**Eleusine indica** L. possesses antioxidant activity and precludes carbon tetrachloride (CCl₄)-mediated oxidative hepatic damage in rats

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**Abstract**

Objectives The purpose of this study was to evaluate the ability of aqueous extract of *Eleusine indica* to protect against carbon tetrachloride (CCl₄)-induced hepatic injury in rats.

Methods The antioxidant activity of *E. indica* was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The total phenolic content of *E. indica* was also determined. Biochemical parameters [e.g. alanine aminotransferase (ALT), aspartate aminotransferase (AST), malondialdehyde (MDA), glutathione (GSH), catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase and quinone reductase] were used to evaluate hepatic damage in animals pretreated with *E. indica* and intoxicated with CCl₄. CCl₄-mediated hepatic damage was also evaluated by histopathologically.

Results *E. indica* extract was able to reduce the stable DPPH level in a dose-dependent manner. The half maximal inhibitory concentration (IC₅₀) value was 2350 µg/ml. Total phenolic content was found to be 14.9 ± 0.002 mg/g total phenolic expressed as gallic acid equivalent per gram of extract. Groups pretreated with *E. indica* showed significantly increased activity of antioxidant enzymes compared to the CCl₄-intoxicated group (*p* < 0.05). The increased levels of serum ALT and AST were significantly prevented by *E. indica* pretreatment (*p* < 0.05). The extent of MDA formation due to lipid peroxidation was significantly reduced (*p* < 0.05), and reduced GSH was significantly increased in a dose-dependently manner (*p* < 0.05) in the *E. indica*-pretreated groups as compared to the CCl₄-intoxicated group. The protective effect of *E. indica* was further evident through decreased histopathological alterations in the liver.

Conclusion The results of our study indicate that the hepatoprotective effects of *E. indica* might be ascribable to its antioxidant and free radical scavenging property.

**Keywords**  *E. indica* · Antioxidant activity · Hepatoprotective effects · Oxidative stress · Carbon tetrachloride

**Introduction**

The liver is considered to be one of the most vital organs, functioning as a centre of nutrient metabolism, such as carbohydrates, proteins and lipids, and waste metabolite excretion. It also handles the metabolism and excretion of drugs and other xenobiotics from the body, thereby providing protection against foreign substances by detoxifying and eliminating them. The bile secreted by the liver has, among other functions, an important role in digestion. Liver cell injury caused by various toxicants, such as carbon tetrachloride (CCl₄), thioacetamide, chronic alcohol consumption and microbes, is well-studied [1].

Oxidative stress is defined as elevated levels of free radicals or other reactive oxygen species (ROS) which can elicit either direct or indirect damage to the body [2]. The generation and subsequent involvement of free radicals in a large number of diseases, such as myocardial ischemia, carcinogenesis, liver damage, inflammatory diseases, cataract formation and Alzheimer’s disease, are recognized [3–5]. Under normal circumstances, ROS are efficiently kept in check by the body’s complex antioxidant defence system, and there is an equilibrium between ROS formation
and degradation. However, an overproduction of ROS and/or inadequate antioxidant defence disturbs this equilibrium in the favour of a ROS upsurge that results in oxidative stress. A deficiency in the body’s natural antioxidant defence mechanisms has been implicated as the etiological or pathological factor in several clinical disorders. The onset or progression of these disorders can therefore be held in check or delayed by supplementation therapy with antioxidants. This has led to scientific research in the field focusing on identifying safe and effective antioxidant compounds. Plant extracts and plant-derived antioxidant compounds potentiate the body’s antioxidant defence or act as antioxidants. Such natural products are the antioxidants of choice because of their better safety profile in comparison to synthetic counterparts. The World Health Organization (WHO) has estimated that more than 75% of the world’s total population are dependent on herbal drugs for their primary healthcare needs. Therefore, there is a major research emphasis on discovering plants that protect against various kinds of injuries or diseases with antioxidant potential that may be used for human consumption [6].

CCl4 intoxication in the rat is an experimental model widely used to study necrosis and steatosis of the liver. The toxic signs of CCl4 in isolated rat hepatocytes have been described by several researchers who have found a suitable correlation with induced cell injury in vivo [7]. Liver injury induced by CCl4 is the most intensively studied system of xenobiotic-induced oxidative hepatotoxicity. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) serum enzyme activities serve as parameters to demonstrate the extent of hepatotoxicity in the rats. Furthermore, increases in the level of lipid peroxidation and the breakdown of the glutathione (GSH)-dependent antioxidant defence system can be seen along with the liver damage induced by CCl4 [8]. The antioxidant property is possibly one of the mechanisms by which plant native substances confer their hepatoprotective effect against the hepatotoxicity caused by CCl4 [9].

Eleusine indica or Wiregrass (grass family Poaceae), also known as Sohinatad by the local people in the area of Tambunan, Sabah, Malaysia, is a native plant of the tropics and subtropical regions and an invasive species. The whole plant, especially the root, is used in traditional medicine as a diuretic, anti-helminthic, diaphoretic and febrifuge and for treating cough and other ailments. The decoctions of the boiled plant are consumed as anti-helminthic and febrifuge treatments [10]. The seed is sometimes used as famine food and also used in the treatment of liver complaints. Many herbal products, such as one studied in this article, have traditional uses that are now being investigated to create an evidence base that will facilitate their inclusion in general medical practice. Studies have also found that C-glycosylflavones from E. indica have anti-inflammatory effects on lipopolysaccharide-induced lung airway inflammation in mice. The infusion of aerial parts of E. indica is used in Brazil against airway inflammatory processes, such as influenza and pneumonia [11]. Plants produce a higher number of naturally occurring secondary metabolites, many of them with unique pharmacologic activities. These metabolites include flavonoids, phenols, phenolic glycosides, saponins, cyanogenic glycosides, unsaturated lactones and glucosinolates [1, 2, 6–14]. To date, only a few studies on E. indica have been reported, including a study on the phytochemical content of its sterol glucoside forms [12] and the anti-inflammatory activity of its C-glycosylflavone [11]. However, the hepatoprotective activity of E. indica and its mechanism of action have not yet been investigated. Therefore, the aim of this study was to evaluate the hepatoprotective effects of E. indica and its mechanism of action. Since E. indica has been shown to inhibit various kinds of injuries and neoplasm, particularly those mediated through the generation of ROS, it is possible that the pretreatment of animals with E. indica may suppress CCl4-mediated oxidative damage. We report herein the in vivo protective effects of E. indica against CCl4-induced oxidative hepatic damage in rats.

Materials and methods

Chemicals

Tris HCl, thiobarbituric acid (TBA), oxidized glutathione, reduced GSH, β-nicotinamide adenine dinucleotide phosphate reduced (NADPH), Folin–Ciocalteu reagent (FCR), 2,2-diphenyl-2-picrylhydrazyl (DPPH), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione reductase, 5,5′-dithio-bis-2-nitrobenzoic acid, sulfosalicylic acid, bovine serum albumin (BSA), hydrogen peroxide (H2O2), flavin adenine dinucleotide, 2,6-dichloroindophenol, trichloroacetic acid, Tween 20, sodium hydroxide (NaOH), sodium carbonate (Na2CO3), sodium chloride (NaCl), ethylenediamine tetraacetic acid and sodium azide (NaNO3) were purchased from Sigma–Aldrich (St. Louis, MO). All other solvents and chemicals used were either of analytical grade or of the highest purity commercially available.

Collection and extraction of E. indica

Fresh E. indica plants were obtained from the local market at Gaya Street, Kota Kinabalu, Sabah, Malaysia. The plants were identified to the species level by Mr. J. Gisil of the Institute of Tropical Biology Conservation, Universiti Malaysia Sabah, Malaysia based on the morphological characteristics of the plants. The samples were washed thoroughly with tap water, air dried at room temperature.
for 2 weeks and then transferred to an oven and kept at 35°C for 3 days. The dried samples were then ground into coarse powder form using a heavy duty blender and the powdered samples boiled in distilled water with stirring on a hot plate for 10 min. The ratio of sample to the amount of distilled water was 1:10. Each of decoctions were removed from the heat and cooled at room temperature for 1 h, and the aqueous extract was then filtered using a tea strainer into a flask to remove coarse residues. The filtrate was filtered once again using Whatman No. 1 filter paper (Whatman, Maidstone, Kent, UK). The pure filtrate was frozen at −80°C and freeze-dried. The products obtained were kept at −20°C until further analysis.

Animals

Adult Sprague–Dawley male rats (8–12 weeks old) weighing 150–200 g were raised from infants through natural breeding in the animal house facility of the Biotechnology Research Institute, University Malaysia Sabah, Malaysia. All animals were treated in a humane manner and well maintained under standard ethical principles according to university regulations and federal laws governing experiments on animals. All animals were acclimatized for 1 week before the onset of the experiment in plastic (polypropylene) cages with paddy husk bedding at room temperature (25 ± 1°C) and 50 ± 5% humidity. The animals were allowed free access to water and chow diet until the start of the experiment.

Determination of total phenolic content

The total phenolic content of E. indica was determined according to the method of Velioglu et al. [13] using gallic acid as the standard. Six different concentrations of gallic acid were prepared (10, 20, 40, 80, 100, 200 μg/ml) in triplicate from a 1 mg/ml stock solution. A 1.5-ml aliquot of FCR was then added to each tube; the solution was then mixed and left in dark at room temperature for 5 min. Subsequently, 1.5 ml of Na2CO3 solution was added to each tube; the solution was mixed and left in dark at room temperature for 90 min. After 90 min, the absorbance of the solutions in each tube was measured at 725 nm on a spectrophotometer. A graph of absorbance against concentration was plotted as the standard. A 1 mg/ml stock solution of plant samples was prepared. Triplicates of plant samples were then prepared with 200 μl stock solution, and 1.5 ml FCR was added to each tube; the solution was mixed and left in the dark at room temperature for 5 min. Thereafter, 1.5 ml of Na2CO3 solution was added to each tube; the solution was then mixed and left in dark at room temperature for 90 min. The average absorbance (725 nm) of each plant sample was compared to the standard graph of gallic acid to determine total phenolic content of the plant sample.

Determination of antioxidant activity (DPPH test)

The antioxidant activity was determined according to the method used by Hatano et al. [14]. A 5 mg/ml stock solution of plant sample was prepared and distributed into eight different concentrations (10, 25, 75, 150, 300, 600, 1200, 2400 μg/ml) in triplicate by adding up the volume in each tube to 300 μl with distilled water. DPPH solution (2.7 ml) was then added to each tube, and the tube vigorously shaken using a vortex mixer and left in dark for 60 min. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm using a spectrophotometer. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: %RSA = [(Acontrol × Asample)/Acontrol] × 100, where Acontrol is the absorbance of the solution without the extract and Asample is the absorbance of the solution containing extract with different concentration. The extract concentration giving 50% inhibition (IC50) was calculated from the graph of RSA percentage against extract concentration.

Experimental protocol

To study the effect of E. indica on hepatic oxidative stress and antioxidant enzymes and for the histopathological studies, we divided 16 adult Sprague–Dawley male rats age (8–12 weeks old; weight 150–200 g) randomly into four treatment groups of four animals each: (1) Group 1, received saline; (2) Group 2, received CCl4 [1.2 ml/kg body weight (b.w.), oral dose (p.o.))] on days 13 and 14; (3) Group 3, received E. indica (150 mg/kg b.w. p.o.) for 14 days + CCl4 (1.2 ml/kg b.w. p.o.) on days 13 and 14; (4) Group 4, received E. indica (300 mg/kg b.w. p.o) for 14 days + CCl4 (1.2 ml/kg b.w. p.o.) on days 13 and 14.

All animals were killed 24 h after the last dose of plant extract or saline within a period of 1 h. The blood and liver of these animals were taken immediately. Blood was centrifuged at 2000 g to obtain serum, whereas livers were cleaned free of extraneous material and perfused immediately with ice cold saline (0.85% NaCl, w/v) and kept at −80°C until further biochemical, haematological and histopathological investigations to assess disturbances in liver functioning.

Preparation of post-mitochondrial supernatant

A modified version of the standard procedure of Mohandas et al. [15] was adopted for the preparation of tissue
fractions for all biochemical estimations. Livers were quickly removed, cleaned free of extraneous material, perfused immediately with ice-cold saline (0.85% NaCl, w/v) and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17% w/v) at the ratio of 1 g in 10 ml buffer (Polytron PT 1200E homogenizer; Kinematica AG, Lucerne, Switzerland). The homogenate was centrifuged at 2000 \( g \) for 10 min at 4°C in a refrigerated centrifuge (model Avanti J-E; Beckman Coulter, Fullerton, CA) to separate the nuclear debris. The aliquot so obtained was centrifuged at 10 000 \( g \) for 30 min at 4°C to obtained the post-mitochondrial supernatant (PMS) that was used as the source of enzymes and also to determine malondialdehyde (MDA) and reduced GSH content.

Biochemical assays

Reduced GSH in the liver was determined by the method of Jollow et al. [16]. Hepatic lipid peroxidation in the PMS was performed following the method of Buege and Aust [17], as described by Iqbal et al. [18], by measuring the rate of production of thiobarbituric acid reactive substances (TBARS; expressed as MDA equivalents). Glutathione peroxidase (GPX) activity was measured according to the procedure of Mohandas et al. [15], as described by Iqbal et al. [19]. Glutathione reductase (GR) activity was determined by the method of Carlberg and Mannervik [20], as described by Iqbal et al. [19]. Catalase (CAT) activity was determined by the method of Claiborne [21], as described by Iqbal et al. [19]. Glutathione-S-transferase (GST) activity was determined by the method of Benson et al. [24], as modified by Iqbal et al. [18]. Serum ALT and AST were determined by the method of Reitman and Frankel [25].

Determination of protein

Protein concentration in all samples was determined according to the method of Aitken et al. [26] using BSA (1 mg/ml) as a standard.

Histopathological assessment

For the histopathological studies, we excised the mid-sections (thickness: a few millimetres) of the livers from all animals and processed these for light microscopy studies to substantiate the biochemical findings and to ascertain the cause of hepatic cell death. The process involving fixing tissue specimens in 10% neutral buffered formalin solution, preparing the blocks in paraffin, cutting sections 5–6 \( \mu m \) in thickness and staining the sections with haematoxylin and eosin stain (H&E). The sections were scanned and analysed by an expert pathologist who was not aware of sample assignment to experimental groups for the pathological symptoms of hepatotoxicity.

Statistical analysis

The statistical analysis was carried out using the SPSS ver. 17.0 windows statistical package (SPSS, Chicago, IL). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnet’s multiple comparisons test. All data points are presented as the treatment group mean ± standard error of the mean (SEM). \( p \) values < 0.05 were regarded as significant.

Results

Total phenolic content

Total phenolic content of the aqueous extract of \textit{E. indica} was found to be 14.9 ± 0.002 mg/g total phenolic expressed as gallic acid equivalent per gram of extract (GAE; mg/g of extract).

Effect of \textit{E. indica} on DPPH radical scavenging

\textit{Eleusine indica} extract was able to reduce the stable DPPH in a dose-dependent manner. As shown in Table 1, a dose-dependent response was observed for the DPPH RSA of \textit{E. indica} extract; the half maximal effective concentration (IC50) was found to be 2350 \( \mu g/ml \).

Effect of \textit{E. indica} on \textit{CCl4}-induced hepatotoxicity

The effects of pretreating rats with \textit{E. indica} extract on the \textit{CCl4}-induced elevation of serum ALT and AST are shown in Fig. 1. Treatment with \textit{CCl4} increased ALT and AST levels compared with those of the saline-treated control (\( p < 0.05 \)). In contrast, the group of animals pretreated with \textit{E. indica} at a dose level of 150 and 300 mg/kg b.w. suppressed the elevated levels of ALT and AST (\( p < 0.05 \)) in a dose-dependent manner.

Effect of \textit{E. indica} on lipid peroxidation

The protective effect of \textit{E. indica} on \textit{CCl4}-induced lipid peroxidation is shown in Fig. 2. Treatment with \textit{CCl4} substantially induced lipid peroxidation in rat hepatic tissues, as monitored by TBARS formation. However, pretreatment of rats with the \textit{E. indica} extract significantly
inhibited CCl₄-induced lipid peroxidation in a dose-dependent manner ($p < 0.05$). The percentage of control exerted by E. indica against CCl₄-treated control was 117 and 134% at a dose of 150 and 300 mg/kg b.w., respectively.

**Effect of E. indica on GSH**

There was a remarkable depletion of hepatic GSH after CCl₄ administration. However, relative to the CCl₄-treated group, the E. indica pretreatment resulted in a significant protection against this CCl₄-induced depletion of GSH in a dose-dependent manner ($p < 0.05$), as shown in Fig. 3.

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### Table 1

| E. indica concentration (µg/ml) | Percentage of DPPH free radical scavenging |
|-------------------------------|------------------------------------------|
| 10                            | 1.03 ± 0.01                              |
| 25                            | 10.34 ± 0.02                             |
| 75                            | 13.00 ± 0.01                             |
| 150                           | 16.40 ± 0.01                             |
| 300                           | 20.24 ± 0.02                             |
| 600                           | 25.70 ± 0.02                             |
| 1200                          | 35.75 ± 0.01                             |
| 2400                          | 51.55 ± 0.01                             |

DPPH, 2,2-Diphenyl-2-picrylhydrazyl  
All values are given as the mean ± standard error (SE) of triplicate tubes.  
Experimental conditions are described in the Materials and methods.
the biochemical studies and to demonstrate hepatic cell death caused by CCl₄ during intoxication and the inhibitory effect of *E. indica* on this type of cellular injury. Histological examination of the liver sections of control animals revealed the presence of normal hepatocytes with well-preserved cytoplasm, a prominent nucleus and distinct sinusoidal spaces. In contrast, the liver sections of CCl₄-intoxicated rats contained extensive liver injuries, characterized by moderate to severe hepatocellular degeneration, necrosis and the loss of cellular boundaries caused by the inflammation of hepatocytes and sinusoidal dilatation. However, these histopathological hepatic lesions were markedly improved by the pretreatment of rats with *E. indica* in a dose-dependent manner, as shown in Fig. 4.

**Discussion**

Phenolic compounds are considered to be a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [27–31]. The free radical scavenging effect of plant extracts is not limited to phenolic compounds but also derives from the presence of various other antioxidant secondary metabolites which directly or indirectly contribute to the free RSA activity of the specific extract [32]. In this respect, our study of *E. indica* has demonstrated that such multi-biological activities do exist, suggesting the presence of chemical constituents in *E. indica* that are responsible for such antioxidant behaviour. Plants produce a large number of naturally occurring secondary metabolites, many of which have unique pharmacologic activities. These metabolites include the flavonoids, phenols, phenolic glycosides, saponins, cyanogenic glycosides, unsaturated lactones and glucosinolates [1, 2, 6–14]. Our results show that there was a strong and significant correlation between total phenolic content and DPPH free RSA of the *E. indica* extract. We can therefore state that most of the antioxidant free RSA of *E. indica* was due to its phenolic constituents and also to the presence of other secondary metabolites, such as glucosides and C-glycosylflavone [11, 12]. In our study, we investigated the hepatoprotective effects of *E. indica* and its mechanism of action against CCl₄-induced oxidative hepatic damage in rats.

DPPH is a stable synthetic nitrogen free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule [33]. In the DPPH test, the ability of *E. indica* to act as a donor of hydrogen atoms or electrons in the transformation of DPPH into its reduced form (DPPH-H) was measured spectrophotometrically at 517 nm. The result of DPPH scavenging activity suggest that *E. indica* exhibited a strong and direct free radical scavenging action.
effect which could result in a favourable action against pathological modifications caused by the generated CCl₃ free radical induced by CCl₄. We further tested the ability of *E. indica* extract to inhibit oxidative stress and associated damage in the CCl₄-induced hepatotoxicity model. The amounts of endogenous hepatic antioxidants, such as GSH and antioxidant enzymes, as well as MDA levels showed a clear correlation with CCl₄-induced hepatotoxicity. A 2-week-long pretreatment with *E. indica* extract prevented CCl₄-induced oxidative stress and also inhibited hepatic injury.

Hepatotoxicity induced by CCl₄ is the most common model system used to screen plant extracts for hepatoprotective activity [34]. AST and ALT have been reported to be sensitive indicators of hepatic injury [35]. In this study, a significant increase in serum AST and ALT levels was observed following the administration of CCl₄. However, the increased levels of these enzymes were significantly decreased by pretreatment with *E. indica*, which clearly suggests a significant restoration of liver damage.

The phospholipid bi-layers of cellular and sub-cellular membranes are the major targets of free radicals. The compound that inhibits membrane phospholipid peroxidation seems to exert a pharmacological effect by preventing radical-induced oxidative pathological events [36]. Lipid peroxidation has been implicated in the pathogenesis of liver injury by the free radical offshoots of CCl₄ and is responsible for damage to the cell membrane and the subsequent release of marker enzymes of hepatotoxicity [8]. In our study, significantly elevated levels of MDA, a product of membrane lipid peroxidation, in the CCl₄-treated group indicated hepatic damage. Pretreatment with *E. indica* prevented lipid peroxidation, which could be due to the free radical scavenging antioxidant elements.

Some of the more important hepatic enzymes active in the detoxification of lipid peroxides or ROS are GPX, GR,
and CAT [37]. Under oxidative stress, GSH is largely consumed by glutathione-related enzymes, thereby resulting in the induction of some intoxication [38]. The low levels of GSH in the CCl₄-treated group indicates that intoxication has occurred in the liver cells. The animal groups pretreated with the E. indica extract showed a significantly dose-dependent increase in GSH level. The enzymatic antioxidant defence system is the natural protector against lipid peroxidation, and GR, CAT, GST and GPX are important scavengers of the superoxide ion and H₂O₂. These enzymes prevent the generation of the hydroxyl radical and protect the cellular constituents from oxidative damage [39]. CAT is an enzymatic antioxidant that is widely distributed in all animal tissues, with the highest activity found in the liver. It breaks down H₂O₂ and protects the tissue from highly reactive hydroxyl radicals [40]. QR, a phase II-metabolizing enzyme, removes potentially active electrophiles, thereby preventing them from damaging the nucleophilic group of DNA and ultimately protecting tissue against carcinogenic and toxic compounds [24]. Reduction in the activity of these enzymes may result in deleterious effects due to the accumulation of superoxide radicals and H₂O₂. The CCl₄-treated group showed a significantly lower activity of these enzymes because of excessive free radical formation. Pretreatment with the E. indica extract significantly increased the activity of these enzymes, thereby demonstrating that E. indica has the potential to reduce the oxidative damage caused by CCl₄ in the liver and increase the antioxidant enzyme activities.

The aim of our histopathological studies was to evaluate the protective effect of E. indica against CCl₄-induced hepatic histopathological alterations in order to ascertain the cause of hepatic cell death and to substantiate the biochemical findings. Our histopathological findings support the biochemical findings. The histopathological changes seen in the liver of rats treated with CCl₄ were characterized by massive fatty change, necrosis of hepatocytes, inflammatory infiltration and sinusoidal dilatation. However, most of these changes were alleviated by the prophylactic treatment of the animals with E. indica. Our results therefore confirm the previous findings of other researchers who had found degenerative changes in the liver of rats exposed to CCl₄ [34, 35].

In conclusion, we found that E. indica has significant hepatoprotective effects against hepatotoxicity induced by CCl₄ in rats. The precise mechanism(s) of inhibitory effects of E. indica are still incompletely understood; it is likely that the antioxidant and free radical scavenging actions of E. indica may, at least in part, may be related to the modulation of CCl₄-induced oxidative hepatic damage. Further research is needed to elucidate whether the hepatoprotective effect is specific for CCl₄ and to identify the active constituents of E. indica. This study could serve as a constructive reference to allow future exploitation of E. indica as a novel preventive and remedial measure for the prevention and treatment of oxidative stress-induced hepatic injury.

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Conflict of interest None.

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