coli and Pseudomonas aeruginosa by agar disc diffusion method. The result possesses the alcoholic extract of leaves of Pz shows maximum antimicrobial activity against all microbial strain 56.

Similarly, Petroleum ether, ethanol and aqueous extract of leaves and stems of Pz investigated against bacteria by paper disc method. The result suggests that the maximum activity was observed in ethanol extract against Micrococcus luteus (12mm) and minimum activity was observed in petroleum ether extract against Staphylococcus aureus and Micrococcus luteus at inhibition range was observed (7mm) 57. Another in-vitro antimicrobial evaluation was carried out by using chloroform, acetone and ethanol extract of same species of plant Pz collected from two different Eastern Himalayan regions by agar disc diffusion method and it was performed on gram-negative bacteria Salmonella typhi and Pseudomonas aeruginosa and gram-positive bacteria Bacillus subtilis and Staphylococcus aureus. The result possesses that the ethanolic extracts of both region plants showed significant antibacterial activity against all the organisms, while chloroform and acetone fractions showed moderate activity 58. Patwardhan et al., 2015 59 studies showed that the root extracts cured plasmid-encoded antibiotic resistances from the clinical isolates and reference strains at curing efficiencies of 4 to 100%. Petroleum ether root extract of Pz demonstrated higher plasmid curing activity than the known plasmid curing agents like ethidium bromide or acridine orange. A study carried out by (Shweta and Dubey, 2015 60). In this study aqueous, methanol extract, and acetone extract of Pz leaves were tested against various bacteria (gram +Ve, & gram –Ve bacteria) and fungus. The result shows that the aqueous extract of Pz is more effective in all micro-organism as compared to methanol extract and acetone extract. Antimicrobial evaluation of crude extract of Pz against S. aureus ATCC, MRSA, S. pneumoniae ATCC, S. pneumoniae (clinical) (gram positive bacteria), K. pneumoniae (clinical), S. boydii ATCC, E. coli ATCC, E. coli
(clinical) (gram negative bacteria) and C. albicans (clinical) fungus were showing a higher antimicrobial activity 61. A study carried out to screen the antibacterial potentials of plant extract Pz extract was prepared in methanol, hexane and chloroform by Soxhlet extractor and tested for its antibacterial activity against clinical pathogens i.e. Enterococcus, E. coli, Klebsiella pneumonia, Lactobacillus acidophilus and Staphylococcus aureus by well diffusion method. Results revealed that plant extracts possess potential antibacterial activity against the tested organism but methanol extract showed more inhibitory effect than the other plant extracts 62. Another study carried out to determine the antimicrobial activities of methanolic extracts of the stem and the leaves of Pz by using the agar well diffusion method against six bacterial species and nine fungal species. The result reveals that Pz leaf extract demonstrated maximum antimicrobial activity against both Staphylococcus aureus and Fusarium oxysporum. The stem extract was found to be more antimicrobial against the Pseudomonas aeruginosa and the Penicillium expansum species 63. An in vitro research, plumbagin was investigated against methicillin-resistant Staphylococcus aureus (MRSA) for anti-microbial activity. Approximately 100 MRSA isolates which included multidrug-resistant phenotypes displayed consistent behaviour with a narrow minimum inhibitory concentration (MIC) range of 4–8 μg/ml. The time-kill study revealed a 99% kill of a reference MRSA strain, 8 h after exposure to plumbagin 64. A study carried out to investigate the anti-fungal activity of Pz against four pathogenic fungal species Fusarium oxysporum, Rhizoctonia solani, Alternaria sp. and Sclerotium rolfsii. The excellent inhibitory activities were observed against Alternaria spp. (98%), F. oxysporum (68%), R. solani (97.5%) followed by S. rolfsii (93.4%) at 62.5 μg/ml 5.

8. CONCLUSION

Medicinal plants are the primary source of effective herbal medicines for treating various diseases. Herbal products have been used over the past decade because of their numerous pharmacological actions. Pz has been used in Ayurvedic and Unani medicine for centuries to improve longevity and vitality. It is the most significant medicinal plant used widely in herbal formulations. It is chemically rich with its diverse content of active compounds, such as plumbazin, dihydrozlanone, zeylanone and many useful naphthoquinone constituents as a multipurpose medicinal agent were present. Pz is one of the important medicinal plants which have several pharmacological properties such as anticancer activity, antimicrobial activity, antioxidant activity, anti-inflammatory activity etc. This also gives the opportunities to the researchers in this area for potential research and development. This is an effort to collect and record information on Pz’s anti-inflammatory and antimicrobial activity and to highlight the needs for research and development.

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Conflict of interest

There is no conflict of interest to declare.

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