Original Article

Protective Effect of Pomegranate (*Punica granatum*) Extract against Diabetic Changes in Adult Male Rat Liver: Histological Study

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Abstract

Background: Diabetes mellitus could be result from disorders in insulin secretion or receptors, characterized by hyperglycemia. Natural antioxidants including pomegranate have hypoglycemic effect. **Aim of the Work:** The present research was designed to evaluate the possible protective role of pomegranate peel extract (PPE) against diabetic-induced hepatic complication. **Materials and Methods:** Forty-eight male Wistar rats, weighed 200–250 g and aged 3 months, were sorted into four groups: Group 1: Used as control, Group 2: Normal rats received PPE (200 mg/kg bw/day) given orally for 11 consecutive weeks. Group 3: Streptozotocin (STZ)-diabetic rats, injected with 55 mg/kg bw of STZ, and Group 4: Normal rats received PPE for 11 weeks and then rats were injected with STZ (55 mg/kg/bw). Effectiveness of the PPE was assessed by measuring serum glucose and histopathology of liver tissue. Liver enzymes were also assayed. PPE was found to control diabetic hyperglycemia and decrease in body weight. Histological examination showed that pretreatment with PPE provided preservation against diabetes-induced hepatic histological changes (necrotic and apoptosis). **Result:** Alanine aminotransferase, alanine phosphatase, and aspartate aminotransferase levels were significantly elevated in Group 3 diabetics and decreased in Group 4 which confirmed histological finding. **Conclusion:** This study confirmed the hypothesized possible protective effect of PPE against diabetic-induced histological and functional alteration of rat liver and advised its use by diabetic patients.

Keywords: Diabetic rat, hepatic histopathology, liver enzymes, pomegranate peel extract, preventive

Introduction

Diabetes mellitus (DM) could result from abnormal secretion of insulin, or altered its receptors is diagnosed by the presence of hyperglycemia. It is a widely spread disease; a report prepared by the World Health Organization estimated the patients with diabetes to rise from 171 million 2000 up to 399 million by 2030. It is also expected that numbers will exceed 552 million by 2030.[1]

Chronic hyperglycemia resulting from diabetes is accompanied with functional failure of many organs, including kidneys, heart, liver, and blood vessels, thus increasing the possibility of diabetic nephropathy and liver diseases.[2]

One of the major organs affected by long-term hyperglycemic damage is the liver, which has an effective role vital role body metabolism. Therefore, involvement of liver in DM has unfavorable impact on person health. The range of liver implication in diabetes varies from nonalcoholic fatty liver to cirrhosis or even hepatocellular carcinoma.[3]

To induce insulin-dependent DM, experimental animals are injected with streptozotocin (STZ) which was known to be diabetogenic, causing an irreversible oxidative damage to beta-cells, with subsequent defect in insulin secretion.[4]

The attempt to find out products with antidiabetic effects in the past two decades has involved several natural products such as herbal extracts that were conventionally used as medicine for diabetes.[5]

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Pomegranate peel extract (PPE) has been shown in several studies to have hypoglycemic and antioxidant properties.[6] Pomegranate products also contribute in the inhibition of carbohydrate digestive enzymes. It may also have an antihyperglycemic effect due to its phenolic content in the flower and peel of *Punica granatum* (PG). Another important factor is that the methanolic extract of PP which can inhibit oxidative stress and histopathological alterations in the liver and kidneys, an effect relates to antiapoptotic and antioxidant activities.[7]

It was recommended to use PPE to study its antioxidant activity as it is 10 times greater than that of the pulp.[8]

The main objective of the present study was to evaluate the possible protective role of PPE against diabetic-induced hepatic complication. Histological study and essay of liver enzymes were used for evaluation.

Materials and Methods

Drugs and chemicals

Pomegranate fresh fruits were obtained from local market in Jeddah, Saudi Arabia. Methanol was purchased from Sigma-Aldrich, Chemie GmbH, Germany. STZ was obtained from Sigma-Aldrich Corp, St. Louis, MO, USA. Mouse alanine transaminase (ALT) ELISA Kit was obtained from Geno Technology, Inc., USA. Rat total alkaline phosphatase (TALP) ELISA kit and rat aspartate aminotransferase (AST) ELISA kit were purchased from My BioSource, Inc., San Diego, CA, USA.

Experimental animals

This study included 48 adult (3 months) male Wistar rats (200–250 g). They were purchased from Mansour Scientific Foundation for Research and Development, Jeddah, Saudi Arabia. Animal care in KFMRC House Animal, KAU University, was provided, according to the guidelines for animal research approved by the Unit of Biomedical Ethics Research Committee, Faculty of Medicine, King Abdulaziz University. The rats were caged for about 10 days before use in the experiment under standard conditions (temperature of 25°C, a 12/12-h light/dark cycle, and 22 CO humidity). Rats were fed with laboratory pellet chow and water *ad libitum*. They were equally divided into four groups, 12 rats each.

Methods

Pomegranate peel extract production and administration

Peel of fresh pomegranate fruit was removed, dried first in shade for 10 days, and then put in a freeze dryer (Martin Christ, Gefriertrocknungsanlagen GmbH, Germany) for complete dryness. Dried peels were crushed, and an amount of 50 g from the resulted powder was pulverized, shaken in 500 ml of methanol (absolute) for 24 h at 22 CO, and extracted using ULTRA-TURRAX disperser (T 50 basic IKA-Werke, Germany). The pooled methanolic extract was filtered through four layers of gauze and evaporated under vacuum using rotary evaporation (Rotavapor, BUCHI, Switzerland) at 45°C. Crude PPE (reddish residue, 500 g) was obtained and kept at −20°C. PPE (200 mg/kg) was given orally in aqueous solution once per day for 11 executive weeks.

Experimental induction of diabetes

In normal and PPE-pretreated rats, animals were fasted overnight with only permission for free access to water before induction of diabetes by injection single dose STZ (55 mg/kg bw) via intraperitoneal route dissolved in 1 ml of 0.05 M citrate buffer (pH 4.5) and freshly prepared immediately before use mentioned.[9] 5% glucose was added to drinking water to overcome the drug-induced hypoglycemia. Diabetes was diagnosed by blood glucose level determination in fasting rats treated with STZ. Citrate buffer alone was also injected to control rats. On the 3rd day after STZ injection, animals blood glucose was measured.[10] Rats with blood glucose level 300–350 mg/dl were considered diabetics mentioned.

Experimental design

The rats were randomly divided into the following four groups (12 each): Group 1: Control group (C), given normal saline solution for 11 consecutive weeks. Group 2: Pomegranate-treated group (P), normal rats received PPE (200 mg/kg bw/day) for 11 consecutive weeks. Group 3: Type 2 diabetic rats (D) were injected with 55 mg/kg bw of STZ. Group 4: Protective group normal rats pretreated with PPE before STZ-induced diabetes (PD). Initially, rats were given PPE for 11 weeks, and later, rats were injected with STZ (55 mg/kg/bw).

The following parameters were evaluated in all animal groups

Body weight

Body weight of all animals groups was recorded, and the end of the experiment before animal sacrificed and subjected to statically analysis.

Blood glucose levels

At day of animals sacrifice, blood samples were collected from the retro-orbital venous plexus of lightly anesthetized animals. Blood samples were left to clot and centrifugation at 4°C, 3000 rpm for 10 min for serum collection, which was kept at −20°C until used for the biochemical analysis.[11] Glucose level was measured weekly all through the experiment using a blood glucometer (ACCU-CHEK; Roche Mannheim, Germany).

Estimation of liver enzymes

Serum was assayed in all groups for liver enzymes; ALT, AST, and ALP. All measurements of the biochemical parameters were carried out in Mansour Scientific Foundation for Research and Development, Jeddah, Saudi Arabia.

Histopathological study

Liver specimens (2 m × 2 m) were taken immediately from dissected liver and were placed in 10% neutral-buffered formalin for further embedding in paraffin. Sections 3–5 μ thickness were cut and stained for general histology by to the hematoxylin and eosin stain.[12] Stained sections were examined

and photographed using a light microscopy provided by a digital camera.

Statistical analysis

Statistical analysis using "IBM SPSS statistics ver. 20.0" (SPSS 20,IBM, Armonk, United States of America) was applied to evaluate and test the hypothesis. Data were presented as means ± standard error (SE). One-way analysis of variance was used to find the significant differences between the four groups' means followed by a *post hoc* test and Tukey's HSD for multiple comparisons. Results were considered statistically significant when $P \leq 0.05$.

Results

Body weight

Data presented in Figure 1 clearly illustrated that rats injected with STZ showed significant decrease in body weight level comparison to the control group, $P < 0.01$. No significant difference in body weight level between pomegranate-treated group and the control group. Pretreatment with PPE (protective group) showed significant increase in body weight compared to diabetics, $P < 0.01$.

Blood glucose levels

The findings revealed that diabetic rat group showed a significantly higher glucose level compared with the control group, $P < 0.0$. There were no significant changes in fasting blood glucose level between pomegranate-treated and control groups. In PPE diabetic protective group, the decrease in blood glucose level was highly pronounced, $P < 0.01$, [Figure 2].

Microscopical examination

Observation of the hepatic tissue of the untreated nondiabetic control rat showed histological feature similar to what was described in the previous literature. The main features are the radially arranged hepatocytes around the central vein. The cells have acidophilic cytoplasm and rounded central vesicular euchromatic nuclei with well-defined nucleoli. The hepatocytes plates are separated by thin-walled blood sinusoids lined by flat endothelial cells. Hepatic tissue of diabetic rat showed an increased in apoptotic hepatocytes (shrunken, dark-stained cells with small degenerated nuclei). The liver tissue from PPE treatments rat showed nearly normal radially arranged hepatocytes around the central vein. Blood sinusoidal spaces and their Kupffer cells are similar to those on control. Interestingly, the hepatic tissue of the diabetic rats pretreated with PPE protective group revealed relatively normal hepatic structure, radially arranged hepatocytes around the central vein. Kupffer cells lining sinusoidal spaces were evident in Notice the normal appearance of sinusoidal spaces and their lining Kupffer cells as exhibited in Figure 3. Estimation of liver enzymes. Data presented in Figure 4, 5, 6 clearly illustrated that, rats injected with STZ showed significant increase in serum ALT, AST, ALP level comparison to the control group $P \leq 0.01$. (Protective group) showed in significant difference to the control group. in significant difference between Pomegranate-treated group with the control group.

Discussion

DM-associated hyperglycemic usually results from altered insulin secretion or its receptors. One of the major organs affected by long-term hyperglycemic damage is the liver which plays a vital role in metabolism of both endogenous (lipid, hormones, carbohydrate, etc.) and exogenous substances and drugs.[13]

Recent interest research is raising in using natural antioxidant present in many food stuffs for preventing diseases caused by oxidative stress including diabetes. Natural antioxidants are well known for its safety and low costs.[14]

In the present study, preventive effect of PPE methanolic extract was evaluated regard its effectiveness in lowering blood glucose level in STZ-diabetic rat and found preserving normal liver architecture.

Results of the present study showed significant increase in blood glucose in STZ-injected rats indicating the existence of diabetes. Similar results were observed by Radovits *et al.*[15] This was most probably result from oxidative damage of beta-cells with alteration in insulin secretion and sensitivity with subsequent hyperglycemia and generation of free radicles that result in depletion of normal body antioxidant defense system.[15]

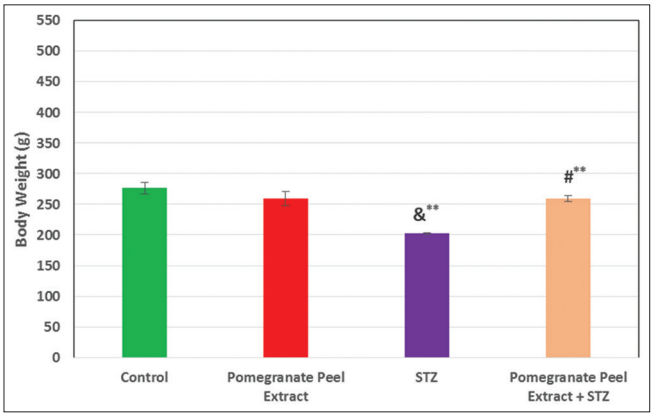

Figure 1: Graph for statistical data of body weight in control and all experimental groups

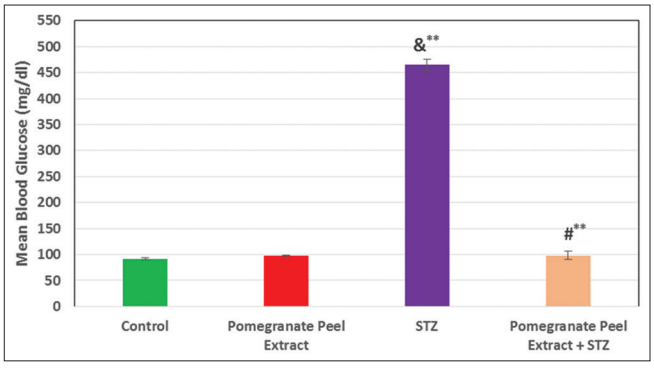

Figure 2: Graph for statistical analysis of blood glucose in control and all experimental groups

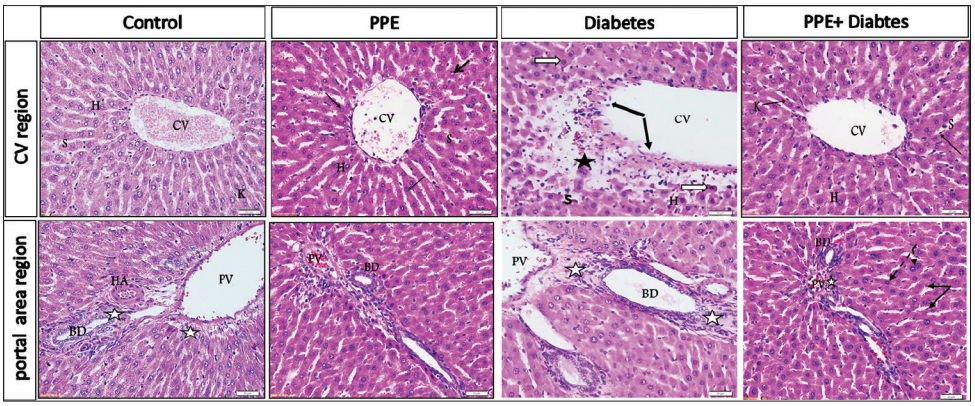

Figure 3: Photomicrographs of rat liver. Group 1: Control showing normal hepatocytes (H) near central vein (CV) and portal area (PA). Blood sinusoids (S) are of normal appearance and showed endothelial and Kupffer (K) cells. Portal vein (PV), bile duct (BD), and hepatic artery (HA) surrounded by scanty connective tissue with few mononuclear cells and fibroblasts (stars). Group 2: PPE showing no alteration in normal structure of hepatic tissue. Hepatocytes (H) showed active vesicular nuclei (arrows). Slight dilation of blood sinusoids(s) could be observed. Portal area showed normal triad (PV, HA, and BD). Group 3: STZ diabetes: Focal hepatocyte necrosis (black star) is seen around the central vein (CV) which showed dilation and damage of its lining epithelium (black arrows). Sporadic (apoptotic) cells with dark stained acidophilic cytoplasm and small dark (pyknotic) nuclei (white arrows). Portal area showed dilated numerous bile ducts (BD) portal vein dilation (PV) surrounded by inflammatory cells (stars). Group 4: PPE before STZ diabetes: showing marked protection against diabetic induced changes. Hepatocytes looked normal with rounded central vesicular active nuclei (dotted arrows). Blood sinusoids (S) are normal and showing prominent Kupffer cells (K, black arrows). Portal area showed normal bile duct (BD), hepatic artery (HA). Connective tissue around them showed few inflammatory cells (white star) (H and E). STZ: Streptozotocin

Decrease in body weight in diabetics was well known in Type 1 diabetes in human diabetes. In experimental animals, decrease in body weight was also reported in STZ-induced diabetes. Decrease in body weight is usually result from disturbance of glucose metabolism and uptake by body cells with subsequent shifting to adipose tissue and muscles as sources of energy that result in weight loss.[16]

On the other hand, rats administered with PPE for 11 weeks before induction of diabetes showed higher body weight gains. These results hypothesized that the using PPE could be the underlying factor for improving appetite and enhanced weight gain, as well as decreasing possible complications of DM.

In the present study, STZ-induced diabetes was found to alter normal histology of rat liver; hepatocyte necrosis was found in perivascular region of central vein. Apoptotic changes, bile duct proliferation, and inflammatory cell infiltrate were also observed around portal blood vessels and bile ducts. Those histological finding were previously described in similar papers.[17]

PPE given alone to normal rats was found to have no effect of liver parenchyma, where hepatocytes, sinusoids, and portal areas looked similar to those of control.

Bassiri Jahromi *et al*.[18] found that administration of PEE (repeated doses of 0.5, 1.9, and 7.5 mg/kg body weight) to mice did not produce any irritation to oral mucosa or respiratory passage. Further, single intradermal injection (224 mg//kg) of the extract was found to be safe without any manifestation of skin allergy.

Based on the previous literature using pomegranate products as hypoglycemic natural agent,[19] it was here as a preventive therapy against STZ-induced diabetes in rat. Both blood glucose and body weight were kept near-normal levels in rats given PPE before diabetes induction. Similar administration of PPE before induction of diabetes result in prevention of hyperglycemia compared to nontreated.[11]

Many researches have been studied PP, which was to possess effect compared to other pomegranate parts.[20] Most pomegranate products as were mentioned previously were also proved to have hypoglycemic effect both in experimental animals and human studies.[19]

Histological changes induced by diabetes in rat liver were not observed in the group administrated PPE before diabetes induction. Animals showed normal liver architecture where hepatocytes are present as normal sheets around the central vein. Bile duct proliferation and portal vein congestion are less compared to those observed diabetic liver.

Hepatic protective effect of PP against hepatotoxic conditions were available in literature.[21] Aqueous extracts of root and peel of PG were reported by Khan *et al*.[22] to have hepatoprotective effect against carbon tetracholoride induced toxicity in Wistar rats. Similar effect against carbon tetrachloride toxicity by PG seed oil was also reported by Gram *et al*.[23] Amiri *et al*.[24] found that administration of PG leaf extract prevents fatty changes in liver of rat receiving high fat diet.

The present results were similar also to what was reported in a study conducted by Amiri *et al*.[24] on rats in which aqueous PPE of the same dosage used in this study (200 mg/kg) was found to lower glucose concentrations in the serum. Diabetic rats treated for 10 days with 200 mg/bw of PPE, rich in polyphenols, showed lower fasting serum glucose and higher insulin levels as well as antilipid peroxidation effects.

Blood glucose control by PPE was reported by many authors,[25] which confirm the effect of PPE in this study where its

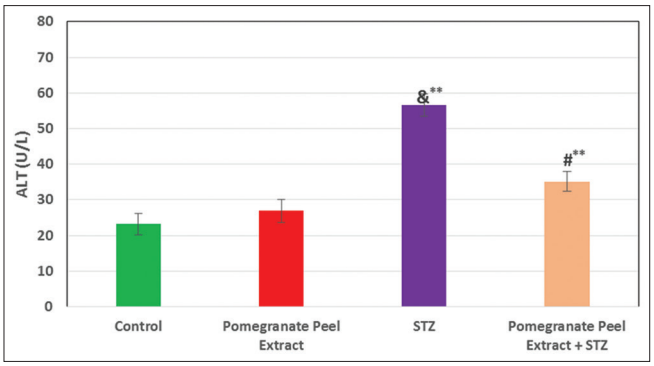

Figure 4: Effect of pomegranate peel extract on serum ALT. STZ (D) significant increase to the control. (PD) in significant difference to the control. (P) in significant to the control. (&** Significant from control $P \leq 0.01$, #** Significant from STZ $P \leq 0.01$). STZ: Streptozotocin, ALT: Alanine aminotransferase

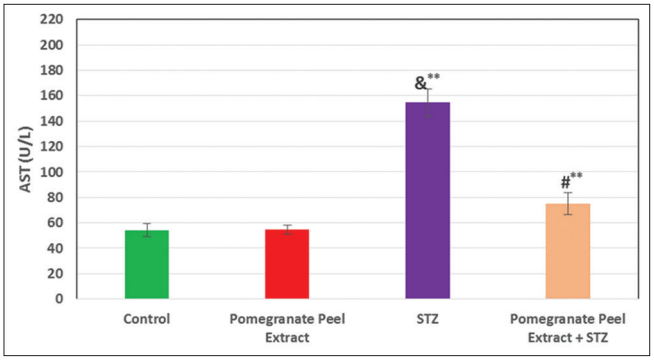

Figure 5: Effect of Pomegranate peel extract on serum AST. STZ (D) significant increase to the control, $P < 0.01$. (PD) in significant difference to the control. (P) in significant difference to the control. (&** Significant from control $P \leq 0.01$, #** Significant from STZ $P \leq 0.01$). STZ: Streptozotocin, AST: Aspartate aminotransferase

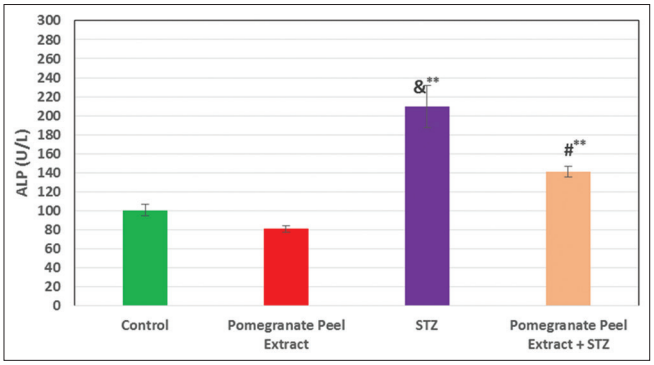

Figure 6: Effect of pomegranate peel extract on serum ALT. STZ (D) significant increase to the control. (PD) in significant difference to the control. (P) in significant difference to the control. (&** Significant from control $P \leq 0.01$, #** Significant from STZ $P \leq 0.01$). STZ: Streptozotocin, ALT: Alanine aminotransferase

administration prior to diabetes induction keep blood glucose near-normal levels, preventing hyperglycemia adverse effects on different organs including liver.

Administration of PP water extract was found by Elsaid *et al.*[26] to be hypoglycemic and hepatoprotective and explained by the authors to related to related inhibition of glucose transporter 2 genes liver of diabetic rats with the protection of hepatocytes from hyperglycemic effects.

Hyperglycemia was reported by Saengboonmee *et al.*[27] to enhance proliferation of bile duct epithelium, so the absence of bile duct proliferation in protective group could be due to lowering blood glucose level by PPE.

Review on using PG parts including peels showed its effective use cases suffering from type 2 diabetes; however, the authors reported that the mechanism is still not well understood. Moreover, more work is needed to define the exact active ingredients of PPE responsible for its antioxidant protective activity against diabetic complication including hepatic involvement.[28]

Biothermal study concerning liver enzymes demonstrated that administration of PEE (200 mg/kg bw/day) showed insignificant difference compared to control, thus PPE could be considered safe causing no damage to liver parenchyma structure and function.

Most enzymes significantly increased in STZ-induced diabetic rats and decreased by PEE given before induction of diabetes. Such biochemical results explained histological in both untreated diabetic and preventive groups. Necrosis of hepatocytes is well known to result in release of enzymes and increase in their serum levels.[29]

Oxidative stress induced by STZ and increased blood glucose were most probably linked to increase liver enzymes[29] and explained their decrease by the antioxidant effect of PEE.

The present results showed that pomegranates decreased the liver toxicity by enhancing enzymatic and nonenzymatic antioxidant defense systems.[30] The presence of polyphenolic a compound in PPE was proved to play a role in the protective activity[31] that seemed to be linked to its effective antioxidant activity both *in vitro* and *in vivo*.

AST is a hepatic enzyme that catalyzes transamination reaction of alanine amino acid. AST is present in highest concentration mainly in heart muscles compared to what was present in other tissues such as skeletal muscle, kidney, and liver.[32] AST is well known of tissue damage resulting from oxidative stress. However, ALT and ALP are considered to be more specific than AST in monitoring liver functions.[32] The current study showed elevation in AST enzymes in Group D which was significantly ameliorated by the administration of PP in Group PD when compared to diabetic. Those results that are similar could be attributed to antioxidant effect of PPE that protect.

Conclusion

Together all previous results clearly showed that administration of PPE exerted potential protective effect on reducing blood glucose level and protect against hyperglycemic-induced hepatic changes. Protective effect was most probably linked to its antioxidant activity of polyphenol content.[32,33] Further

study on the same samples are running to confirm this mechanism.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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