

Biochemical effects of *Calotropis procera* on hepatotoxicity

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Abstract— Introduction: *Calotropis procera* commonly known as 'Sodom apple' is a 6-meter high shrub that belongs to the Asclepiadaceae plant family and is commonly found in West Africa and other tropical places. In Saudi Arabia the plant is commonly used in traditional medicine for the treatment of variety of diseases including fever, constipation, muscular spasm and joint pain. **Aim:** In the present study *C. procera* were investigated for the hepatoprotective activity. **Material and Methods:** Carbon tetrachloride is used to produce hepatotoxicity. Forty-two male albino rats, weighting 150-200 gm divided into seven groups, each consisted of 6 rats. Carbon tetrachloride 2 ml/kg was administered twice a week to all of the groups of animals except group I, which served as control and given the normal saline. Group II served as carbon tetrachloride control. Group III received Silymarin at 100 mg/kg/day dose, Group IV received aqueous leaves extracts *C. procera* 200 mg/kg, Group V received chloroform leaves extracts *C. procera* 200 mg/kg, Group VI received ethanol leaves extracts *C. procera* 200 mg/kg, Group VII received latex of *C. procera* 200 mg/kg. The effect of aqueous, chloroform, ethanol leaves extract and latex *C. procera* on biochemical parameters of liver was measured. **Results:** The results showed that the aqueous, chloroform, ethanol leaves extract and latex *C. procera* produced significant decrease in acid phosphatase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein, albumin and total bilirubin levels compared to the CCL₄ treated group II. **Conclusion:** *Calotropis procera* appears to have hepatoprotective activity and these may be due to enrich of the plant by phytoconstituents that activate and hence a pharmacological response of different parts of the body and this study need further studies to show the complete properties of the plant.

Keywords: *Calotropis procera*, hepatoprotective, biological activity, medicinal potentials

INTRODUCTION

From olden days, plants sources have been used to treat and prevent diseases. A lot of research have been done on plants sources and became the main supply of drugs for use in medicine during the modern era. A large number of medicinal plants affirm the significance of herbs as medicinal remedies. Hence, standardized experimental procedures should be employed to test the efficacy of the drugs. Liver is a very important organ of the body which is a place where lots of metabolic reactions occur. Hence any injury to liver may affect all these metabolic reactions and may lead to serious health problems to the human body (Halilu et al., 2012).

Commonly known as 'Sodom apple', *Calotropis procera* is a 6-meter high shrub that belongs to the Asclepiadaceae plant family and is commonly found in West Africa and other tropical places in the region (Irvine, 1961). The plant is described as large, tall, erect and perennially branched with milky latex throughout. The root bark secretions have key traditional uses in India such as skin ailments treatment, cure for abdominal viscera enlargements and de-worming agent (Parrotta, 2001). Cutaneous mycosis (ringworm), leprosy and syphilitic sores are treated with the milky latex of the plant (Burkill, 1985). In Nigeria, *C. procera* is regarded a vital traditional medicine that can be solely used or in combination with other herbal remedies and is applied for the management of febrile diseases, indigestion, rheumatism, eczema, diarrhea and common colds. Latex mixed with honey is good for rabies treatment as well as for cough and toothache (Burkill, 1985). The plant's leaf extracts, latex and chopped leaves have shown potential as an anti-nematodes both *in vivo* and *in vitro* (Khirstova and Tissot, 1995). The leaves of *C. procera* as an alternative for water treatment and its effect to diminish total viable count have been indicated (Shittu, 2004).

West African traditional doctors have been utilizing the plant in curing numerous diseases (Ayoola et al., 2008). Based on the available morphological studies, the leaves of *C. procera* are characterized as broadly ovate, obovate, ovate-oblong or elliptical, measuring 6-15 cm by 4.5-8 cm, subsessile, acute, pubescent when young and as they mature turn into glabrous on both sides (Alam and Ali, 2009).

MATERIALS AND METHODS

Collection of Plant material

The fresh leaves and latex of *calotropisprocera* were collected from the Dawadmi Kingdom of Saudi Arabia.

Calotropisprocera leaves were air dried under shed and blend into powder form by using electric blender (Moulinex). This powder was stored in glass air tight jar and kept under room temperature.

The fresh latex of *calotropisprocera* was collected in sterile 250 ml conical flask by making incision at stem near the leave attachment and stored in ice bag to maintain homogeneity and activity during transport.

Preparation of different plant extracts by cold maceration method

A stock solution of 5% (5 grams of leaves powder in 100 milliliters of aqueous, Chloroform or Ethanol) was prepared and kept in Stoppard bottles. Mixing was carried out in a conical flask on a magnetic stirrer for 6 hours with the flask shaken every 30 minutes. After 24 hours, the solution was filtered through cotton wool, and used for the study.

The latex centrifuged (3000-4000 rpm) at room temperature (25°C) 15 min to remove inter coagulum. Supernatant was then filtered through a membrane filter and the filtrate (the pure latex) was kept in cool place for the study.

Animals

Male albino rats 150-200 g were used in this study. They were kept under light and dark cycles of 10 and 12 h respectively for one week before and during the experiments. Standard diet was given to animals and food was withdrawn 18-24 h before the experiment. Animal experiments were carried out according to the guidelines of the Animal Ethical Committee of the Institute.

Preliminary phytochemical screening

Preliminary phytochemical screening has done as per the methods mentioned in Practical pharmacognosy (Kokate, 1994).

Acute toxicity test

Acute toxicity test has done for the leaves and latex of *Calotropis procera* as per the standard method (OECD No: 425). Six albino female rats were used in this study. The dose of aqueous, chloroform, ethanol

leaves extracts and latex of *C. procera* were prepared with 1% gum acacia and was administered orally.

Hepatoprotective studies

Carbon tetrachloride 2 ml/kg body weight was injected subcutaneously twice a week in a 7-day period to induce hepatotoxicity in all groups of animals except group I, which received the normal saline 5ml/kg once daily. Group II served as Carbon tetrachloride control. Group III treated with Silymarin at 100 mg/kg/day dose, Group IV treated with aqueous leaves extracts *C. procera* 200 mg/kg, Group V treated with chloroform leaves extracts *C. procera* 200mg/kg, Group VI treated with ethanol leaves extracts *C. procera* 200 mg/kg, Group VII treated with latex of *C. procera* 200 mg/kg (Reddy et al., 1993).

Blood samples were withdrawn and serum separated. Serum was used for the estimation of alkaline phosphatase, (ALP), total protein (TPL), aspartate aminotransaminase, (AST), alanine aminotransaminase (ALT), acid phosphatase (ACP), albumin (Alb) and total bilirubin (TB) levels. Biochemical parameters were assessed in method of calorimetric enzymatic using commercial kits (Boehringer Mannheim Diagnostica GmbH, Mannheim, Germany).

Statistical evaluation

One-way analysis of variance (ANOVA) followed by the Dunnett multiple comparisons test was used for the statistical analysis. The data presented as mean \pm SEM, n= 6. $P < 0.01$ was considered to be significant.

RESULTS

Preliminary phytochemical test

Phytochemical screening showed that *C. procera* contains glycosides, saponins and terpenes, alkaloids, flavanoids.

Acute toxicity test

Acute toxicity test showed that there was no any significant changes in the behavioral and neurological responses up to 2500 g/kg b. wt. and non-toxic nature of the aqueous, chloroform; ethanol leaves extracts and latex of *Calotropis procera*.

Hepatoprotective study

The carbon tetrachloride-treated animals showed that there was increase in serum SGOT, SGPT, ACP, ALP, total protein, Albumin and total bilirubin levels. The

groups treated with aqueous, chloroform; ethanol leaves extracts and latex of *Calotropis procera* showed significant decrease in the level of serum marker enzymes as compare with CCl₄ treated group II.

From the tables (1-2) and figures (1-7), we can observe significant hepatoprotective effects of the aqueous, chloroform; ethanol leaves extracts and latex of *Calotropis procera* (Table 1-2, Fig. 1-7). The level of acid phosphatase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein, albumin and total bilirubin were analyzed in serum samples of different groups of albino rats, in the group II CCl₄ treated animals showed the level of marker enzymes were significantly high when compared to the normal group I animals, but there was significant decrease of the enzyme level ($p < 0.01$) in the aqueous, chloroform; ethanol leaves extracts and latex of *Calotropis procera* treated animals group IV, V, VI and VII respectively.

DISCUSSION

Calotropis procera is well known plant in many different region worldwide including United Arab Emirates, Yemen and Saudi Arabia (Chatterjee and Pakrashi, 1994).

The previous studies showed that the plant leaves contain calotropin and calotropenone while the latex contain uzarigenin, and terpenol ester, in addition *Calotropis procera* contain cardiac glycosides and many other compounds that have different pharmacological activity including insecticidal, antifungal and anticancer (Ahmed et al., 2006), analgesic activity of dry latex (Saber et al., 1969), antifertility activity ethanolic extract of the roots (Saxena and Saxena, 1979) antitumor potential of the root extracts (Mathur et al., 2009), anthelmintic of the flowers (Larhsini et al., 1997). This study aims to test some medicinal potentials and biological activity of crude aqueous, chloroform and ethanolic extract of *Calotropis procera* leaves in addition to its latex.

The activity of any herbal plant is related on its ability to either reduce the deleterious effects or in maintain the normal function of liver. CCl₄ is a well-known animal model for hepatotoxicity and can be justified by increased level of liver function maker enzymes (Reddy et al., 1993).

Table 1. Effect of leaves extracts and latex of *Calotropis procera* on biochemical parameters

Groups	Treatment mg/kg	AST LEVEL (IU/L)	ALT LEVEL (IU/L)	ACP LEVEL (IU/L)	ALP LEVEL (IU/L)	TP LEVEL (g/dl)	Alb LEVEL (g/dl)	TB LEVEL (mg/dl)
I	Normal control	38.07 ±1.45	35.11±1.32	1.63±0.31	41.25±2.5	6.59±1.25	2.8±0.21	0.594±0.25
II	CCL ₄	275.36±25.65	229.62±5.56	4.32±0.51	559.72±25.2	4.25±1.29	1.8±0.32	1.255±0.29
III	Silymarin + CCL ₄	165.45±6.67	136.78±5.33	2.3±0.12	220.14±15.3	5.75±1.15	2.1±0.40	0.751±1.15
IV	Aqueous extract + CCL ₄	298.34±6.43	180.34±2.54	3.22±0.34	350.12±19.6	5.68±1.36	1.99±0.28	0.982±0.36
V	Chloroform extract + CCL ₄	175.6±13.34	159.59±5.67	2.95±0.98	250.34±14.4	5.67±1.15	2.23±0.27	0.978±0.15
VI	Ethanol extract + CCL ₄	178.7±2.39	156.6±2.23	2.09±1.43	248.35±11.5	5.22±1.25	2.3±0.35	0.802±0.25
VII	Latex + CCL ₄	168.9±4.19	149.5±2.65	2.01±1.15	225.41±13.9	5.16±1.23	2.1±0.40	0.765±0.23

Values are expressed as mean ± S.E.M. (n=6) for the groups of 6 animals in each group. * *P* < 0.05 significant with respect to II Group.

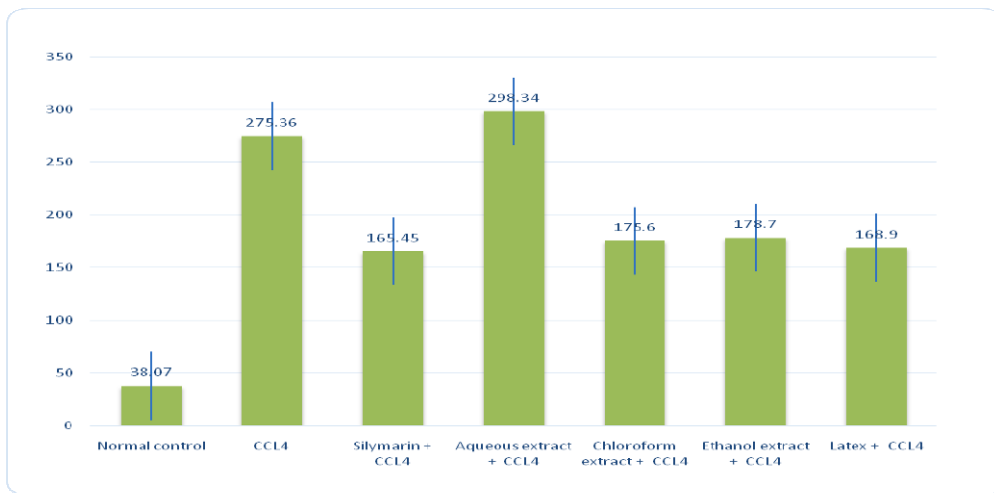


Figure 1. The mean level of SGOT in different groups.

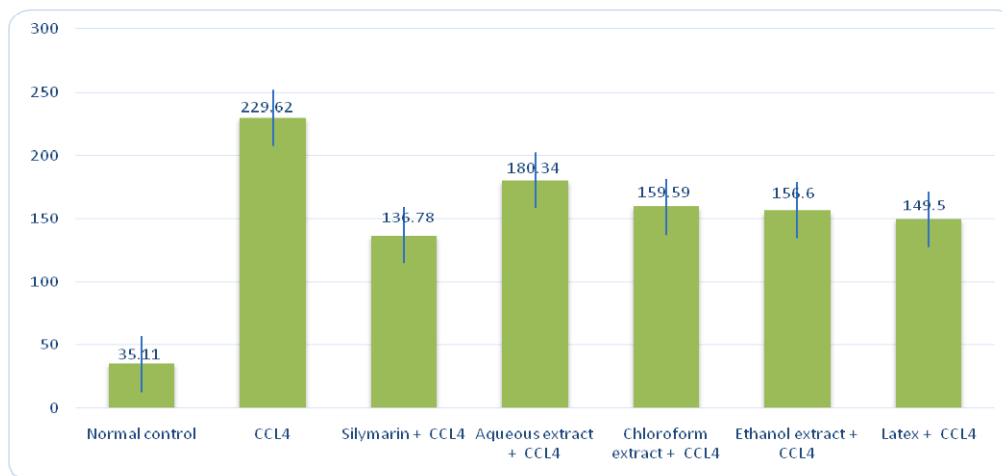


Figure 2. The mean level of SGPT in different groups.

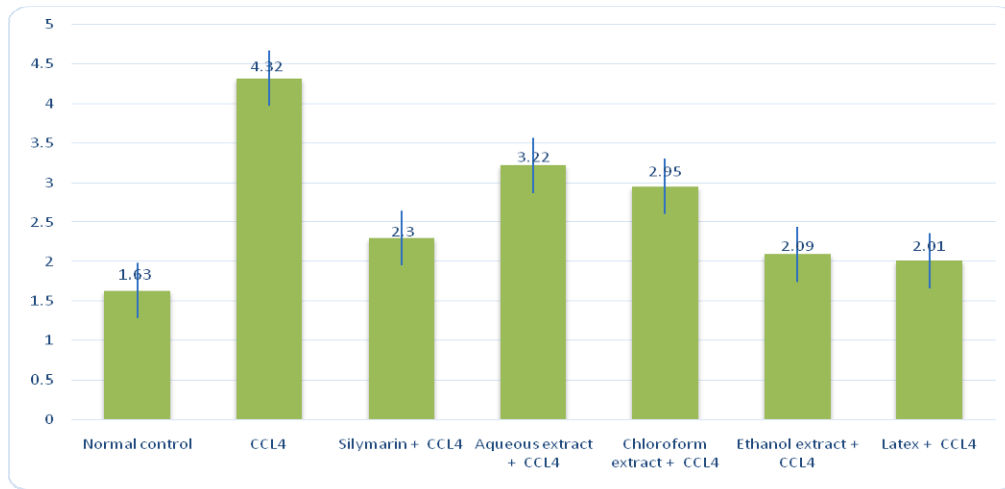


Figure 3. The mean level of Acid phosphatase (ACP) in different groups.

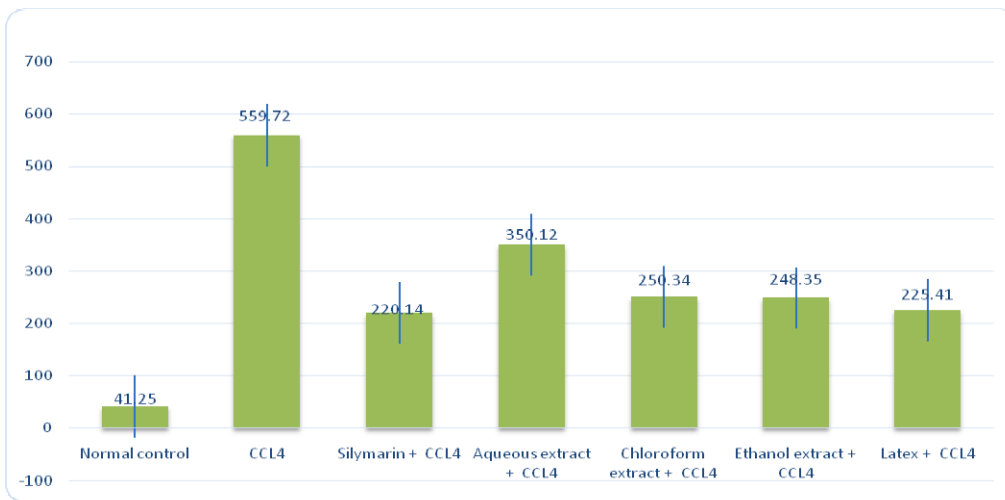


Figure 4. The mean level of Alkaline phosphatase (ALP) in different groups.

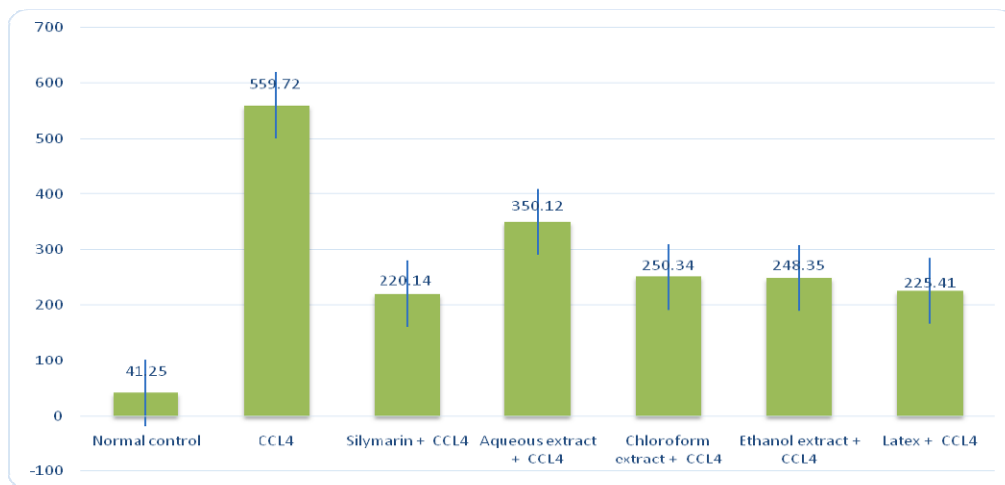


Figure 5. The mean level of total protein in different groups.

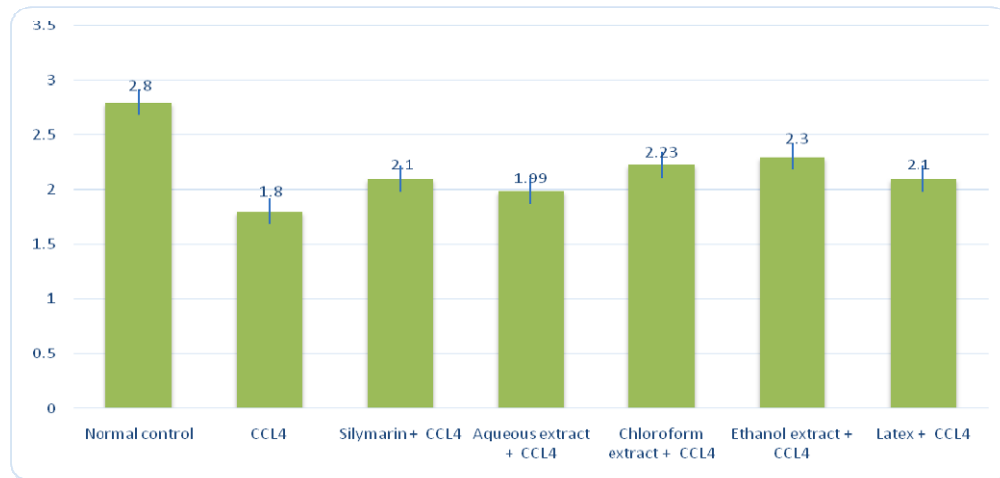


Figure 6. The mean level of Albumin in different groups.

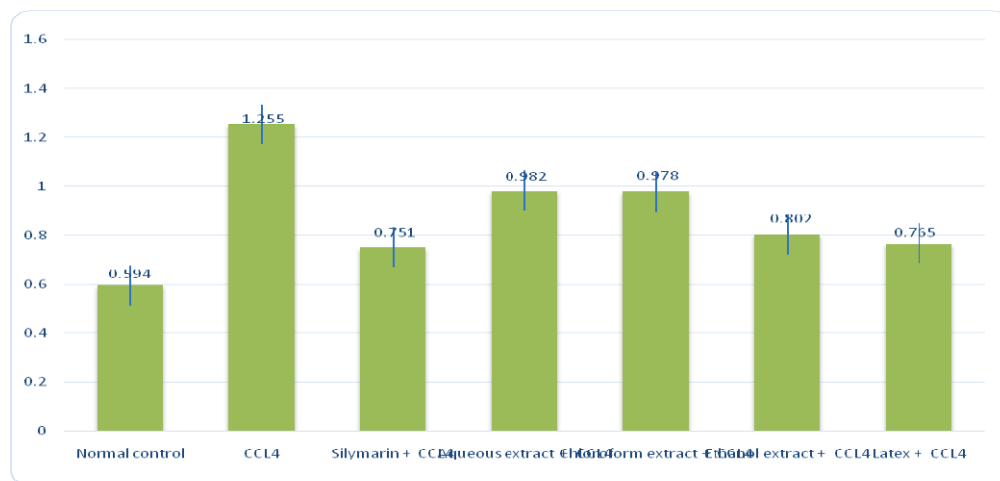


Figure 7. The mean level of Total bilirubin (TB) in different groups.

Orally administered doses of aqueous, chloroform; ethanol leaves extracts and latex of *Calotropis procera* produced significant decrease in acid phosphatase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total protein, albumin and total bilirubin levels. The activity of the latex of *Calotropis procera* is found to be more than to the aqueous, chloroform; ethanol leaves extracts of *Calotropis procera*. Administration of aqueous, chloroform, ethanol leaves extracts and latex of *Calotropis procera* showed recovery against the toxic effects of CCL₄. The hepatoprotective efficacy of *Calotropis procera* may be due to the presence of alkaloids, flavanoids, glycosides, saponins and terpenes which have hepatoprotective

properties. The result of this investigation indicated that the aqueous, chloroform, ethanol leaves extracts and latex of *Calotropis procera* possess hepatoprotective activity against CCL₄ induced liver damage in rats. Further research is to be needed for better understanding the mechanism of its hepatoprotective efficacy.

Previous study by (Ramachandra Setty S1, 2007) showed that hydro-ethanolic extract (70%) of *Calotropis procera* flowers find to have hepatoprotective effect against paracetamol-induced hepatitis in rats through enhancing the SGPT, SGOT, ALP, bilirubin and cholesterol levels and these agree with the our results .

Calotropis procera aqueous root extract that was also

found to contain phenolics (15.76±1.52) mg PGE/g) and flavonoids (1.62± 0.05) mg QE/g) that enable the plant to defense against free radical mediated damage (Kumar et al., 2013). These may explain the enhancement that observed in activity of the plant against CCl₄ induced liver damage in our study.

In contrast, another study showed that the ethanolic leaves extract of *Calotropis procera* is detrimental to the integrity of the liver tissues as evident in the necrotic nature of liver based on the histological observations (Buraimoh, 2011).

CONCLUSION

In the present investigation, we have revealed that the aqueous, chloroform, ethanol leaves extracts and latex of *Calotropis* possess hepatoprotective activity in CCl₄ induced hepatotoxicity in rats, which supports the usual use of this plant as well as its prior findings. The replacement of synthetic with traditional antioxidants is superb. Our study showed good hepatoprotective activity.

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Competing interests

The authors declare that they have no competing interests.

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