Anti-hyperlipidemic Investigations of Litchi chinensis in Rats

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ABSTRACT

Litchi chinensis is a plant with a wide variety of ethnic medicinal uses, hence it is planned to screen anti-hyperlipidemic action (based on traditional uses) by taking two models- Triton-X-100 and High Fat-Diet induced hyperlipidemia in rats. The ethanolic extract of pericarp of this plant was used to evaluate on above mentioned models. Anti-hyperlipidemic study was performed on two models: (a) Triton-X-100 (b) High Fat-Diet method taking Atorvastatin as a standard (10mg/kg, p.o.) (Novartis) and by using oral low dose (200mg/kg), high dose (400mg/kg) of extract. During the study of extract no mortality was observed in any animals up to the dose level of 2000 mg/kg, indicating their practically non toxic in nature. The low and high doses of extract (200 and 400mg/kg, p.o.) were found significant in reducing total cholesterol and triglycerides against different models of hyperlipidemia induced in rats. Both the doses of extract provide protection against atherosclerosis. The result provides strong evidence to support that the ethanolic extract of pericarp of Litchi chinensis possess Antihyperlipidemic activity as well as it is entirely safe for longer duration of treatment. However, further molecular based studies will be necessary to clarify its mechanism of action and to characterize the active principles.

Keywords: Litchi chinensis, Antihyperlipidemic activity, Triton-X-100 and High Fat-Diet induced hyperlipidemia.

INTRODUCTION

Hyperlipidemia is a significant reason for atherosclerosis and the atherosclerosis-related conditions, for example, coronary illness, ischemic cerebrovascular ailments and fringy vascular infection. Despite the fact that the occurrence of the atherosclerosis related occasions has declined in the US, these condition despite everything represents most of grimmness and mortality among moderately aged and more established grown-ups, the rate and supreme number of yearly occasions will increment throughout the following decade in view of pestilence of heftiness and maturing of the U.S populace.1, 2 Dyslipidemia, including hyperlipidemia and hypercholesterolemia and low degree of high thickness of lipoproteins cholesterol HDL are significant reason for expanded atherogenic hazard; both hereditary issues and way of life diet high in calories, immersed fat, and cholesterol add to dyslipidemia seen in created nations around the world.3 Hyperlipidemia is normally known as hypercholesterolemia or hypertriglyceridemia. Despite hypercholesterolemia is the most well-known hyperlipidemia. In an essential term of the hyperlipidemia is the reason for atherosclerosis, and accordingly expanded the danger of coronary illness and stroke. The danger of coronary illness in future additionally relies upon different components that influence the strength of an individual’s veins and blood circulation.4 Diverse plant substance constituents having polyphenolic structures have been recognized as food supplements for creating better medical care during present day years.5 Herbal arrangement of medication (indigenous to India) obviously expresses the utilization of restorative plants for treating different issues with no risky impact on human just as creatures. The individuals who lives in India has a ton of information on plant treatment from Ayurveda arrangement of medication treatment and different kinds of natural medications details are readied and they are assessed logically. Keeping this perspective, the Litchi chinensis is one of the most potential plants which produce foods grown from the ground different pieces of Litchi chinensis might be utilized in the different sorts of malady and issues with no harmful impacts.6 Litchi chinensis is generally utilized in neuralgic confusion, orchitis, lumbago, hernia, tumors, ulcers, gastralgia and remembers hacking. The organic product strip of Litchi chinensis utilized in the treatment of, smallpox eruption and looseness of the bowels. Leaves of the plant is utilized to treat or fix the skin sicknesses, heat stroke, diabetes, dyspepsia, corpulence, diuretic, carminative, hostile to febriles, remembers neural torment, diarrhea, swellings, pain relieving, antipyretic and haemostatic in humans.7
MATERIALS AND METHODS

Plant material

The plant material (fruits) of Litchi chinensis were taxonomically identified collected and authenticated by qualified botanist. The fruit collection was done in the month of June from, Mahakaleshwar Dham, NH-24, Delhi road, behind Hotel Regency, Moradabad, (UP) India. Plant authentication was done by a team of botanist under supervision of Dr. Ashok Kumar, Head of Department, Botany, IFTM University, Moradabad, (UP) India. The reference no. of the plant was given as 2017/SOS/BOT/49. Collected fruits were dried in shade at room temperature for 20 days.

Preparation of extract

The dried fruits (Litchi chinensis) material was powdered and passed through a 20-mesh sieve. The fruit material was defatted with petroleum ether (40-60g) and then extracted with ethanol (95% v/v) and by using a Soxhlet apparatus. The extracts of the plant (Litchi chinensis) were filtered and concentrated by distilling off the solvents and evaporated to dryness using rotatory vacuum evaporator. The % yield was calculated as-

\[
\text{Observed yield (g)} = \frac{x \times 100}{\text{Total weight of dry powder (g)}}
\]

Requirements

Experimental animals

Wister albino rats of either sex of body weight (150g-200g)

Drugs and Chemicals

Atorvastatin (Watson Pharmaceutical and India Ranbaxy laboratories), Acetic acid (Otto Chemie Pvt. Ltd, Mumbai), Ethylacetate (SD fine chemicals Mumbai), Silica gel (CDH Ltd, New Delhi), Acetone (CDH Ltd, New Delhi), Formaldehyde (CDH Ltd, New Delhi), Sterile water for injection (Nirlife Health Care, Mumbai), Methanol (SD fine chemicals, Mumbai), Hydrochloric acid (SD fine chemicals Mumbai).

Grouping of Animals

Six groups of animals were used for the study.

Group 1: Normal group.

Group 2: Control group

Group 3: Test group treated with low dose (EELC-1).

Group 4: Test group treated with high dose (EELC-2).

Group 5: Standard drug. (Atorvastatin).

Group 6: Standard drug + low dose (Atorvastatin + EELC-1).

Toxicity Study

Acute oral toxicity study (LD_{50})

For the acute oral toxicity, the starting dose for animal was selected from the fixed dose toxicity levels of 5, 50, 300 and 2000 mg/kg as a portion expected to deliver evident harmfulness in the creatures. Without any data, the beginning portion for the trial creature was chosen by 2000 mg/kg body weight.

The test substance (Litchi chinensis) was administered in a single dose by using a suitable oral feeding tube. Animals were fasted before stared the dosing (for example if there should arise an occurrence of rodents, food was retained yet not water retained over-night. Past the time of fasting, the animals were gauged and the test substance (Litchi chinensis) was directed. After the test substance (Litchi chinensis) was controlled, food was retained for a further 3-4 hours in rodents. Where a portion of test substance (Litchi chinensis) was controlled to the animals in partitions over some undefined time frame, it might be important to give the animal with food and water contingent upon the length of the period. A time of at any rate 24 hours was suggested between the dosing of each exploratory animal. All test animals utilized in the investigation were watched for in any event next 14 days.8

IN-VIVO STUDY

Antihyperlipidemic activity

Triton-X-100 induced hyperlipidemic activity

In this model, hyperlipidemia was induced in Wister albino rats by using single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in normal saline solution after overnight fasting for 18 hr. The test animals were divided into five groups and each group contain of six animals. The group 1st was received standard pellet diet, water and orally administered with 5% CMC orally. The group 2nd was received a single dose of Triton-X-100 (100mg/kg, i.p.) After 72 hours of Triton-X-100 injection, this group received dose of 5% CMC (0.5mg/kg, p.o) for 7 days. The group 3rd received a daily dose of 200mg/kg and group 4th received a daily dose of 400 mg/kg of EELC dissolved in sterile water, p.o., for 7 days, after inducing hyperlipidemia in rats. The group 5th was received the standard Atorvastatin 10mg/kg, p.o. The group 6th was received the Triton-x-100 + Standard drug + EELC low dose (Atorvastatin 10 mg/kg, + 200mg/kg p.o.) for 7 days.

On the 8th day, blood sample were collected by retro orbital sinus cut, under gentle ether anaesthesia. The collected blood samples were centrifuged for around 10 minutes. At that point serum sample were gathered and utilized for different biochemical examinations. Assurance of serum cholesterol, fatty substance, LDL, HDL and VLDL levels of blood test of rodents (was gathered by retro-orbital method) utilizing Autoanalyzer/semianalyzer (Robert Reille photometer) with the assistance of demonstrative kit.9,10,11
Grouping and treatment of animals is as follows;

**Group 1:** Vehicle (0.5% CMC in distilled water 10 ml/kg, p.o)

**Group 2:** Triton-x-100 100 mg/kg, i.p.

**Group 3:** Triton-x-100+ EELC- low dose (200 mg/kg, p.o.)

**Group 4:** Triton-x-100+EELC- high dose (400 mg/kg, p.o.)

**Group 5:** Triton-x-100+ standard drug (Atorvastatin10 mg/kg, p.o.)

**Group 6:** Triton-x-100+ Standard drug + EELC low dose (Atorvastatin 10 mg/kg, + 200mg/kg p.o.)

**High fat diet induced hyperlipidemic activity**

High fat was prepared by mixing Indian vanaspati ghee and coconut oil in the ratio of 3:1(v/v). It was given to the rats at a dose of 3ml/kg body weight per day.12 The chronic hyperlipidemia induced in wistar albino by feeding HFD once a day for 30 days. The Group 1st was received normal diet once a day for 30 days. Group 2nd was received HFD (High fat diet) once a day for 30 days. Group 3rd was received HFD with EELC 200mg/kg. Group 4th was received HFD with EELC 400mg/kg. Group 5th was received HFD with standard dose of Atorvastatin 10mg/kg.13 Group 6th was received HFD +standard drug + EELC low dose (Atorvastatin10 mg/kg, + 200mg/kg p.o.) once a day for 30 days.

On the 31 day, blood samples were collected by retro orbital sinus puncture, under gentle ether anesthesia. The collected samples of the blood were centrifuged for around 10 minutes. At that point serum sample were gathered and utilized for different biochemical experiments. Assurance of serum cholesterol, triglyceride, LDL, HDL and VLDL levels of blood sample of rats was gathered by retro-orbital method utilizing Autoanalyzer/semianalyzer (Robert Reille photometer) with the assistance of demonstrative kit.5,10,11

Grouping and treatment of animals is as follows;

**Group 1:** Normal diet

**Group 2:** HFD 3ml/kg, p.o.

**Group 3:** HFD+EELC- low dose (200 mg/kg, p.o.)

**Group 4:** HFD +EELC- high dose (400 mg/kg, p.o.)

**Group 5:** HFD +standard drug (Atorvastatin10 mg/kg, p.o.)

**Group 6:** HFD +standard drug + EELC low dose (Atorvastatin10 mg/kg, + 200 mg/kg p.o.).

**Statistical analysis**

Differences in all hematological parameters, body and organ weight, water and food consumption, and for all treated and control rats were resolved utilizing a Two- route Analysis of Variance (ANOVA) trailed by Bonferroni test. A p estimation of 0.05 or less (p<0.05) were taken as significant. All informations were communicated as mean ± standard error of the mean (SEM). All these investigation were finished with the assistance of a factual programming — GRAPHPAD PRISM (5.0).14

**RESULTS AND DISCUSSION**

**Toxicity studies (LD_{50})**

**Acute oral toxicity study**

No mortality or morbidity or any signs of behavioral changes or toxicity were observed throughout the 14-day period after single oral administration of EELC up to the dose levels of 2000 mg/kg body weight. Morphological qualities (hide, skin, eyes, and nose) seemed ordinary. No tremors, convulsion, salivation, loose motion, lethargy or unusual behaviors such as walking backward and so forth were observed; gait and posture, reactivity to handling or sensory stimuli, grip strength were all ordinary. There were no significant alteration found in the body weights and relative organ weight of control and treatment groups. From the current investigation, it was discovered that there were no significant alteration in the hematological and biochemical parameters in the EELC treated group contrasted with control group. The histopathological assessments of different organs recolored with haematoxylin and eosin revealed no significant differences. LD50 of this plant was hence assessed to be in excess of 2000 mg/kg.

**In-vivo studies**

**Antihyperlipidemic activity**

**Triton -x-100 induced hyperlipidaemia**

In the Triton-X-100 induced hyperlipidaemia model, the Control group animals were received Triton-X-100 (100 mg/kg i.p.) the levels of TC and TG were showed highly significant, HDL and VLDL were showed non-significant and LDL was showed highly significant effects when compared with the normal group animals on 8th days (Table 1). Group I animals were received Triton -X -100 and EELC low dose (100 mg/kg, i.p +200 mg/kg, p.o.) the levels of TC, TG and HDL were showed highly significant, VLDL and LDL were showed moderately significant and highly significant effects respectively when compared with the normal group animals on 8th days.

Group II animals were received Triton -X -100 and EELC high dose (100 mg/kg, i.p +400 mg/kg, p.o.) the levels of TC, TG and HDL were showed highly significant, VLDL and LDL were showed non-significant and highly significant effects respectively when compared with the normal group animals on 8th days.

Group III animals were received Triton -X -100 and Atorvastatin (100 mg/kg, i.p + 10 mg/kg, p.o.) the levels of TC and TG were showed highly significant, HDL was showed non-significant, VLDL and LDL were showed highly significant effects when compared with the normal group animals on 8th days.
Group IV animals were received Triton-X -100+Atorvastatin+EELC Low Dose (100 mg/kg, i.p + 5mg/kg, p.o. + 200 mg/kg, p.o.), the levels of TC and TG and HDL were showed Moderately significant, non-significant and moderately significant and VLDL and LDL were showed non- significant and highly significant effects when compared with the normal group animals on 8th days.

The effect of EELC as a feed supplement out of two doses, that is 200mg/kg and 400mg/kg, resulted in a dose-dependent reduction in lipid profiles in case of 200 mg/kg (Table 1).

### Table 1: Effect of EELC on lipid profile in Triton-x-100 induced hyperlipidaemia

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>VLDL=TG/5 (mg/dl)</th>
<th>LDL=TC-HDL-VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Distilled water</td>
<td>10 ml/kg, p.o.</td>
<td>82.00±1.58</td>
<td>71.00±1.58</td>
<td>26.00±1.58</td>
<td>14.20±0.31</td>
<td>41.80±3.10</td>
</tr>
<tr>
<td>Negative</td>
<td>Triton-x-100</td>
<td>100 mg/kg, i.p.</td>
<td>157.24±2.38***</td>
<td>149.43±1.58***</td>
<td>28.01±1.92ns</td>
<td>29.88±0.46  ns</td>
<td>99.35±4.18*</td>
</tr>
<tr>
<td>Control</td>
<td>Triton +EELC-low dose</td>
<td>100 mg/kg,i.p.+200 mg/kg,p.o.</td>
<td>96.00±1.58***</td>
<td>75.00±2.07*</td>
<td>30.00±1.92*</td>
<td>15.00±0.61 ns</td>
<td>51.00±3.51*</td>
</tr>
<tr>
<td>I</td>
<td>Triton+HFD-high dose</td>
<td>100mg/kg,i.p.+400 mg/kg, p.o.</td>
<td>101.00±1.58***</td>
<td>90.00±1.92***</td>
<td>29.00±1.92ns</td>
<td>18.00±3.80*</td>
<td>54.00±3.45*</td>
</tr>
<tr>
<td>III</td>
<td>Triton+Atorvastatin</td>
<td>100mg/kg,i.p.+10 mg/kg, p.o.</td>
<td>97.00±1.58***</td>
<td>68.00±2.07*</td>
<td>29.00±1.58ns</td>
<td>13.60±0.31 ns</td>
<td>54.40±1.20*</td>
</tr>
<tr>
<td>IV</td>
<td>Triton+Atorvastatin+ EELC-low dose</td>
<td>100mg/kg,i.p.+5mg/kg+200 mg/kg,p.o.</td>
<td>111.00±1.58***</td>
<td>90.00±2.23***</td>
<td>32.00±1.92***</td>
<td>18.00±0.44*</td>
<td>61.00±1.09***</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM (n=5 animals in each group); EELC: Ethanolic extract of *Litchi chinensis*; TC: Total cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoproteins; VLDL: Very Low Density Lipoproteins. Two-way ANOVA followed by post test Bonferroni. *** highly significant (p < 0.001), ** moderately significant.

### High fat diet induced hyperlipidaemia

In present study, however no favorable changes in body weight were detected after EELC dosing. High fat diet was prepared by mixing Indian vanaspati Ghee and coconut oil in the ratio of 3:1 (v/v). It was given to the rats at a dose of 3 ml/kg/ p.o, body weight per day. The control group animals treated with high fat diet (3ml/kg, p.o.) the levels of TC and LDL were showed highly significant and TG, HDL and VLDL were showed non-significant when compared with the normal group on the 31th day. Control group animals were received HFD (3ml/kg, p.o) the levels of TC, TG and LDL were showed highly significant and TG, HDL and VLDL were showed non-significant when compared with the normal group on the 31th days. Group I animals were received HFD and EELC low dose (3ml/kg, p.o.+200 mg/kg, p.o.), the levels of TC and LDL, were showed highly significant, TG, HDL, and VLDL were showed moderately significant effects when compared with the normal group animals on 31th days. Group II animals were received HFD and EELC low dose (3ml/kg, p.o.+400 mg/kg, p.o.) the levels of TC, TG and LDL were showed highly significant, HDL and VLDL were showed non-significant and significant effects respectively when compared with the normal group animals on 31th days. Group III animals were received HFD + Atorvastatin (3ml/kg, p.o + 10 mg/kg, p.o.) the levels of TC and LDL were showed highly significant, TG showed significant and HDL and VLDL were showed non-significant, non significant , non-significant and highly significant effects respectively when compared with the normal group animals on 31th days. Group IV animals were received HFD + Atorvastatin + EELC Low Dose (3ml/kg, p.o.+5mg/kg, p.o. + 200 mg/kg, p.o.), the levels of TC, TG, HDL, VLDL and LDL were showed highly significant, highly significant, highly significant , significant and highly significant effects respectively when compared with the normal group animals on 31th days.

The effect of EELC as a feed supplement out of two doses, that is 200mg/kg and 400mg/kg, resulted in a dose-dependent reduction in lipid profiles in case of 400 mg/kg (Table 2).
Hyperlipidemia is a significant supporter of medical issues worldwide and drives particularly to atherosclerosis, bringing about coronary heart infections (CHD). The significance of restorative plants in the treatment of hyperlipidemia was tentatively concentrated lately, where oxidative pressure instigated apoptosis in fat tissue was noticed.15

In screening natural products for the pharmacological activity, assessment of the harmful qualities of medicinal product (extract, confined compound and definition) is typically a fundamental advance. During such assessment, the assurance of LBSO is typically an underlying advance to be directed. The acute oral toxicity study may provide initial information on the mode of toxic action of an agent, acts as the basis for classification and labeling, helps in deciding the dose of novel compound in animal studies. In this investigation, EELC at a dose of 2000 mg/kg had no unfavorable impact on the treated animals as long as 14 days of observation. There were no critical changes in the weight and the organs of the animals. The hematological parameters among control and treated groups, demonstrated concentrate was non-poisonous to the haemopoietic system.16

In this study, EELC was chosen to screen for it against hyperlipidemic action in Triton X (100 mg/kg) instigated hyperlipidemic rats, which was practically tantamount to that of the standard atorvastatin drug utilized in the treatment. EELC obviously shows that, at a dose of 200 and 400 mg/kg altogether significantly lowered the TGs and cholesterol levels. The decrease of TGs by the EELC was demonstrated concentrate was non-significant. The decrease of TC and LDL was demonstrated significant (p<0.05), ** moderately significant (p<0.01) and highly significant (p < 0.001) vs Control.

The counter hyperlipidemic activity of EELC (200 and 400 mg/kg) against Triton X-100 and High fat diet regimen indicated a significant decrease in TC, TG, LDL-C, VLDL and significant increases in HDL-C in a dose dependent manner by contrasting with standard atorvastatin treated group. Moreover, the combination study of extract and standard allopathic drug atorvastatin didn’t create any convincing outcomes in decreasing hyperlipidemia. In any case, there is a need for additional examination to work for more understanding in to the potential components.

HFD-fed hyperlipidemic rat model has prior been accounted for as an ideal in-vivo model for testing anti-hyperlipidemic drugs. Havel and Rapport announced that fatty diets cause elevation of plasma TC and LDL cholesterol. Elevated levels of TC and above all LDL cholesterol are indicators of atherosclerosis.18

<table>
<thead>
<tr>
<th>Group</th>
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<th>TC (mg/dl)</th>
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<td>26.00±1.58</td>
<td>14.20±0.31</td>
<td>41.80±3.10</td>
</tr>
<tr>
<td>Negative</td>
<td>HFD</td>
<td>3 ml/kg, p.o.</td>
<td>156.22±1.58***</td>
<td>166.31±1.92***</td>
<td>28.04±1.58** ns</td>
<td>33.20±0.3 ***</td>
<td>94.76±1.88***</td>
</tr>
<tr>
<td>Control</td>
<td>HFD +EELC-low dose</td>
<td>3 ml/kg, p.o.+200 mg/kg, p.o.</td>
<td>110.00±1.92***</td>
<td>92.00±1.58***</td>
<td>36.00±1.58** *</td>
<td>18.40±0.31**</td>
<td>55.60±1.93***</td>
</tr>
<tr>
<td>II</td>
<td>HFD +EELC- high dose</td>
<td>3 ml/kg, p.o.+400 mg/kg, p.o.</td>
<td>110.00±1.58***</td>
<td>65.00±1.58***</td>
<td>39.00±1.58** *</td>
<td>13.00±1.74 ns</td>
<td>58.00±4.07***</td>
</tr>
<tr>
<td>III</td>
<td>HFD + Atorvastatin</td>
<td>3 ml/kg, p.o.+10 mg/kg,p.o.</td>
<td>69.00±1.58***</td>
<td>48.00±1.92***</td>
<td>25.00±1.92** *</td>
<td>9.60±0.38***</td>
<td>34.40±0.59***</td>
</tr>
<tr>
<td>IV</td>
<td>HFD + Atorvastatin+ EELC- low dose</td>
<td>3 ml/kg, p.o.+5mg/kg+200mg/kg,p.o.</td>
<td>86.00±1.58**</td>
<td>68.00±1.58** ns</td>
<td>22.00±1.92**</td>
<td>13.60±0.31 ns</td>
<td>50.40±2.79***</td>
</tr>
</tbody>
</table>

EELC: Ethanolic extract Litchi chinensis; TC: Total cholesterol; TG: Triglycerides; HDL: High Density Lipoprotein; LDL: Low Density Lipoproteins; VLDL: Very Low Density. Values are expressed as Mean ± SEM (n=5 animals in each group); using Two-way ANOVA followed by post test Bonferroni test. ns: not significant;* significant (p<0.05), ** moderately significant (p<0.01) and highly significant (p < 0.001) vs Control.

Table 2: Effect of EELC on lipid profile in HFD induced hyperlipidemic rats

CONCLUSION

The ethanolic extract of Litchi chinensis was investigated for anti-hyperlipidemic activity in rats along with its toxicological profile. Two doses of the ethanolic extract of leaves of Litchi chinensis (200, 400 mg/kg, p.o.) were administered. The Antihyperlipidemic activity in two models (a) Triton - x - 100 (100 mg/kg). (b) High fat diet (3ml/kg) vanaspati ghee + coconut oil ratio is (3:1) and standard drug using Atorvastatin (10 mg/kg) were studied.
The ethanolic extract possessed the antihyperlipidemic activity in a dose dependent manner as well found to have preclinically nontoxic in nature for chronic use. This study thus, supports the acclaimed use of the plant in the management of hyperlipidemia.

The research further needs more evidences to provide an authentic information over its anti-hyperlipidemic activity.

Acknowledgement: It’s my privilege to express my obligation and to acknowledge heartfelt gratitude to my supervisor Mr. Mayur Porwal, Assistant Professor, Faculty of Pharmacy, IFTM University, Moradabad (U.P) for providing his valuable excellent guidance, continuous encouragement and support through the project work and shared intelligent thoughts, criticism, and incultated discipline.

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Conflict of Interest: None declared.

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