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COMPARISON OF ANTIDIABETIC, HYPOLIPIDEMIC AND PROTECTIVE EFFECTS HYDROALCOHOLIC EXTRACTS OF LEAVES, BARKS AND ROOTS OF *SALACIA OBLONGA* IN ALBINO WISTAR RATS

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ABSTRACT

Salacia oblonga (SO) has been used for thousands of years for the treatment of obesity and diabetes. The present study was undertaken to evaluate antidiabetic, hypolipidemic and toxicological assessments of SO extracts obtained from root, bark and leaves. The extracts were administered to streptozotocin (STZ) rats once in a day for a period of fifteen days at 100 mg/kg dose. Blood glucose levels and body weight changes at different days (1st, 5th, 10th and 15th days) were measured during experiment. Serum lipid profiles were also estimated to investigate the hypolipidemic potential. Various oxidative stress and hepatic biomarker enzymes in serum were also determined to investigate the toxicity potential of extracts. Results collectively suggested that all extracts at 200 mg/kg dose produced significant reduction in blood glucose level and restored body weight, signifying antidiabetic action. Hypolipidemic action was observed via reduction of lipid and increase of HDL in serum during administration of extracts. Various oxidative stress biomarkers and hepatic enzymes levels were normalized with respect to diabetic control. The extracts demonstrated good antidiabetic, hypolipidemic and antioxidant properties in STZ diabetic rats.

Keywords :*Salacia oblonga*, Antidiabetic, Hypolipidemic, Antioxidant

INTRODUCTION

Lifestyle-related diseases, predominantly diabetes, are promptly growing in innovative nations owing to change in lifestyle and communal environment. According to World Health Organization (WHO) prognostications, almost 300 million of the diabetic population is likely to expand by the year 2025. At present, the approachable rehabilitations for diabetes consist of oral antidiabetic agents causing innumerable deleterious adverse effects; as a consequence, it is still a challenge to accomplish diabetes without any side effects [1].

In the meantime, the risk of enduring diabetic consequences can be overwhelmed by strict glycemic control, devoted to the medications derived from Ayurvedic antidiabetic herbs. The WHO appraisals that presently 80 percent of the world population consume herbal medicine for certain aspect of primary health care [2]. They have listed 21,000 plants, which are consumed for medicinal resolutions throughout the world. Amongst them, 2500 species can be accessible in India, out of which 150 species are exploited commercially on a legitimately large scale [3].

Salacia, species are traditionally practiced for the management and treatment of diabetes [4]. In India, the whole plant has been exploited in Ayurvedic antidiabetic formulations and marketed as antioxidant, antilipidemic and anti-hypoinsulinemic agents. Several study revealed that the aqueous extract of the root bark partakes evidenced hypoglycaemic activity [5].

Moreover, the roots and stems of *Salacia oblonga* have been expended extensively in Ayurveda and traditional Indian medicine for the management for diabetes. Concurrently, the root extracts of the plant have been sold as a commercial food and food supplement for several years in Japan, for the treatment of diabetics and obesity [6, 7].

Oral management of the *Salacia oblonga* root extract diminishes the cardiac triacylglycerol (TG) and the fatty acid (FA) contents [8]. The objective of this study is to assemble and equate the disseminated scientific information on the root, stem and leaves of the herbs and to deliver the potent status of the plant on which antidiabetic activity has been performed.

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MATERIAL AND METHODS

Procurement of Salacia oblonga extracts (SOE)

The standard hydroalcoholic extract of root (SO_{RT}, Batch no. HN/SOL/1401), stem (SO_{BK}, Batch no. HN/SOL/1402) and leaf (SO_{LF}, Batch no. HN/SOL/1403) were obtained from Herbo Nutra™, New Delhi along with the certificate of analysis which stated that all these extracts complied with all the morphological specifications like appearance, odor, taste, extract ratio, loss on drying, sieve analysis, bulk density, heavy metals, ash value, total plate count and microbial load of yeast, mold, Escherichia coli. Later, various phytochemical tests such as carbohydrates, alkaloids, glycosides, flavonoids, fixed oils and fats were performed, and the results were in conformity with the previously reported literature.

Experimental animals

Healthy adult albino Wistar female rats (125 – 150g) were used for the study and feed a standard pellet diet and had free access to water during acclimatization under 12h day and night light condition. Experimental animal model were procured from CSIR-CDRI, Lucknow and protocol was approved by Institutional Animal Ethical Committee (Approval no. UIP/IAEC/2014/FEB/10/R2). Rats were housed in polypropylene cages in standard environmental conditions (temperature 25±5°C, relative humidity 55±10%). All the animals were acclimatized in laboratory condition for 7 days.

Induction of hyperglycemia

Hyperglycemia was induced by previously well stabilized reported protocol [9]. Briefly single dose of streptozotocin (STZ) solution in normal saline at 50 mg/kg was administered intraperitoneally after 12h fasting of animals. On the 5th day after STZ administration, the blood sample was collected through tail vein puncture and the fasting blood glucose level was measured using one touch select Glucometer (Johnson & Johnson, India) strips. Rats with a fasting blood glucose level of 250 mg/dL were considered for hyperglycemic condition.

Experimental design and protocol

Diabetic animal model were randomly divided into nine groups, having 6 animals in each group (n=6) [9]. First group was allocated as normal control and treated orally with 0.25% carboxy methyl cellulose (CMC) suspension at dose of 2.0 mL/kg. Second group was assigned as a diabetic control and treated orally 0.25% CMC at dose of 1.0 mL/kg. Third group was treated orally with glibenclamide as a standard marketed drug at dose of 10 mg/kg. Fourth and fifth group of animal were treated with SO_{RT} at dose of 100 and 200 mg/kg, respectively. In similar fashion, sixth and seventh group of animal were treated with SO_{LF} at dose of 100 and 200 mg/kg, respectively. Furthermore, eighth and ninth group of animals were treated orally with SO_{BK} at dose of 100 and 200 mg/kg, respectively. All these doses were administered after the 5thday of STZ administration (except first group) and were given for fifteen days. The blood glucose and body weight were measured on the 1st, 5th, 10th and 7thday of treatment. Serum was collected for further lipid profiling. Animals were ethically sacrificed by cervical dislocation and organs like pancreas and liver were dissected out and rinsed with ice cold saline and stored at -20°C for further studies.

Serum lipid profile

Serum lipid concentrations were measured spectrophotometrically (Labtronics UV-Vis spectrophotometer, Australia) by using a lipid profile kit (Agape Diagnostic Ltd., Kerala, India) which was published previously [9]. Total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) were estimated through this lipid profile kit. Low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated using Friedewald's formula [9].
LDL (mg/dL) = TC – HDL – (TG/5) and VLDL (mg/dL) = TC – HDL – LDL

Determination of oxidative stress parameters

The oxidative parameter, like thiobarbituric acid reactive substances (TBARS) was measured in the liver tissue and which was published previously [10]. Additional parameters, such as superoxide dismutase (SOD), tissue catalase (CAT), and glutathione (GSH) levels were estimated in the pancreatic tissue in the similar experiment. The total protein content of each sample was measured using the Bradford reagent and bovine serum albumin (BSA) was used as a standard.

Determination of liver function test

Liver function biomarkers like aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were also measured in the serum using commercially available kits from Transasia bio-medicals ltd. India. AST and ALT assay were performed as per the manufacturer protocol. 100 µl of serum sample was added in 1 mL of working reagent which provided in the manufactured kit separately for both parameters. The resultant sample was subjected to determine the absorbance at 340 nm whereas the absorbance of working regent was considered as a blank.

The general formula for converting absorbance change into international Units (IU) of activity is:

$$\frac{IU}{L} = \left(\frac{\Delta A}{min}\right) \times T.V. \times 10^3 / S.V. \times Absorptivity \times P$$

Where:

T.V. = Total reaction volume (µl)
S.V. = Sample volume (µl)
Absorptivity = millimolar absorptivity of NADH at 340 nm = 6.22
P = Cuvette lightpath (cm) = 1 cm

Activity of AST/ALT= $\Delta \frac{Abs}{min} \times 1768$

Statistical analysis

Statistical analysis was carried out using Graphpad Prism 5.0 (San Diago, CA, USA). All the results were expressed as mean standard deviation (SD). The data was analyzed by a one-way ANOVA (analysis of variances) followed by Bonferroni Multiple Comparison Test. For biochemical estimations, statistical significance differences were considered with respect to second group (diabetic

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control), where the level of significance was denoted as a ($p< 0.001$), b ($p< 0.01$) and c ($p< 0.05$).

RESULTS

The effect of Salacia oblonga extract (SOE) on fasting blood glucose level and body weight in experimental rats

The antidiabetic-promoting activity was dignified as the ability of the extracts (**SO_{RT}**, **SO_{LF}** and **SO_{BK}**) to obstruct the initiation of blood glucose level via refining insulin level. The **SO_{LF}** revealed remarkably strongest antidiabetic activity among all at the 200 mg/kg dose (Table 1). Table 2 indicated substantial decrease in body weight in diabetic rats, whereas positive control at 100 & 200 mg/kg and glibenclamide at 10 mg/kg administration restored the changes in body weight near to normal.

The effect of SOE on lipid content in experimental rats

The effect of oral administration of SOE for 15 days on lipid contents in liver of control group and experimental groups are rendered in Table 3. Associated to normal control group, the level of total cholesterol was expressively reduced in untreated diabetic group. Animal groups processed with standard drug (glibenclamide at 10mg/kg), **SO_{RT}**, **SO_{LF}** and **SO_{BK}**, each at 100 & 200 mg/kg, rendered noteworthy reduction in total cholesterol level when equated with normal group. Alike tendency were noted for total cholesterol, where the reduction in level was monitored in comparison to diabetic control group.

The effect of SOE on oxidative stress parameters and plasma enzyme levels in experimental rats

Oxidative stress is subsidized markedly to the pathogenesis of diabetic complications due to fabrication and amputation of reactive oxygen species [10]. The results of SOD for **SO_{RT}**, **SO_{LF}** and **SO_{BK}** are collapsed in Table 4**.**

The streptozocin treatment was resulted in substantial diminution of the level of tissue antioxidant biomarkers such as SOD, CAT and GSH when compared with the control group. After 2 week from the administration of the **SO_{RT}**, **SO_{LF}** and **SO_{BK}**, significant intensifications were notified in the level of SOD, CAT and GSH in a dose dependent manner with respect to control untreated mice. On the other hand, **SO_{RT}**, **SO_{LF}** and **SO_{BK}** significantly reinstated the elevated level of TBARS reasonably in STZ induced diabetic rats to non-diabetic rats. The decline in TBARS level in liver tissue in extract dealt group certifies the antioxidant potential of the *Salacia oblonga*.

Furthermore, serum transminases AST and ALT are deliberated as sensible biomarker of liver injury. Table 5 indicated various levels of AST and ALT in serum of control and experimental rats. Augmentation in serum level of AST and ALT has been endorsed to the discredited structure of liver tissue. In the diabetic control rats, serum AST and ALT levels were raised with respect to normal control rats. The levels of both enzymes were substantially decreased in diabetic control rats dealt with **SO_{RT}**, **SO_{LF}** and **SO_{BK}** and glibenclamide. It entails normal functioning of liver and affirms hepatoprotective nature of *Salacia oblonga.*

Table 1: Effect of isolated materials from *Salacia oblonga* (Root, stem and leaves) on blood glucose level (mg/dL) on STZ induced diabetic rats.

Groups	1st day	5th day	10th day	15th day
N Control	93.67±7.53	97.45±5.78	103.43±6.26	99.76±7.34
D Control	310.29±8.78	307.77±3.85	311.93±7.71	309.64±6.82
D + G	258.34±5.39	235.12±4.91	173.64±5.88	155.63±4.84
D + SO_{RT}100	426.00±4.24[a]	333.50±2.12[a]	203.00±8.49[a]	175.50±6.36[a]
D + SO_{RT}200	329.33±9.45[a]	276.00±5.66[a]	215.50±6.36[a]	159.00±7.07[a]
D + SO_{BK}100	389.67±5.13[a]	221.00±6.08[a]	178.00±9.85[a]	122.33±6.11[a]
D + SO_{BK}200	313.33±5.03[a]	265.00±4.58[a]	169.00±5.57[a]	102.33±4.16
D + SO_{LF}100	393.33±9.02[a]	255.33±8.14[a]	189.33±5.51[a]	112.33±7.57[c]
D + SO_{LF}200	385.67±5.51[a]	203.33±5.86[a]	194.67±8.50[a]	115.00±4.00[b]

Data represented as mean±SD (n=6). Statistically significant differences were observed between D control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; [(a) $p<0.001$, (b) $p<0.01$, (c) $p<0.05$].

Table 2: Effect of isolated materials from *Salacia oblonga* (Root, stem and leaves) on body weight (g) on STZ induced diabetic rats.

Groups	1st day	5th day	10th day	15th day
N Control	99.45±3.89	105.45±4.15	104.12±3.67	108.34±3.22
D Control	120.44±2.79	115.67±3.76	111.88±4.78	103.56±3.87
D + G	104.23±4.98	102.55±2.83	105.62±2.90	106.13±4.45
D + SO_{RT}100	89.50±4.95[b]	93.50±2.12[a]	92.00±2.83[a]	100.00±1.41[a]
D + SO_{RT}200	100.67±4.16	110.00±1.41	111.50±2.12[b]	111.50±0.71
D + SO_{BK}100	86.67±5.03[a]	88.67±3.51[a]	84.00±4.00[a]	91.33±2.52[a]
D + SO_{BK}200	135.67±1.53[a]	136.00±4.36[a]	134.00±2.65[a]	135.00±4.00[a]
D + SO_{LF}100	85.67±4.16[a]	92.33±3.21[a]	94.33±2.08[a]	93.67±3.06[a]
D + SO_{LF}200	91.67±1.53[c]	104.00±3.61	114.00±2.65[a]	94.33±2.52[a]

Data represented as mean±SD (n=6). Statistically significant differences were observed between D control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; [(a) $p<0.001$, (b) $p<0.01$, (c) $p<0.05$].

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Table 3: Effect of isolated materials from *Salacia oblonga* (Root, stem and leaves) on total cholesterol in the liver serum on STZ induced rats.

Groups	TC (mg dL^{-1})	TG (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
N Control	46.87 ± 1.43	65.76 ± 0.96	79.12 ± 1.12	6.70 ± 0.45	25.55 ± 0.45
D Control	106.54 ± 2.65	136.32 ± 9.81	21.11 ± 1.05	30.24 ± 1.06	55.19 ± 0.77
D + G	50.12 ± 0.98[a]	77.43 ± 5.09[c]	56.39 ± 3.16[b]	14.23 ± 2.34	7.96 ± 0.37[a]
D + SO_{RT}100	54.60 ± 1.03[a]	92.46 ± 10.89	53.55 ± 7.98	27.89 ± 2.67	27.57 ± 5.87
D + SO_{RT}200	55.99 ± 1.50[a]	90.45 ± 10.99	58.80 ± 6.48	20.24 ± 1.75	18.50 ± 3.78
D + SO_{BK}100	56.61 ± 0.63[a]	98.99 ± 8.71	54.08 ± 6.74	31.92 ± 1.05	29.74 ± 0.49[a]
D + SO_{BK}200	65.70 ± 1.22[a]	96.48 ± 7.42	47.78 ± 8.74	21.62 ± 4.56	9.25 ± 3.48
D + SO_{LF}100	63.49 ± 1.03[a]	107.20 ± 8.54	43.75 ± 7.61	34.97 ± 1.87	15.23 ± 2.99
D + SO_{LF}200	66.81 ± 1.36[a]	116.58 ± 9.32	44.80 ± 4.62[b]	40.81 ± 1.67	18.24 ± 2.45

TC; Total cholesterol, TG; triglycerides, HDL; high density lipoprotein, LDL; low density lipoprotein; VLDL; very low density lipoprotein. Data represented as mean±SD (n=6). Statistically significant differences were observed between D control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; [(a)p<0.001, (b) p<0.01, (c) p<0.05].

Table 4: Effect of isolated materials from *Salacia oblonga* (Root, stem and leaves) on SOD, CAT, GSH in pancreas and TBARS in liver on STZ induced rats.

Groups	SOD (U/mg of Protein)	CAT mM H_2O_2 decomposed/min/mg of protein	GSH (µM/mg of Protein)	TBARS (nM of MDA/mg of protein)
N Control	0.39 ± 0.05	3.23 ± 0.41	9.01 ± 0.48	0.24 ± 0.02
D Control	0.13 ± 0.01	0.95 ± 0.01	4.65 ± 0.31	0.48 ± 0.03
D + G	0.33 ± 0.02[a]	2.73 ± 0.06	8.87 ± 0.24[a]	0.31 ± 0.04[a]
D + SO_{RT}100	0.31±0.02[a]	2.81±0.45[b]	7.39±0.17[a]	0.30±0.02[a]
D + SO_{RT}200	0.31±0.01[a]	2.38±0.49[b]	8.96±0.43[a]	0.28±0.02[a]
D + SO_{BK}100	0.32±0.01[a]	1.21±0.75[b]	6.26±0.23	0.25±0.08[a]
D + SO_{BK}200	0.31±0.01[a]	1.72±0.09[a]	6.75±0.06	0.25±0.02[a]
D + SO_{LF}100	0.32±0.01[a]	1.42±0.95[b]	7.00±0.19	0.28±0.03[a]
D + SO_{LF}200	0.27±0.01[a]	1.32±0.09[a]	6.76±0.04	0.34±0.04[a]

SOD; superoxide dismutase, CAT; catalase, GSH; glutathione, TBARS; thiobarbituric acid reactive substances. Data represented as mean±SD (n=6). Statistically significant differences were observed between D control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; [(a)p<0.001, (b) p<0.01, (c) p<0.05].

Table 5: Effect of isolated materials from *Salacia oblonga* (Root, stem and leaves) on AST and ALT in serum in STZ induced rats.

Groups	AST (U/dL)	ALT (U/dL)
N Control	46.18 ± 1.33	57.22 ± 1.43
D Control	136.64 ± 2.98	106.41 ± 2.18
D + G	56.23 ± 1.54[a]	66.47 ± 0.67[a]
D + SO_{RT}100	75.58 ± 5.63	71.60 ± 1.25[a]
D + SO_{RT}200	86.19 ± 4.56[b]	69.39 ± 4.63[b]
D + SO_{BK}100	91.05 ± 4.25[b]	85.75 ± 3.25[b]
D + SO_{BK}200	97.68 ± 3.63[b]	82.21 ± 5.25[c]
D + SO_{LF}100	99.45 ± 2.88[a]	80.89 ±11.63
D + SO_{LF}200	92.82 ± 5.50[c]	87.96 ± 6.67

Data represented as mean±SD (n=6). Statistically significant differences were observed between D control and test groups [one way-ANOVA followed by Bonferroni multiple comparison test; [(a)p<0.001, (b) p<0.01, (c) p<0.05].

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DISCUSSION

Diabetes mellitus is one of the preeminent cause of death, illness and economic loss all over the world, associated with hyperglcaemia, hyperlipidemia and co-morbidites such as obesity, hypertension [11, 12]. Intervention with oral hypoglycemic agents is consorted with various side effects [13]. Properly administered plant-derived products unremarkably do not bring out any side effects [14]. Secondary metabolites from various plants like flavonoids, alkaloids and triterpinoids accounted to assume effective antidiabetic properties. Commonly, Salacia species are copious source of naturally occurring flavonoids and phenolic compounds that plays a vital role in antidiabetic, hypoglycemic and antilipidemic activity [15].

Administration of STZ was chosen for potent diabetogenic action through single intraperitoneal injection. STZ is accredited for its selective retrogression and necrosis of β-cells of islets of Langerhans, moreover it is less toxic and has an irreversible effects on pancreatic beta cells. STZ producing diabetic conditions simultaneously elevates blood glucose level and ultimately higher declining in the body weight of animal modal. For many years, glibenclamide has been used to provoke the release of insulin from the β-cells and thus panoptical used to treat diabetes mellitus [12-14, 16-18].

In the present study, it was promulgated that after administration of SO_{RT}, SO_{LF} and SO_{BK}, blood glucose level was diminuend significantly when equated to diabetic control group animals. It was estimated that animals treated with SO_{RT} at a dose of 200mg/kg depicts significantly prominent consequences with respect to SO_{LF} ($p<0.001$) and SO_{BK} ($p<0.001$). In STZ induced diabetic rats, SO_{RT}, SO_{LF} and SO_{BK} administration significantly increased the body weight when compared with the D control group animals. Increase in body weight in STZ induced diabetic rats and potent hypoglycemic activity of $SO_{RT,}$ are mainly attributed to higher active chemical constituents with respect to SO_{LF} and SO_{BK}.

In the current scenario, substantial interest has been targeted towards the lipid profile conventions as it is creditworthy to cardiovascular risk in diabetes mellitus [11]. When we focalize the hypolipidemic activity of *Salacia oblonga,* our study directs that evocation of diabetics in rats along with STZ has enhanced the propagating lipid profile, for instance triglycerides and total cholesterol. Oral administration of SO_{RT}, SO_{LF} and SO_{BK} to the STZ induced animals had significantly diminuend the circulating lipid levels which have prophylactic function for the diabetes ramifications. Additionally, it is estimated that animals treated with SO_{RT} at a dose of 200mg/kg depicts significantly prominent consequences with respect to SO_{LF} ($p<0.001$) and SO_{BK} ($p<0.001$).

Previous findings suggests STZ induced animals exposes to the most of diabetic ramifications raised through oxidative stress [17]. Elevation in the level of the lipid peroxidation might be revelatory of a reduction in enzymatic antioxidant defense biomarkers[19]. In addition to GSH, SOD, CAT and TBARS are other antioxidant defense mechanisms whose actions impart to eradicate superoxide, hydrogen peroxide and hydroxyl free radicals. In our study, oral administration with SO_{RT}, SO_{LF} and SO_{BK} enhanced the level of SOD, CAT and GSH in STZ-induced liver impaired rats to preclude assemblage of unreasonable free radicals and to defend the liver from many deleterious effects [20]. Unlikely, the administration of SO_{RT}, SO_{LF} and SO_{BK} repressed the level of TBARS when compared with D control rats. Altogether, in case of antioxidant defense mechanisms, animals treated with SO_{RT} at a dose of 200mg/kg depicts significantly prominent consequences with respect to SO_{LF} ($p<0.001$) and SO_{BK} ($p<0.001$).

Prominent accomplishments of serum aminotransferases are creditworthy to production of ketone bodies, a usual sign of liver disease and complications in diabetics [13]. A noteworthy diminution in the liver enzyme biomarkers AST and ALT levels was acknowledged following oral administration of SO_{RT}, SO_{LF} and SO_{BK} with respect to D control groups. Comparatively, SO_{RT} at a dose of 200mg/kg is significantly higher effective than those of SO_{LF} ($p<0.001$) and SO_{BK} ($p<0.001$).
When, we compared the activity between SO_{LF} and $SO_{BK,}$ minute differences were noticed; briefly, in case of increase in body weight and decrease in blood glucose, SO_{LF} was found to be more effective, whereas in case of all other parameters studied, SO_{BK} was found to be more effective.

CONCLUSION

In this study, the leaf, bark and root of the *Salacia oblonga* possessed potent hypoglycemic, hypolipidemic and antioxidant activities with prominent effects in roots. The comparison between leaves and stem was found to be ambiguous; yet to a good extent, in case of increase in body weight in STZ induced diabetic rats and decrease in blood glucose levels, SO_{LF} was found to be more effective, whereas in case of all other parameters studied, SO_{BK} was found to be more effective. Finally, we observed that *Salacia oblonga* had moral antidiabetic activity and minor toxic potential which might be favorable for forthcoming drug design perception.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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