RESEARCH ARTICLE

Benefit of cinnamon (*Cinnamomum burmannii*) in lowering total cholesterol level after consumption of high-fat containing foods in white mice (*Mus musculus*) models [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

**Background:** Hypercholesterolemia is a condition where cholesterol levels in the body exceed the normal range. In Indonesia, the development of traditional medicine is carried out by examining plants known for their medicinal qualities, including cinnamon (*Cinnamomum burmannii*). There are many nutritional components contained in cinnamon, such as cinnamaldehyde. This has been suggested as a substance that can reduce cholesterol and triglyceride levels. This study was conducted to determine the efficacy of cinnamon in reducing total cholesterol levels of mice (*Mus musculus*) given high-fat feed.

**Methods:** This is an experimental study with a pre-post control study design. The groupings were performed by a simple random sampling method. There were five groups (n=6/group): 1) Negative control (aquades); 2) positive control of high-fat containing food (HFC; quail’s yolk); 3) HFC + cinnamon extract (CE; dose 2mg/20g body weight (BW)); 4) HFC + CE (dose 4mg/20gBW); 5) HFC + CE (dose 8mg/20gBW). The study was conducted for 28 days. The intervention of CE started on day 15 and ended on day 28. Measurement of total cholesterol and BW of mice was performed on days 0, 14 and 28.

**Results:** A decrease in the total cholesterol of mice (p = 0.001) was found in the groups that consumed CE. However, there was not a significant change between groups in mice BW (p = 0.419).

**Conclusions:** Providing cinnamon extract (*Cinnamomum burmannii*) for 28 days could decrease total cholesterol levels in mice compared to those not given cinnamon extract when consuming high-fat containing foods.

**Keywords**
hypercholesterolemia, cinnamon, high-fat feed, quail’s yolk, body weight of mice
Introduction

Hypercholesterolemia is a state where the cholesterol level in the body exceeds the normal range. Hypercholesterolemia can increase the risk of atherosclerosis, coronary artery disease, pancreatitis, diabetes mellitus, thyroid disorder, liver disease and renal disease1.

Data from the Indonesian Ministry of Health in 2019 reported it’s estimated that coronary artery disease to be the first killer, 26.4% of all deaths in Indonesia1. Out of the 17 million the premature death (under the age of 70) due to non Communicable disease, most cases are in low-and middle income countries, and 37% are caused coronary heart disease and 6.7 million of these were triggered by stroke (WHO, 2015).2

In Indonesia, the development of traditional medicine is performed by exploring herbs known to be beneficial for health, and this information is passed from generation to generation. One such herb is such as gambier (Uncaria gambir Roxb.), which has antioxidant effects and lowers blood glucose when treating type 2 diabetes mellitus. There is also bangun-bangun leaves (Coleus amboinicus), which are believed to be effective in relieving pain when distilled as ethanol and water extract3. Traditional medicine that comes from plants is used commonly by many people in Indonesia. One of these medicines is cinnamon (Cinnamomum burmannii,) which is often eaten daily with food. Cinnamon is a native plant in Indonesia and can be found abundantly in Central of Java, (Karanganyar), West Sumatra (Padang), Jambi (Kerinci), etc. Cinnamon has been shown to reduce total cholesterol, low-density lipoprotein and triglyceride levels, as well as increasing high-density lipoprotein levels4. Cinnamon is a flavoring ingredient that has been used in daily routines as a spice. Data from various literature on cinnamon reveal that it mainly contains essential oils and important compounds such as cinnamaldehyde, eugenol, cinnamic acid, and cinnamate5.

This study was conducted to determine the efficacy of cinnamon in reducing total cholesterol levels of mice (Mus musculus) given high-fat feed. Mice were used in this study because of they have a similar biology to humans, and can therefore be a model for human hyperlipidemia5–10.

Methods

This is an experimental study in an animal model with a pre-post control study design. This study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara, Indonesia.

Ethics

This study was approved by the Health Research Ethical Committee of Universitas Sumatera Utara (No:55/TGL/KEPK FK USU-RSUP HAM/2019).

This study used mice that were given high-fat containing food (HFC) to provide a hyperlipidemic mouse model. The intervention was carried out by giving different CE doses to find the dose that reduces the total cholesterol level of the mice. Cholesterol total levels were obtained from the blood by cutting the mice’s tail. To ensure the experimental animals remained in a comfortable condition, the mice were given ketamine anesthesia intraperitoneally (80 mg/kgBW) before cutting of the tail11.

Experimental animals

The experiments took place at the Laboratory of Pharmacology – Universitas Sumatera Utara (USU). The animals were purchased from the Department Biology of Mathematics and Scientific Faculty USU. In total, 30 male white mice (Mus musculus), Swiss Webster strain, 10 to 12 weeks old, and weighing 25 – 40 g were used. Before conducting the study, the mice were adapted to their cages (plastic (30 × 20 × 10 cm) and covered with fine wire mesh; base of the cages was covered with rice husks as thick as 0.5 - 1 cm and replaced every day during the study) for 2 weeks before the experiment started. They had 12 hours of daylight (6:00 A.M. – 6:00 P.M.) and 12 hours of dark (6:00 P.M. – 6:00 A.M.). The mice were fed with standard feed (CP 551) from PT Charoen Pokphand-Indonesia and water was given ad libitum. Room temperature and humidity were kept at normal ranges. They were weighed once a week to avoid stress.

Allocation and treatment groups

After the adaptation period, the mice were randomly divided into five groups, with each group consisting of 6 mice.

The sample size was calculated according to Federer’s formula12:

\[(t-1) (n-1)>15\]

\[t = \text{the number of groups}\]
\[n=\text{the number of samples}\]

All 30 mice were given a number (1–30) using a SPIDOL marker pen, then randomized by putting the numbers in an envelope and dividing them into 5 groups according to the numbers are taken from the envelope12.

Groups were as follows: 1) K0, negative control group/placebo, no treatment or high-fat food (only given aquadest 0,2 cc); 2) K1, positive control group, diet of HFC (quail yolk); 3) K2, HFC + cinnamon extract (CE) dose 2mg/20g body weight (BW); 4) K3, HFC + CE dose 4mg/20gBW; 5) K4, HFC + CE dose 8mg/20gBW.

The study was conducted for 28 days.

Cinnamon extract. CE was given to the mice every morning at 8:00 am. Animals were kept during research in the mice laboratory in the Pharmacology laboratory. Before being given CE, it was dissolved with aquadest. The extract was given to the mice orally using feeding tubes. CE interventions began on the 15th day until the 28th day.

Cinnamon extract (Cinnamomum burmannii) was obtained as Herbilogy Cinnamon Extract Powder® (PT.Phytochemindo Reksa, Bogor, Indonesia; batch no. 033CT).

High-fat containing food. The quail egg yolk was used to induce hypercholesterolemia, because the concentration of the
lipid in quail egg yolk is higher than chicken egg yolk. A dosage of 0.5 ml/day was given until day 28. The administration of quail egg yolk was done orally using feeding tubes as per the administration of CE.

Determination of dose of cinnamon. Based on research by Vanessa et al. in 2013, who saw a decrease in total blood cholesterol levels in white rats (Rattus norvegicus) by administering instant cinnamon powder drink (Cinnamomum burmannii Bl.) at a dose of 14.4 mg and 43.2 mg for 14 days. In their research, it was found that a dose of 14.4 mg can reduce cholesterol levels in rats. Therefore, we used a dose of 14.4 mg, because it was effective and efficient. We converted its dose to mice and increased it with variation doses of 2, 4 and 8 mg. The value converted from 200 g rat to 20 g mice is 0.14.

Rat dose = 14.4 mg
Conversion to mice = 0.14 × 14.4 mg = 2.01 mg
~ 2 mg/20 gBW mice

Laboratory analysis
Measurement of total cholesterol and BW of mice was performed on days 0, 14 and 28 (every two weeks). Ketamin 80 mg/kgBW mice intraperitoneally injected for anesthesia before the tail of the mice was cut.

Mice tails were cut 1-2 cm to draw blood, which was then assessed for total cholesterol using the digital autocheck® cholesterol measurement tool. Mice were weighed using a digital scale.

Statistical analysis
Data were analyzed using SPSS 24. The number average of sample data presented as mean ± SD. The one-way ANOVA statistical analysis to indicate the effects of treatments for all group. If the result is significant (p < 0.05), then bootstrapping was performed using post-hoc Bonferroni for differentiating between each group.

Results
Figure 1 shows that groups K0 (negative controls), K1 (positive controls), K2 (CE 2 mg/20 gBW), K3 (CE 4 mg/20 gBW), and K4 (CE 8 mg/20 gBW), had a decrease in total cholesterol levels. There is a significant difference in total cholesterol among the groups (p = 0.001 between groups). A post-hoc Bonferroni test was performed to see the difference in total cholesterol averages within each group.

It was seen that there was a difference in average total cholesterol levels between K0 (107.5 ± 1.87 mg/dl) vs K1 (120.3 ± 5.53 mg/dl), (p = 0.001). This shows that in the K1 group (positive control) given quail yolk succeeded in increasing cholesterol in mice with a significant difference compared to the K0 group (negative control), which was only given aquadest. In addition, differences in total cholesterol levels in the K1 group (120.3 ± 5.53 mg/dl) compared with K2 (107.3 ± 3.61 mg/dl), K3 (106.8 ± 4.57 mg/dl) and K4 (106.7 ± 0.51 mg/dl) groups showed that a significant decrease in total cholesterol levels (p = 0.001). This proves that CE is efficacious in reducing total cholesterol levels. Between groups K2, K3, and K4, which were all given CE, there was not a significant difference between total cholesterol levels (p > 0.05).

Figure 2 shows that there were increases in BW in all five groups. The largest increase of BW was in group K2, which was the positive control group who were given HFC quail egg yolk at 0.5 ml/20 gBW. The smallest increase in BW was found in group K5, which was the group provided with HFC.
quail egg yolk and CE with the dose of 8 mg/20gBW (highest dose in this study). One-way ANOVA showed that there was no significant difference in BW between groups \( (p=0.419) \), which could be inferred that the action of giving CE gave no effect in increasing BW in the mice.

Discussion

The aim of the study to investigated cholesterol levels was lowered in mice using CE and quail egg yolks for a high-fat diet for 28 days. In this study, it was proven that giving 0.5ml quail egg yolk for 28 days increased the total cholesterol level between the negative and positive control groups \( \text{(post-hoc Bonferroni } K0 \text{ vs } K1; p=0.001) \).

There was a significant difference in the decrease of total cholesterol after treatment among all groups in this research. However, this is not consistent with Vanessa et al. (2013) who stated that there was a decrease, but the difference was not statistically significant. Their study was only conducted for 14 days, whereas our study was conducted for 28 days, leading to differing results.\(^\text{16}\)

Cholesterol is formed by the action of HMG-CoA reductase enzyme \( (3\text{-hydroxy-3-methylglutaryl-CoA}) \). If hypercholesterolemia is left without implementing proper diet or treatment, it can cause occlusion in a blood vessel. To treat hypercholesterolemia, some medicines, such as simvastatin, could be given\(^\text{15}\). The use of statins will competitively block HMG-CoA reductase and efficiently reduce serum LDL cholesterol. But, treatment using simvastatin can cause rhabdomyolysis\(^\text{16}\). Thus, it needed research on traditional or herbal medicine, e.g. CE, that needs developed and have minimal adverse effects.

Cinnamon \( (Cinnamomum burmannii) \) has cinnamaldehyde as its biggest compound. Cinnamaldehyde, a phenolic compound abundantly found in \( Cinnamomum \). Bandara et al. (2011) stated that cinnamon had the ability to be an antioxidant, antivirus, antifungal, antimicrobe, antitumor, and can lower cholesterol and blood pressure with low fat compounds. Cinnamon is believed to have a direct role in lipid metabolism and preventing hypercholesterolemia and hypertriglyceridemia, as well as in preventing free fatty acids with its strong lipolytic activity.\(^\text{17}\) In the present study we believe that the increase of cholesterol levels due to quail egg yolk could be decreased with cinnamon by its ability to block HMG-CoA reductase enzyme and suppress lipid peroxidation through increased antioxidant enzyme activity\(^\text{18}\). In research by Pai et al. (2013), it was stated that cinnamaldehyde could lower cholesterol and triglyceride levels by the action of some enzymes secreted in certain amounts, which might contribute to bile acid synthesis\(^\text{19}\).

Our study showed that there was no difference in BW of the mice between groups \( (p = 0.419) \). This is not consistent with Vafa et al. (2012) who stated that consuming 3 grams of cinnamon for 8 weeks could decrease some biochemical and anthropometric variables compared to previous states significantly, i.e. a decrease of 1.19% body weight, 1.54% body mass index and 1.36% body fat. In addition to lowering BW variables, it could also decrease fasting blood glucose level by about 9.2%, and 6.12% of HbA1c and 15.38% of triglyceride levels.\(^\text{20}\)

Leaf and Antonio (2017) stated that the higher the food intake, the higher the increase in BW\(^\text{21}\). In the present study, in addition to standard food intake, giving hypercholesterol and inter-
Conventional food should increase BW, because the mice had increased energy intake. The amount of food intake will affect the amount of energy intake, which will be saved as fat and impact on the mice’s BWs, since energy intake is inversely proportional to physical activity. A high-fat diet group has low sensitivity to leptin, which results in increased appetite and food intake, thus increases the BW\(^2\). However, this was not the case in our study.

**Conclusion**

In the present study, a high-fat containing food used in combination with cinnamon extract (Cinnamomum burmannii) for 28 days could decrease total cholesterol levels in mice. Cinnamon extract is believed to have direct effects in lipid metabolism and prevent hypercholesterolemia and hypertriglyceridemia, as well as decreasing free fatty acids by its strong lipolytic activity.

**Data availability**

**Underlying data**

Figshare: data Analysis-cholesterol and bodyweight of mice.docx, https://doi.org/10.6084/m9.figshare.11901174.v2\(^2\)

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Acknowledgments**

The author gives appreciation and thanks to the Pharmacology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara for allowing the author to use the facility for collecting data in this research. In addition, thanks to all lecturers and examiners who had given advice and suggestions to the author, and all other parties who had contributed to this research.

**References**

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Mourad Akdad  
Department of Biology, Faculty of Science and Technology Errachidia (FSTE), Errachidia, Morocco

This study aimed to evaluate the effect of cinnamon extract (C. E) on cholesterol levels in mice with hypercholesterolemia. Authors demonstrated that C. E is useful to decrease cholesterol level, so its a promising plant to explore in the treatment of hypercholesterolemia and the related illness (hyperlipidemia, diabetes, atherosclerosis…).

1. Cinnamon has been shown to reduce total cholesterol, low-density lipoprotein and triglyceride levels, as well as increasing high-density lipoprotein levels. » precise the model of the study.

2. In introduction section, you must conclude by the aim of your research, this phrase “Mice were used in this study because of they have a similar biology to humans, and can therefore be a model for human hyperlipidemia8–10. must be include in discussion.

3. Avoid repetition such as: “This study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara, Indonesia. » « The experiments took place at the Laboratory of Pharmacology Universitas Sumatera Utara (USU). »

4. What is the type of extract (aqueuous, ethanolic…..), and the part of plant by which industry do extraction.

5. Try to explain why you don’t have a dose-dependent effect in your experiment.

6. « Figure 1 shows that groups K0 (negative controls), K1 (positive controls), K2 (CE 2 mg/20gBW), K3 (CE 4 mg/20gBW), and K4 (CE 8 mg/20gBW), had a decrease in total cholesterol levels. » why all groups have a decrease of Total Cholesterol, you don’t precise the days of this results you must clarify the idea.

7. In the section results, days of measurement must be righted in the description to clarify the idea.

8. The smallest increase in BW was found in group K5 », K5 ??, you would like to say K4?
9. In the present study we believe that the increase of cholesterol levels due to quail egg yolk could be decreased with cinnamon by its ability to block HMG-CoA reductase enzyme and suppress lipid peroxidation through increased antioxidant enzyme activity. The mechanism involved in the antihypercholesterolemic effect must be declared with precaution, 'may be'.

10. Our study showed that there was no difference in BW of the mice between groups (p = 0.419). This is not consistent with Vafa et al. (2012) who stated that consuming 3 grams of cinnamon for 8 weeks could decrease some biochemical and anthropometric variables compared to previous states significantly, i.e. a decrease of 1.19% body weight, 1.54% body mass index and 1.36% body fat. In addition to lowering BW variables, it could also decrease fasting blood glucose level by about 9.2%, and 6.12% of HbA1c and 15.38% of triglyceride levels. Model used by the study cited must be declared.

11. In the present study, a high-fat containing food used in combination with cinnamon extract (Cinnamomum burmannii) for 28 days could decrease total cholesterol levels in mice. This conclusion must be reformulated, high-fat containing food was used only to have the model for studying the effect of CE on cholesterol level, so the conclusion must be related only to CE.

References

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Ethnopharmacology, medicinal plants, diabetes, hypertension

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
Tahereh Farkhondeh
Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran

The present study investigated the effect of cinnamon extract on body weight and cholesterol levels of mice-fed HFD. It is a good idea.

Some revisions should be done:

Abstract
1. In the method section several point should be cleared:
   1. Type of cinnamon extract.
   2. Female or male mice.

Introduction
1. The aim of study should be better mentioned. It was not clear the question about the effect of plant on the lipid profile. In the past paragraph, the author described the lipid lowering effect of extract. Did this evidence extracted from traditional medicine reports?

Method
1. Did you used xylazine with ketamine for analgesic effect? It should be mentioned. Animal ethics did not accept this anesthesia procedure.

2. Please more about cholesterol measurement (technique, instrument, kit).

Conclusion
1. If you tested the quail egg yolks as a high-fat diet that induces hypercholesteremia. It is necessary in the abstract and introduction in the aim section.

2. In the first paragraph, you should about your findings without analytic indicator such as p-value.

3. You did not measure the changes in TG or FFA therefore, you should not concluded about in the conclusion section.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: metabolic disorder

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

RESPONSE to review’s Tahereh Farkhondeh
Cardiovascular Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran
The present study investigated the effect of cinnamon extract on body weight and cholesterol levels of mice-fed HFD. It is a good idea.

Some revisions should be done:

Abstract
1. In the method section several point should be cleared:
   1. Type of cinnamon extract.
   
   Answer:
   Type of cinnamon extract used in this study had been explained part of METHODS (page 3 manuscript in F1000research).
   We used Cinnamomum burmannii in powder extract.
   
   2. Female or male mice.
   
   Answer:
   The sample animal were male mice. This is one of the inclusion criteria of this study. Its mean to homogenized (make smaller of the differences in each samples) for prevent BIAS. As we known, there are differences between male and female will increasing number of lipid and dysfunction metabolic, including of hyperlipidemia. It related to level of hormones.

Introduction
1. The aim of study should be better mentioned.

   Answer:
   Thank you for your comment, we would confirm our statement on the aim of this study.
   The aim of the study to determine cinnamomum burmannii effective in reducing total cholesterol levels of mice (Mus musculus) given high fat feed.
   
   It was not clear the question about the effect of plant on the lipid profile.
Answer:
We had explained about the effect of plant on the lipid profile related to few references. Please see in part of DISCUSSION (page 5 manuscript in F1000research). Cinnamon is believed to have a direct role in lipid metabolism and preventing hypercholesterolemia and hypertriglyceridemia, as well as in preventing free fatty acids with its strong lipolytic activity17.

In the present study we believe that the increase of cholesterol levels due to quail egg yolk could be decreased with cinnamon by its ability to block HMG-CoA reductase enzyme and suppress lipid peroxidation through increased antioxidant enzyme activity18.

In research by Pai et al. (2013), it was stated that cinnamaldehyde could lower cholesterol and triglyceride levels by the action of some enzymes secreted in certain amounts, which might contribute to bile acid synthesis19.

(This study needed continuos some research in the future to measured number of cinnamaldehyde in Cinnamon Extract Powder® per 1 mg CE used. Then we'll do new research to prove how CE mechanism as anti hypercholesterolemia, ls it blocked by HMG-CoA reductase enzyme and suppress lipid peroxidation through increased antioxidant enzyme activity or dysfunction of bile acid synthesis or both mechanism) ?

In the past paragraph, the author described the lipid lowing effect of extract. Did this evidence extracted from traditional medicine reports?
Answer:
Yes, the explain about “this has been suggested as a substance……triglyceride levels” better moves to part of DISCUSSION, because we took from one of reference. We will add inform about the quail egg yolks as a high-fat diet that induces hypercholesterolemia in part of ABSTRACT. We can not add inform in the AIM of study. Because, if we added in the Aims of study, it was not consequence to the title of study. If we changed title "Benefit of cinnamon (Cinnamomum burmannii) in lowering total cholesterol level after consumption of the quail egg yolk as a high-fat diet that induces hypercholesterolemia". It will make the title very long and not in accordance to the rules of journals F1000Research.

Method
1. Did you used xylazine with ketamine for analgesic effect It should be mentioned. Animal ethics did not accept this anesthesia procedure.

Answer:
Yes, we did. We corrected uncomplete in methods ketamin with xylazine. We choosed of Injectable Anesthetic Agents, we used Ketamine/xylazine* We follow to GUIDELINES - PREPARATION OF KETAMINE/XYLAZINE COCKTAIL FOR MICE. Dose Ketamine 80-100 mg/kg intra peritoneal (IP) and Xylazine 10-12.5 mg/kg IP.(reference 11. Vertebrate Animal Research. The University of Iowa).

The administration of effective anaesthetic agents to avoid impact/effects of the experimental procedures when we did cut off mice tail to collect blood sample with anesthesia to make minimize pain and prevent distress caused of pain in mice.

1. Please more about cholesterol measurement (technique, instrument, kit).

Answer:
In Laboratory analysis study showed how was technique to take blood from tail correction in methods study cut of mice tail.

“Mice tails were cut 2 – 5 mm to draw blood, which was then assessed for total cholesterol using the digital autocheck® (merck of digital cholesterol measured tool). We used stick cholesterol to measured total cholesterol of mice.

Regarded to follow National Centre for the Replacement, Refinement & Reduction (NC3Rs) of Animals in Research to measured (page 4 manuscript in F1000research).

Conclusion

1. If you tested the quail egg yolks as a high-fat diet that induces hyperchloestromia. It is necessary in the abstract and introduction in the aim section.

Answer:
Yes you right. We had tried to add in abstract before, but it was more than 250 words, it was not allow with Journal F1000research rules. But we will try again to put in your comment in abstract.

1. In the first paragraph, you should about your findings without analytic indicator such as p-value.

Answer:
I'm sorry, I can’t catch what you mean in this statement. Would you like to describe more detail?

1. You did not measure the changes in TG or FFA therefore, you should not concluded about in the conclusion section.

Answer:
Yes, thank you for your comment. We are agree with you.

Conclusion:
The cinnamon extract (Cinnamomum burmannii) proved it can be decrease of total cholesterol levels of for 14 days in mice consuming high-fat containing compared to group without given CE.

Competing Interests: No competing interests were disclosed
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