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Effect of ethanolic fruit extract of *Solanum xanthocarpum* Schrad. & Wendl. against D-galactosamine-induced hepatopathy in experimental rodents

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ABSTRACT

The present study was designed to evaluate hepatoprotective potential of the ethanolic fruit extract of *Solanum xanthocarpum* against D-galactosamine-induced hepatopathy induced liver toxicity in experimental animals. In the present study, *in- vivo* hepatoprotective effect of 50% Ethanolic fruit extract of *Solanum xanthocarpum* (SXE, 100, 200 and 400 mg/kg body weight) was evaluated using two experimental models D-galactosamine (D-GalN) (200 mg/kg, body weight, i.p.) induced hepatotoxicity in experimental animals. The hepatoprotective activity was assessed using various biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatise (ALP), γ-glutamyl transferase (γ-GT) and total bilirubin. Meanwhile, *in vivo* antioxidant activities as lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were screened along with histopathological studies. Obtained results demonstrated that the treatment with SXE significantly ($P<0.05$-$P<0.001$) and dose-dependently prevented chemically induced increase in serum levels of hepatic enzymes. Furthermore, SXE significantly (up to $P<0.001$) reduced the lipid peroxidation in the liver tissue and restored activities of defence antioxidant enzymes GSH, SOD and catalase towards normal levels. Histopathology of the liver tissue showed that SXE attenuated the hepatocellular necrosis and lead to reduction of inflammatory cells infiltration. The results of this study strongly indicate the protective effect of SXE against acute liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

INTRODUCTION

A large number of plants have been used in India since ancient times, which claim the efficient cure of hepatic disorder. One of the reputed plants is *Solanum xanthocarpum* Schrad. and Wendl. (Family: Solanaceae), commonly known as Yellow Berried Nightshade (syn-Kantakari), found throughout India, mostly in dry places like a weed on roadsides and wastelands. The fruits are glabrous, globular berries, green, and white strips when young but yellow when mature. The fruits are known for several medicinal uses such as anthelmintic, antipyretic, laxative, anti-inflammatory, anti-asthmatic, diuretic, urinary stone, and aphrodisiac activities. The stem, flowers, and fruits are prescribed for relief in burning sensation in the feet accompanied by vesicular eruptions[1]. The fruits are reported to contain several steroidal alkaloids like solanacarpine, solanacarpidine, solancarpine, solasonine, solamargine and other constituents like caffeic acid, coumarins like aesculetin and aesculin, steroids carpesterol, diosgenin, campesterol, daucosterol and triterpenes like cycloartanol and cycloartenol were reported from the fruits[2]. Formulations containing *S. xanthocarpum* are being promoted for use in conditions like irregular menses, menopause, breast cancer and infertility[3]. The antispasmodic, cardiotonic, hypotensive, anti-anaphylactic, arbuda tumour and anti-urolithiatic activities were

also reported. Solasodine is present in a number of Solanum species (Solanaceae) such as Solanum khasianum, *Solanum xanthocarpum, Solanum nigrum,* Solanumgracile, Solanum laciniatum etc. Lupeol, apigenin and solamergine exhibited Solasodine anticancer property, anti-nociceptive, antioxidant activities of the chloroform extract and hypoglycaemic. The flavanoids quercitrin and apigenin glycosides are the major chemical constituents which are present in the fruits of *S. xanthocarpum* constituents which are present in the fruits of *S. xanthocarpum*[4]. To the best of our knowledge there was lack of scientific reports available in support of its traditional claim of hepatoprotective potential. So far, there has been only few researches had been reported on hepatoprotective effect, against paracetamol[5], carbon-tetrachloride[6] and anti-tubercular drug[4] induced liver toxicity. Therefore, present study was designed to demonstrate the effect of *Solanum xanthocarpum* fruit extract (SXE) against D-galactosamine induced hepatic damage in experimental animals.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade and procured from Sigma chemicals Co., USA and Qualigens fine chemicals, Mumbai, India.

Animals

Wistar rats weighing (150-170 g) of either sex were procured from Animal house of Shri Ram Murti Smarak College of Engineering and Technology, Bareilly. They were kept in departmental animal house in well cross ventilated room at 22±2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given *ad-libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 222/2000/CPCSEA).

Preparation of plant extract

Fresh and matured fruits were collected from campus garden of National Botanical Research Institute, Lucknow, India in June 2014. The plant material was identified and authenticated and the voucher specimen number NBRI-SOP-222 was deposited in the institutional herbarium. The freshly collected fruits (2 kg) of *Solanum xanthocarpum* were dried and powdered. The powdered plant a material (900 g) was macerated with petroleum ether, the marc was exhaustively extracted with of 50% ethanol for three days. The extract was separated by filtrations and concentrated on rota vapour (Buchi,USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure. The yield obtained was 198.40 g of solid residue (yield 22.04% w/w). The extract obtained was further subjected to preliminary phytochemical screening and pharmacological investigation.

Acute oral toxicity studies

Acute toxicity study was performed according to OECD guidelines No. 423[7]. Swiss albino mice of either sex were divided into six groups with six animals each. SXE was administered orally as a single dose to mice at different dose levels of 250, 500, 1000, 1500 and 2000 mg/kg *b.w*. Animals were observed periodically for the symptoms of toxicity and death within 24 h and then daily for 14 days.

D-Galactosamine (D-GalN)-induced hepatotoxicity

The rats were randomly divided into six groups of six animals each. Group I (control) animals were administered a single daily dose of sodium carboxymethyl cellulose (0.3%, 5 ml/kg body weight, p.o. body weight). Group II was served as D-GalN treated control and received the vehicle. Groups III-V was treated with SXE at the dose levels of 100, 200 and 400 mg/kg body weight. Group VI was treated with standard drug silymarin at 100 mg/kg body weight. All these treatments were given orally for 8 days. On the last day of the treatment, the animals of groups IIVI received a single dose of D-GalN in distilled water at 200 mg/kg body weight *i.p* after 1 h of the vehicle, SXE or standard silymarin treatments. On the 9th day, the animals were anesthetized by anesthetic ether the liver samples were dissected and blood was collected [8].

Assessment of hepatoprotective activity

The collected blood was allowed to clot and serum was separated at 2500 rpm for 15 min and the biochemical parameters like serum enzymes: aspartate aminotransferase (AST, U/L), glutamate pyruvate transaminase (ALT, U/L)[9], alkaline phosphatase (ALP, U/L)[10], total bilirubin (mg/dL)[11] and gamma glutamyl transferase (γ-GT) using assay kits[12].

Assessment of antioxidant parameters

Assessment of lipid peroxidation (LPO)

The dissected out liver samples were washed immediately with ice cold saline to remove as much blood as possible. Liver homogenized (5%) in ice cold 0.9% NaCl with a Potter-Elvenhjem glass homogenizer. The homogenate was centrifuged at 800 for 10 min and the supernatant was again centrifuged at 12,000 for 15 min and the obtained mitochondrial fraction was used for the estimation of LPO[13]. A volume of the homogenate (0.2 ml) was transferred to a vial and was mixed with 0.2 ml of a 8.1% (w/v) sodium dodecyl sulphate solution, 1.5 ml of a 20% acetic acid solution (adjusted to pH 3.5 with NaOH) and 1.5 ml of a 0.8% (w/v) solution of thiobarbituric acid (TBA) and the final volume was adjusted to 4.0 ml with distilled water. Each vial was tightly capped and heated in a boiling water bath for 60 min. The vials were then cooled under running water. Equal volumes of tissue blank or test samples and 10% trichloroacetic acid were transferred into a centrifuge tube and centrifuged at 1000g for 10 min. The absorbance of the supernatant fraction was measured at 532 nm (Beckman DU 650 spectrometer). Control experiment was processed using the same experimental procedure except the TBA solution was replaced with distilled water[14]. Malonyldialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen thiobarbituric acid reactive substance. 1, 1, 3, 3-tetra ethoxypropan was used as standard for calibration of the curve and is expressed as nmole/mg protein.

Assessment of catalase and superoxide

The liver tissue was homogenized (5%) and mitochondrial fraction was prepared as described above. Decomposition of H_2O_2 in presence of catalase (CAT) was followed at 240 nm[15]. One unit (U) of catalase was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per min, at 25 °C and pH 7.0. Results are expressed as units (U) of CAT activity/mg protein. Superoxide dismutase (SOD) activity was estimated by the inhibition of nicotinamide adenine dinucleotide reduced)-phenazine methosulphatenitrobluetetrazolium reaction system as described

by Nishikimi et al.[16] and as adapted by Kakkar et al.[17]. One unit of the enzyme is equivalent to 50% inhibition in the formazan formation in 1 min at room temperature (25 ± 2°C) and the results have been expressed as units (U) of SOD activity/mg protein.

Assessment of reduced glutathione (GSH) activity

The concentration of GSH was determined by the method of Anderson[18] based on the development of a yellow colour when 5,5-dithiobis (2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups. The reaction mixture contained equal volumes of 4% sulfosalicylic acid and tissue samples homogenized in 4 volume of ice cold 0.1 m/l phosphate buffer (pH 7.4). The method used for estimating GSH in this study also measures non-protein sulfhydryl concentration inclusive of GSH. However, 8090% of the non-protein sulfhydryl content of the cell represents free endogenous GSH. Enzyme activity was expressed as milligram per hundred grams[19].

Histopathological studies

For histological studies, the liver tissues were fixed with 10% phosphate buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (5M) were cut and stained with routine hematoxylin and eosin stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Statistical analysis

The values were represented as mean ± S.E.M. for six rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software (Version 3.0) for the determination of level of significance. The values of $p<0.05$ was considered statistically significant.

RESULT

Acute toxicity studies

Solanum xanthocarpum produces no mortality at 2000 mg/kg. Therefore, one-tenth of the maximum no mortality dose of extract were selected as therapeutic middle dose (200 mg/kg) and just double as well as half dose of it as highest (400 mg/kg) and lowest dose (100 mg/kg) respectively, in this study.

Effect of SXE on AST, ALT, ALP, γ-GT and total bilirubin against D-GalN induced liver injury in rats

The effect various doses of SXE were studied on serum marker enzymes and total bilirubin in D-GalN intoxicated animal. Hepatic injury induced by D-GalN caused significant changed in marker enzyme as AST by 90.62%, ALT by 50.48%, ALP by 163.10%, γ-GT by 218.98% and total bilirubin by 57.71% compared to control group. The percentage protection in marker enzyme of treated group at 100, 200 mg/kg as AST 3.87 ($P< 0.05$), 15.67 ($P< 0.001$), ALT 2.36 (ns), 9.61 ($P< 0.01$), ALP 2.22 ($P< 0.05$), 13.86 ($P< 0.001$), γ-GT 13.49 (ns), 38.89 ($P< 0.001$) and total bilirubin 11.92 ($P< 0.05$), 18.34 ($P< 0.01$) compared to toxic group while maximum percentage protection in marker enzyme at the dose of 400 mg/kg and silymarin (100mg/kg) as AST 36.87 ($P< 0.001$), 43.37 ($P< 0.001$), ALT 20.89 ($P< 0.001$), 29.45 ($P< 0.001$), ALP 42.47 ($P< 0.001$), 53.70($P< 0.001$), γ-GT 42.52 (P<

Table 1. : Effect of SXE on serum AST (U/L), ALT (U/L), ALP (U/L), Total Bilirubin (TBL) level (mg/dl), γ-GT (U/L) against D-galactosamine induced liver toxicity in rats.

Groups	AST	ALT	ALP	TBL	γ-GT
Control	146.38 ±3.25	81.21 ±2.1	162.75 ± 3.0	0.70±0.06	2.16 ± 0.13
D-GalN.	279.04±4.38†	122.21±2.47†	428.21±3.89†	1.09±0.03†	6.89±0.65†
SXE 100	268.24±3.21^{a}	119.32±2.97^{n}	418.67±3.01^{a}	0.96±0.02^{a}	5.96±0.21^{n}
SXE 200	235.31±3.01^{c}	110.46±2.08^{b}	368.86±3.09^{c}	0.89±0.02^{b}	4.21 ± 0.42^{c}
SXE 400	176.21±2.95^{c}	96.68±2.01^{c}	246.31±2.49^{c}	0.76 ±0.04^{c}	3.96 ±0.31^{c}
SYL 100	158.01±2.95^{c}	86.21±1.96^{c}	198.24 ± 2.4^{c}	0.72± 0.02^{c}	2.36 ± 0.30^{c}

Values are mean ± S.E.M. of 6 rats in each group
n : non significant
P values: †<0.001 compared with respective control group I
P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with group II (D-galactosamine)

Table 2. : Effect of SXE on liver LPO (MDA nmole/min/mg of protein), GSH (nmole/mg of protein), SOD (unit/mg of protein) and CAT (units/mg of protein) against D-galactosamine induced liver toxicity in rats.

Groups	LPO	GSH	SOD	CAT
Control	1.31 ± 0.17	1.26 ± 0.02	26.22 ±1.96	51.21 ± 3.1
D-GalN.	0.32 ± 0.02†	0.38 ± 0.01†	9.26 ±1.08†	29.12 ± 2.2†
SXE 100	0.59 ± 0.01^{n}	0.52 ± 0.03^{a}	11.24 ±1.28^{n}	38.61 ± 2.1^{a}
SXE 200	0.64 ± 0.02^{a}	0.60 ± 0.04^{b}	17.28 ± 2.01^{a}	41.27 ± 2.2^{b}
SXE 400	0.89 ± 0.07^{c}	0.98 ± 0.05^{c}	21.21 ± 2.02^{c}	46.21 ± 2.9^{c}
SYL 100	1.21 ± 0.01^{c}	1.04 ± 0.06^{c}	23.06 ± 2.9^{c}	49.71 ± 3.1^{c}

Values are mean ± S.E.M. of 6 rats in each group
n : non significant
P values: †<0.001 compared with respective control group I
P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with group II (D-galactosamine)

0.001), 65.74 (P< 0.001), and total bilirubin 30.27 (P< 0.001), 33.94 (P< 0.001) which is almost comparable to the group treated with silymarin, a potent hepatoprotective drug used as reference standard (Table 1).

Estimation of LPO, GSH, SOD and CAT against D-GalN induced liver injury in rats

The results in table 2 showed clear significant percentage change in the levels of LPO in D-GalN intoxicated rats as 75.57 (P<0.001) compared to control group. Treatment with SXE at the doses of 100, 200 and 400 mg/kg significantly prevented this heave in levels and the percentage protection in LPO were 84.37 (ns), 100 (P< 0.05) and 178.12 (P< 0.001) respectively. The GSH, SOD and CAT content had significantly increased in SXE treated groups whereas D-GalN intoxicated group had shown significant decrease in these parameters compared to control group. The percentage changed of GSH, SOD and CAT in *D-GalN* intoxicated group were as 69.84 (P< 0.001), 64.68 (P< 0.001) and 43.13 (P< 0.001) respectively. The percentage protection in GSH as 36.84 (P< 0.05), 57.89 (P< 0.01), 157.89 (P< 0.001) and SOD 21.38 (ns), 86.60 (P < 0.05), 129.04 (P < 0.001) while in CAT 32.58 (P< 0.05), 41.72 (P < 0.01), 58.68 (P < 0.001) at the doses levels 100, 200 and 400 mg/kg, respectively. In different doses level of SXE, 400 mg/kg has shown maximum protection which was almost comparable to those of the normal control and silymarin.

Histopathological observations

The histological observations basically support the results obtained from serum enzyme assays. Liver section in normal control rats showed central vein surrounded by hepatic cord of cells while in D-Galactosamine treated rats liver showing congestion in central vein, necrosis, and the loss of cellular boundaries. Whereas the SXE treated groups showed absence of cell necrosis, but with minimal inflammatory conditions. The SXE 400 mg/kg, p.o. treated group showed regeneration of hepatocyte around central vein with near normal liver architecture possessing higher hepatoprotective action (Fig.1).

DISCUSSION

As a result of considerable attention that has been devoted to the liver diseases during the last two decades, the vast majority of studies have been concerned with the liver of the experimental animals and the characteristics of animal model's normal liver have been well defined. In the absence of reliable liver-protective drugs in modern medicine, a large number of medicinal plants with long and well-established traditional use have been recommended for treatment of liver disorders[20]. However, their application requires detailed in vivo pharmacological characterization[21], and clinical studies the reafter. In the present investigation, *Solanum xanthocarpum* (SXE) was evaluated for the hepatoprotective activity using D-GalN hepatotoxicity in rat. The hepatoprotective activity of SXE, we investigated whether this extract protects against D-GalN-induced acute liver injury. D-GalN is also a well-established hepatotoxicant, inducing a liver injury witch closely resembling human viral hepatitis in its morphologic and functional features and, therefore, it is very useful for evaluation of hepatoprotection[22,23]. D-GalN hepatotoxicity is considered as an experimental model of acute hepatitis and it does not affect other organs[24]. D-GalN has great liver specificity because hepatocyte has high levels of galactokinase and galactose-1-uridyltransferase, and it disrupts the synthesis of essential uridylate nucleotides. Depletion of these nucleotides ultimately impairs the synthesis of protein and glycoprotein, leads to progressive damage of cellular membranes resulting in a change

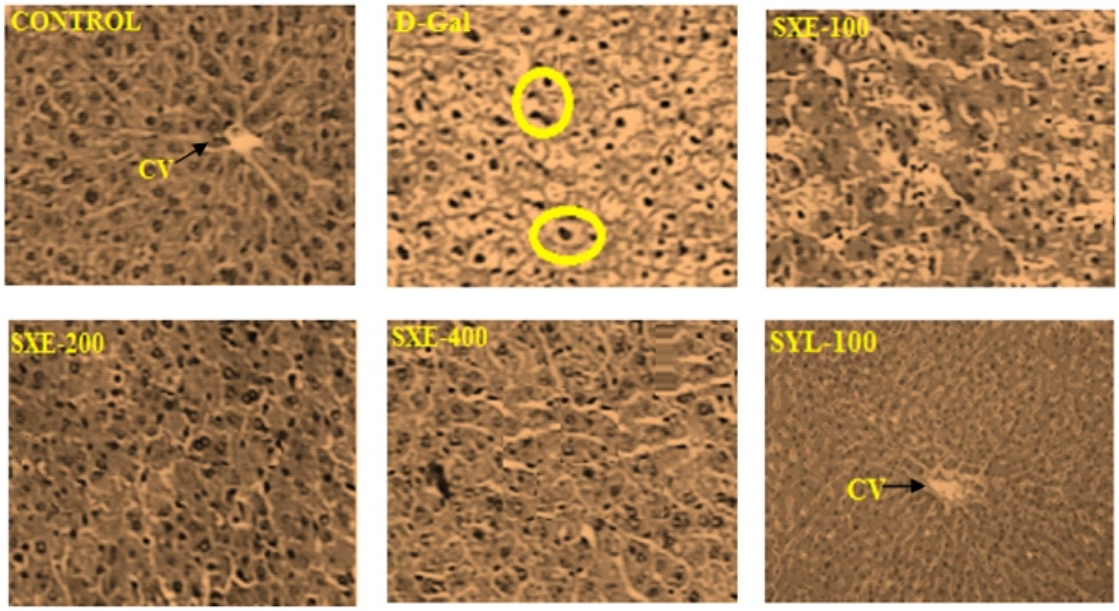

Fig. 1. : CHistopathology of liver tissues. (CONTROL) Liver section of normal control rat shows central vein surrounded by hepatic cord of cells (normal architecture). (D-Gal) Liver section of D-galactosamine treated rats showing congestion in central vein, necrosis, and the loss of cellular boundaries (indicated by arrow). (SXE-100) Liver section of rats treated D-galactosamine and 100 mg/kg of SXE showing inflammatory collections around central vein and focal necrosis with sinusoidal dilatation. (SXE-200) Liver section of rats treated D-galactosamine and 200 mg/kg of SXE absence of necrosis. (SXE-400) Liver section of rats treated D-galactosamine and 400 mg/kg of SXE showing regeneration of hepatocyte's around central vein toward near normal liver architecture. (SYL-100) Liver section of rats D-galactosamine and 100 mg/kg of silymarin showing normal liver architecture.

in permeability of the cellular membrane, and finally with enzyme leakage from the cells[25]. Liver damage induced by D-GalN generally reflects disturbances of liver cell metabolism which lead to characteristic changes in the serum enzyme activities[26]. The increased levels of AST, ALT, ALP, and γ-GT in this study may be interpreted as a result of the liver cell destruction or changes in the membrane permeability indicating the severity of hepatocellular damage induced by D-GalN, which is in accordance with previous reports[25]. Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function[27]. Pre-treatment with SXE extract (at different doses level 100, 200 and 400 mg/kg) attenuated the increased activities of these enzymes (AST, ALT, ALP and γ-GT) in serum caused by D-GalN. Recovery towards normalisation suggests that SXE extract causes parenchymal cell regeneration in liver, thus protecting membrane fragility, thereby, decreasing enzyme leakage and extract mediated suppression of the increased bilirubin level suggests the possibility of the extract being able to stabilise biliary dysfunction. Oxidative stress has been reported as one of the major causes of DGa1N-induced liver damage, excessive production of free radicals resulting from oxidative stress can damage macromolecules as lipids and the D-Ga1N injection decreased liver GSH, SOD and CAT; these results were in agreement with Najmi et al.[28] who indicated that D-Ga1N-intoxicated rats showed an increased TBARS (Thio-barbituric acid reactive substance) level, a typical parameter of lipid peroxidation. Also, Zhou et al.[29] indicated that treatment with DGa1N decreased antioxidative enzyme activities. The current results showed that SXE increasing liver antioxidant parameters and decreased oxidative stress which appeared in decreasing the mean value of liver TBARS and compared to D-GalN. The reduced activities of GSH, SOD and catalase observed point out the hepatic damage in the rats administered with D-GalN but the treated with 100, 200 and 400 mg/kg of SXE groups showed significant increase in the level of these enzymes and normalise the level of LPO, which indicates the antioxidant activity of the *Solanum xanthocarpum*. The hepatoprotective effect of the SXE was further accomplished by the histopathological examinations. SXE at different dose levels offers hepatoprotection, but 400 mg/kg is more effective than all other lower doses. As demonstrated in our study, administration of D-GalN significantly elevated serum levels of hepatic enzymes, indicating considerable hepatocellular damage. Our study confirmed the protective effect of SXE against D-GalN in rats. In rat, SXE hepatoprotective activity is quite similar to silymarin, a reference hepatoprotective agent. On phytochemical screening, SXE revealed the presence of flavonoids, steroidal alkaloids, triterpenes, flavanoids, quercitrin and apigenin glycosides are the major chemical constituents. Hence, it is possible that the mechanism of hepatoprotection of *Solanum xanthocarpum* may be due to its antioxidant property present in these phytochemicals by reducing the oxidative stress imposed by D-galactosamine and other like anti-inflammatory property[30] which may prevent inflammatory hepatic damage.

CONCLUSION

The results of this study strongly indicate the protective effect of SXE against acute liver injury which may be attributed to its hepatoprotective activity, and there by scientifically support its traditional use.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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