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Antidiabetic activity of methanol extract of *Acorus calamus* in STZ induced diabetic rats

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ABSTRACT

Objective: To investigate the antihperglycemic activity of methanol extract of *Acorus calamus* (AC) rhizome in streptozotocin (STZ) induced diabetic rats. **Methods:** Oral glucose tolerance test (OGTT) was performed in normal rats. Male albino rats were rendered diabetic by STZ (40 mg/kg, intraperitoneally). 200 mg/kg of AC extract was administered orally to diabetic rats for 21 days to determine the antihyperglycemic activity by estimating various biochemical parameters. **Results:** Oral administration of AC methanol extract showed significant restoration of the levels of blood glucose level. After 21 days of treatment, blood glucose, lipid profile (total cholesterol, LDL and HDL-cholesterol), glucose 6-phosphatase, fructose 1,6 bis phosphatase levels and hepatic markers enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) were decreased when compared with diabetic control. Plasma insulin, tissue glycogen, glucose-6-phosphate dehydrogenase levels were increased significantly compared to diabetic control. Concurrent histopathological studies of the pancreas showed comparable regeneration by extract which were earlier necrosed by STZ. **Conclusions:** The results exhibited that AC methanol extract possess potent antihyperglycemic activity in normal and STZ induced diabetic rats and so might be of useful in the management of diabetes. The phyto-treatment showed more efficient antihyperglycemic effect than the standard drug glibenclamide.

1. Introduction

Diabetes is one of the most debilitating diseases in the world. It is a condition in which body is unable to metabolize glucose, which can in turn be due to lack of production of insulin or insensitivity towards it[1]. Various therapies are available such as hypoglycemic drugs, insulin and recently cellular therapy, but these therapies have their own limitations[2]. Hence, alternative strategies and formulations are required to encounter this problem. One of the solutions resides in the traditional medicines using medicinal plants which have been effective against various diseases and disorders. One such plant is *Acorus calamus* (AC) which has been widely described in Indian and Chinese medicines.

Acorus calamus belongs to the family of Acoraceae and is generally known as sweet flag.

The rhizomes of AC are considered to possess aphrodisiac, diuretic, antispasmodic, rheumatism, eczema, antihelminthics[3], antiinflammatory and antioxidant properties[4]. It is used to treat dysentery in children, bronchitis, nervous complaints, remittent fever, colic pain and dyspepsia[5]. Morever, it also increases learning performance and reduces memory disorder[6]. Parab and Mengi[7] demonstrated the hypolipidemic activity of AC in managing coronary artery disease. In addition, the radix of AC is widely used in the therapy of diabetes in traditional folk medicine of America[8]. This plant not only possesses antiproliferative and immunomodulatory activity but also has anticarcinogenic properties[9].

With the plant having such beneficiary properties, not much work has been conducted in relation to the field of diabetes. So far, it has been reported that ethyl acetate fraction of AC leaf helps in increasing insulin sensitivity and has antidiabetic effects and this is due to its role in releasing insulin and alpha-glucosidase inhibition which helps in improving postprandial hyperglycemia[10,11]. Due to lack of sufficient literature on the role of AC in diabetes, we, in our current study have checked various parameters

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to ascertain the antidiabetic activity of this plant. Here, we investigated the antihyperglycemic effect of methanol extract of AC rhizome in streptozotocin induced diabetic rats.

2. Materials and methods

2.1. Plant materials

AC rhizome was collected form National Institute of Siddha, Chennai and authenticated by Angelin Vijayakumari, Head, Department of Plant Biology and Biotechnology, Voorhees College, Vellore, India. Shade dried rhizome (250 g) was milled and extracted using methanol (250 mL) in Soxhlet apparatus for 8 h. Then, the extract was evaporated to dryness and the final dry chocolate colour crude extract was stored in dark at −20 ℃ until used for the experiments.

2.2. Chemicals

Streptozotocin was obtained from Sigma Chemicals, Bangalore, India. Kits to estimate alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) were purchased from Qualigens Diagnostics, Mumbai, India. All other chemicals were of analytical grade.

2.3. Experimental animals

Healthy adult Wistar male albino rats with body weight around 170−200 g at 60−70 days from birth were procured from Madavaram Vertinery Medical College, Chennai,Tamil Nadu. They were kept in polycarbonate cage housed in a room with a 12 h light/12 h dark cycle at (25±2) ℃. The rats were fed with standard rat pellet diet (Pranav Agro Industry Ltd, Maharastra) and given water ad libitum.

2.4. Glucose tolerance test (GTT) in normal rats

The rats divided into 4 groups. Group 1 received 1 mL of distilled water only. Group 2, 3 and 4 received methanol extract of AC rhizome at doses of 50, 100 and 200 mg/kg orally, in 1 mL of water suspension, respectively. Glucose (2 g/kg/mL) was administered orally to each rat. Blood samples were collected from the tail vein before and after 30, 60 and 120 min of glucose loading and the blood glucose level were measured using a glucometer (One Touch HorizonTM).

After assessment of antihyperglycemic activity with different doses of AC methanol extract in normal rats, maximum fall in blood glucose level was observed with the dose of 200 mg/kg. Therefore, further studies were carried out with 200 mg/kg dose administered daily for a period of 21 days in STZ-diabetic rats.

2.5. Induction of diabetes

Rats previously fasted for 16 h were given single intraperitoneal injection of 40 mg/kg body wt. streptozotocin (Sigma, USA) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). Animals with fasting blood glucose over 250 mg/dL, three days after streptozotocin administration were considered diabetic and they received treatment similar to that of normal rats.

2.6. Experimental Design

In the experiment totally 24 rats (6 normal and 18 STZ diabetic surviving rats) were used. These rats were divided into four groups of 6 rats each. The AC methanol extract were dissolved in distilled water and administered orally for 21 days.

Group I Normal rats
Group II Diabetic rats
GroupIII Diabetic rats + AC methanol extract (200 mg/kg)
Group IV Diabetic rats + Glibenclamide (600 μg/kg)

2.7. Biochemical analysis

Blood glucose estimation was done using a glucometer on 0, 7, 14, 21 days after treatment with AC extract. At the end of 21 days, the animals were euthanized between 9:00−11:00 am to minimize diurnal variation. Insulin level was estimated in plasma of normal and STZ induced diabetic rats by ELISA method. The glycogen level of liver and skeletal muscles was measured by anthrone method[12]. Carbohydrate metabolising enzymes such as glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, fructose-1,6-bisphosphatase were estimated by the methods of Koide and Oda[13], Bergmeyer[14], Gancedo and Gancedo[15] respectively. Lipid profile (total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride) levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India).The activities of AST, AST and ALP were assayed in the serum using commercial kits purchased from Qualigens Diagnostics (Mumbai, India).

2.8. Histopathological examination of pancreas

Pancreatic tissues from all groups were subjected to histopathological studies. The pancreas was immediately dissected out, washed with cold physiological saline and fixed in 10% formalin, then immediately processed by the paraffin technique, sectioned and stained by hematoxylin and eosin (H & E) for histological examination.

2.9. Statistical analysis

One-way ANOVA and Student's t-test (SPSS program; version 11.5) were carried out to compare the data with the level of significance set at $P<0.05$.

3. Results

3.1. Effect of AC extract on GTT

AC extract, when administered prior to glucose loading produced significant reduction ($P<0.05$) in the rise in blood glucose levels at 60 min after glucose administration. AC extract at doses of 50, 100 and 200 mg/kg produced reduction in blood glucose in a dose dependent manner respectively when compared to vehicle treated group at 60 min (Table 1). 200 mg/kg AC extract showed more significant antihyperglycemic activity than the other two doses (50 and 100 mg/kg). Hence further studies was carried

out with 200 mg/kg AC extract.

3.2 Effect of AC extract on fasting blood glucose in diabetic rats

The effect of repeated oral administration of AC methanol extract on blood glucose levels in STZ-diabetic rats is presented in Table 2. AC extract at the doses of 200 mg/kg to STZ-treated diabetic rats caused significant ($P<0.05$) reduction of blood glucose levels which was related to

Table 1

Effect of GTT of AC methanol extract on blood glucose levels in normal rats ($n=6$).

Groups	0 min	30 min	60 min	120 min
Vehicle control	68.93±2.21	146.36±1.24	133.02±1.44	123.00±1.39
AC (50 mg/kg)	65.21±1.25	134.23±0.44*	119.57±1.19*	102.00±1.53*
AC (100 mg/kg)	65.98±1.02	128.71±1.35*	109.19±1.65*	96.12±0.83*
AC (200 mg/kg)	65.92±1.63	123.57±2.29*	98.94±8.73*	75.13±2.01*
Glibenclamide (600 μg/kg)	65.55±1.32	123.35±0.98*	114.43±1.03*	78.80±1.21*

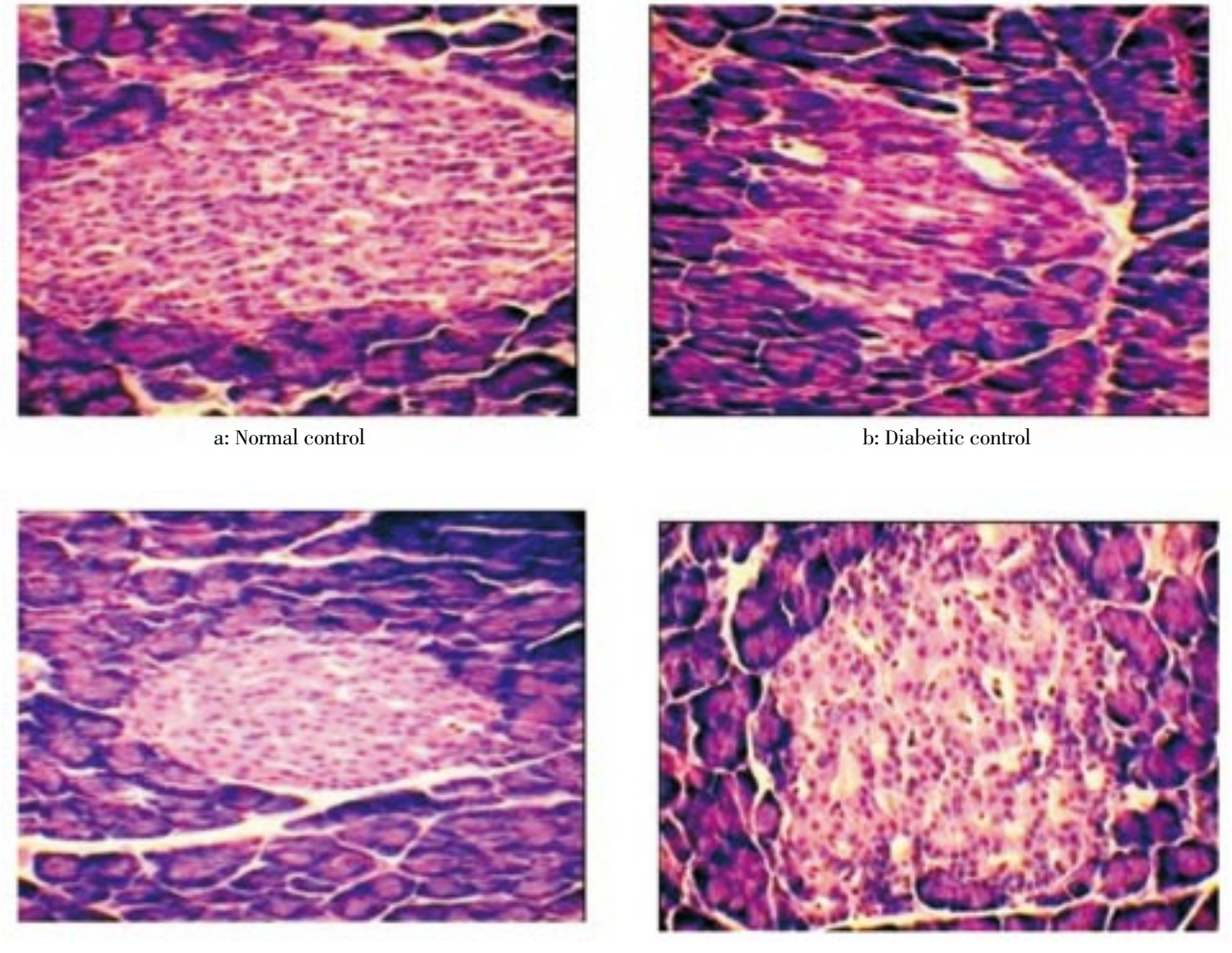
All the values are (mg/dL) mean±SEM for six rats. ** Values deviate very significantly from diabetic control group ($P\leq 0.05$).

Table 2

Effect of AC methanol extract on fasting blood glucose levels in normal and STZ induced diabetic rats ($n=6$).

Groups	0th day	7th day	14th day	21st day
Normal control	73.29±1.13	74.92±6.43	74.77±0.89	72.34±1.08
Diabetic control	267.01±9.59	282.32±6.23	285.66±5.94	288.95±5.20
Diabetic + AC (200 mg/kg)	248.19±2.31	180.79±0.69*	161.65±1.25*	114.24±1.29*
Diabetic + Glibenclamide (600 μg/kg)	243.64±1.86	167.25±3.46*	125.20±1.97*	105.78±1.33*

All the values are (mg/dL) mean±SEM for six rats. ** Values deviate very significantly from diabetic control group ($P\leq 0.05$).



a: Normal control

b: Diabeitic control

c: Diabetic+AC methanol extract (200 mg/kg)

D: Diabetic+Glibenclamide (600 mg/kg)

Figure 1. Histopathological examination of pancreas in normal and STZ induce diabetic rats.

duration of treatment. Maximum reduction was observed on the 21st day. Glibenclamide exhibited a significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

3.3 Effect of AC extract on plasma insulin in diabetic rats

STZ caused a significant decrease in plasma insulin. Administration of AC methanol extract (200 mg/kg) caused significant (P<0.05) increase in insulin levels at the end of the study. The significant increase in plasma insulin level by AC extract (200 mg/kg) was comparable to the increment of plasma insulin level by glibenclamide (Table 3).

Table 3

Effect of oral administration of AC extract on plasma insulin levels in normal and STZ induced diabetic rats (n=6).

Groups	Plasma Insulin (μU/mL)
Normal control	128.97±1.57
Diabetic control	46.50±6.60
Diabetic + AC (200 mg/kg)	113.43±1.54*
Diabetic + Glibenclamide (600 μg/kg)	118.68±0.95*

All the values are mean ± SEM for six rats; *Values deviates significantly from diabetic control (P≤ 0.05).

3.4 Effect of AC extract on glycogen content in diabetic rats

Glycogen content in liver and skeletal muscle decreased significantly (P<0.05) in diabetic control compared to normal control (Table 4). Administration of AC at the doses of 200 mg/kg for 21 days resulted in significant (P<0.05) increase in the glycogen levels in both the liver and skeletal muscle than glibenclamide.

3.5 Effect of AC extract on gluconeogenic enzymes in diabetic rats

Table 5 shows the activities of carbohydrate metabolism enzymes in the liver of normal and STZ induced diabetic rats. The activity of glucose-6-phosphatase and fructose-

Table 4

Effect of oral administration of AC extract on liver and muscle glycogen content levels in normal and STZ induced diabetic rats (n=6).

Groups	Liver glycogen (mg/g wet tissue)	Muscle glycogen (mg/g wet tissue)
Normal control	12.48±0.38	9.52±0.17
Diabetic control	5.01±0.19	1.70±0.10
Diabetic + AC (200 mg/kg)	12.10±0.36*	8.22±0.13*
Diabetic + Glibenclamide (600 μg/kg)	12.63±0.35*	8.58±0.23*

All the values are mean±SEM; *Values deviate very significantly (P<0.05) when compared with diabetic control values.

Table 5

Effect of oral administration of AC extract on gluconeogenic enzyme activities in liver of normal and STZ induced diabetic rats (n=6).

Groups	Glucose 6-phosphatase (U/min/mg protein)[a]	Glucose 6-phosphate dehydrogenase (U/mg protein)[b]	Fructose 1, 6-bisphosphatase (U/h/mg protein)[c]
Normal control	0.160±0.009	4.515±0.022	0.447±0.013
Diabetic control	0.382±0.011	2.140±0.100	0.797±0.004
Diabetic + AC (200 mg/kg)	0.225±0.006*	3.687±0.093*	0.571±0.007*
Diabetic + Glibenclamide (600 μg/kg)	0.190±0.008*	4.075±0.058*	0.557±0.010*

All values are in mean±SEM; *Values deviate significantly (P<0.05) when compared with diabetic control values. a-μmol of Pi liberated per hour; b-μmol of glucose phosphorylated per hour; c-nmol of NADPH formed per min.

Table 6

Effect of the AC methanol extract on total cholesterol, triglycerides and HDL-cholesterol in normal and STZ- induced diabetic rats (n=6).

Groups	Serum lipid profile (mg/dL)		
	Total cholesterol	HDL-cholesterol	Triglycerides
Normal control	50.59±1.40	72.51±1.29	52.72±1.70
Diabetic control	203.06±10.99	24.51±1.41	204.45±1.80
Diabetic + AC (200 mg/kg)	95.18±1.42*	74.84±1.23**	74.07±1.18**
Diabetic + Glibenclamide (600 mg/kg)	97.82±3.60*	72.86±1.12**	74.04±1.52**

All the values are mean ± SEM for six rats,* values deviate significantly from diabetic control ** values deviate very significantly from diabetic control group (P≤ 0.05).

Table 7

Effect of oral administration of AC methanol extract on serum marker enzymes in normal and STZ induced diabetic rats (n=6).

Groups	ALT (μ/L)	AST (μ/L)	ALP (μ/L)
Normal control	25.67±0.66	75.72±1.37	84.69±1.18
Diabetic control	64.87±1.09	127.17±0.73	147.15±1.23
Diabetic + AC (200mg/kg)	30.71±1.38*	94.45±1.09*	97.73±0.83*
Diabetic + Glibenclamide (600 μg/kg)	25.81±0.57*	88.42±1.17*	89.35±0.94*

All the values are mean ± SEM for six rats; ** values deviate very significantly (P≤0.05) when compared with diabetic control values.

1,6-bisphosphatase enzyme increased in diabetic rats whereas the activity of glucose-6-phosphate dehydrogenase decreased when compared with normal control rats. Oral administration of AC extract decreased the activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase enzyme thereby increasing the activity of glucose-6-phosphate dehydrogenase enzyme significantly when compared with diabetic control rats.

3.5. Effect of AC extract on lipid profile in diabetic rats

Table 6 represents the serum lipid profile of normal and diabetic control and diabetic treated rats. Over five fold increases was observed in total cholesterol, triglycerides and two fold decreases in HDL cholesterol level in diabetic control compared to normal control. AC methanol extract treated diabetic rats significantly reduced total cholesterol, triglycerides and increased HDL cholesterol compared to diabetic control.

3.6. Effect of AC extract on hepatic markers in diabetic rats

Table 7 shows the levels of hepatic markers in all the experimental group of rats. Diabetic rats showed elevated activities of AST, ALT and ALP when compared to normal rats. Treatment with AC methanol extract at a dose of 200 mg/kg for 21 days brought back the activities of these serum markers to near normal levels. Similar effects were observed with glibenclamide, but they were less in magnitude when compared with those of AC extract. Even at this high dose there were no gross behavioural changes.

3.7 Histological analysis

Histological examination of pancreas showed normal histology in normal rats and rats treated with AC extract. Pancreas in diabetic rats showed shrinkage of islets and growth of adipose tissue. Pancreas in diabetic rats treated with AC extract or glibenclamide reduced these changes. (Figure 1).

4. Discussion

The increase in number of diabetic patients has motivated the scientists to find new methods to cure diabetes. Inspite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease[16]. The present study is the preliminary assessment of the antidiabetic activity of the methanol extract of Acorus calamus rhizome. The extracts showed a significant fall in fasting blood glucose (FBG) in STZ induced diabetic rats.

From GTT it could be concluded that AC extract at the dose of 200 mg/kg showed the maximum improvement in glucose tolerance. When AC extracts were administered to glucose loaded normal rats, reduction in the blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h. In our study, the difference observed between the initial and final FBG levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group at the end of 21 days experimental period. Administration of AC methanol extract to diabetic rats showed a significant decrease in the FBG and an increase in the serum insulin levels may be due to the presence of saponins, glycosides and sequiterpenoids which possesses hypoglycemic property[7,18,19]. Hence, the possible mechanism by which AC brings about its hypoglycemic action may be by potentiating the plasma insulin effects by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. The hypoglycemic activity of AC rhizome extract was compared with glibenclamide, a standard hypoglycemic drug.

Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. The regulation of glycogen metabolism *in vivo* occurs by the enzymes glycogen synthase and glycogen phosphorylase. The reduced glycogen store in the diabetic rats has been attributed to the reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. This is probably due to lack of insulin in the diabetic state, which results in the inactivation of the glycogen synthetase systems[20]. Skeletal muscle is also a major site of insulin-stimulated glucose uptake[21]. Decrease in both muscle and hepatic glycogen was observed in this study. Oral administration of AC extract (200 mg/kg) for 21 days significantly increased muscle and liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract.

Glucose-6-phosphatase is a crucial enzyme for the final step of gluconeogenesis or glycogenolysis in which it catalyzes the hydrolysis of glucose-6-phosphate to glucose and phosphate. Glucose is transported out of the liver to increase blood glucose concentration. Normally insulin inhibits the hepatic glucose production by suppressing Glucose-6-phosphatase and fructose-1,6-bisphosphatase enzyme activities[22]. In diabetic rats, administration of AC extract at the dose of 200 mg/kg to diabetic rats decreased the activities glucose-6-phosphatase and fructose-1,6-bisphosphatase thereby decreasing gluconeogenesis and also increased the activity of glucose-6-phosphate dehydrogenase.

Since lipid abnormalities accompanying with premature atherosclerosis are the major causes of cardiovascular diseases in diabetic patients therefore ideal treatment for diabetes, in addition to glycemic control, should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes[23].

The liver is regarded as the central metabolic organ in the body, with an important role in glucose and lipid homeostasis[24]. AST, ALT, and, ALP are considered as liver toxicity markers[25].The increase in the activities of plasma AST, ALT and AST indicated that diabetes may be induced hepatic dysfunction .Therefore, the increment of the activities of AST, ALT and ALP, in plasma may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream , which gives an indication on the hepatotoxic effect of STZ[26]. Treatment of the diabetic rats

with AC methanol extract caused reduction in the activity of these enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. Histopathological observation also revealed that the alterations occurred in the architecture of pancreatic islets in streptozotocin-induced diabetic rats. By oral administration of AC extract or glibenclamide for 21 days, the alterations were apparently reverted back to near normal.

In conclusion, our results clearly showed that the methanol extract of AC rhizome possesses potent antihyperglycemic activity in STZ induced diabetic rats and further study is needed to identify the compounds responsible for its promising *in vivo* antidiabetic activity. Our study adds credence to the traditional use of AC to treat diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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