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In-vivo Antidiabetic Activity of Saccharum Spontaneum on STZ- Induced Diabetic Mice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objectives: Saccharum spontaneum is traditionally used in Arunachal Pradesh, India for the treatment of diabetes mellitus. Review of existing literature, found no scientific investigation on this claim. Therefore, this study was aim to evaluate the antidiabetic effect of ethanolic extract of S. spontaneum on STZ induced diabetic mice.
Materials and Methods: Diabetes was induced in male albino mice by single intraperitoneal injection of streptozotocin (100mgkg-1 BW). Initially Starch tolerance test, blood glucose level and area under the curve (AUC) were evaluated after S. spontaneum treatment in diabetic mice. The test extract was administered for three weeks and the antidiabetic activity was evaluated by means of monitoring the change in blood glucose level, body weight and biochemical parameters.
Results: The extract showed a significant decrease in the blood glucose level, slight enhancement of body weight after the treatment. Daily oral treatment of extract also showed a significant reduction in the Serum ALT, creatinine and triglyceride when compared to the diabetic control.
Conclusion: The results provide supports to the traditional claim made on Saccharum spontaneumand provide evidence that this plant might be a potential source of antidiabetic agent.

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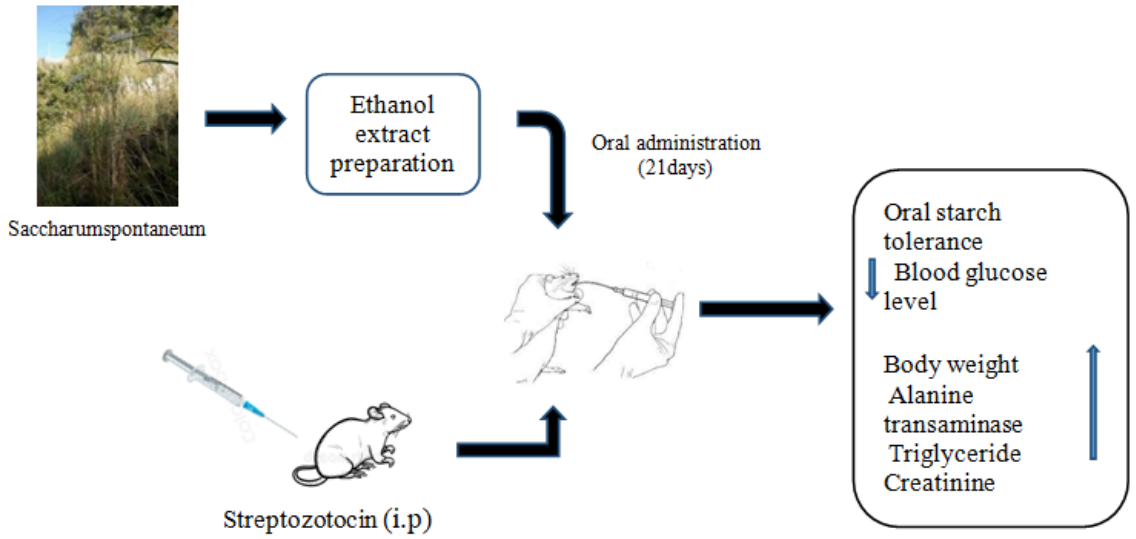
Graphical Abstract



Keywords: Diabetes; streptozotocin; hyperglycemia; saccharum spontaneum.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent diseases. It is a chronic metabolic derangement of protein, fats and carbohydrate caused by an absolute or relative lack of insulin secretion resulting in hyperglycemia which is defined as a clinical characteristic of diabetes [1,2]. Chronic alteration in lipid and protein metabolism may accelerate the development of microvascular and macrovascular complication [3]. Many clinically available anti-diabetic drugs lack the potential to restore normal glucose homeostasis and are associated with adverse side effects [4]. Herbal medicines are gaining importance as they are less expensive and free from adverse side effects when compared to the synthetic drugs. Therefore, medicinal plants with anti hyperglycemic activity offer a promising alternative for the treatment of diabetes [5].

Saccharum spontaneum L. (Family: Poaceae) is a tall perennial grass with deep root and rhizome growing upto a height of 3-4 m [6], grows in the banks of water bodies or along roadsides. It is widely distributed throughout the tropical countries of Asia, Africa, America and Australia [7]. The roots of the plant are astringent, emollient, diuretic, tonic and are use for treating dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles and respiratory troubles [8]. Leaves and whole plant are reported to possess activities like antioxidant [9,10], antidiarrhoeal [11], CNS depressant [12] and antiurolithiatic activity [13].Traditionally, the plant is reported to use for treatment of diabetes [14] however scientific validations of its anti-diabetic effect are not available. Therefore the present work was aimed to evaluate the anti-diabetic activity of ethanolic extract of *Saccharum spontaneum* on streptozocin (STZ) induced diabetic mice.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The young shoot of *Saccharum spontaneum* was collected from Arunachal Pradesh, India. The collected plant was identified and authenticated by Dr. Manish Kandwal, Scientist D, Botanical Survey of India (BSI), Itanagar, Arunachal Pradesh. The sample herbarium specimen was deposited for future reference (HAG-008).

2.2 Preparation of Plant Extract

The air dried plant material was grinded, extracted with ethanol and kept at rotary shaker for 24 h. The filtrate obtained was concentrated in a rotary vacuum evaporator to give solid residue. The residue was stored in airtight

container in refrigerator for subsequent experiments.

2.3 Chemicals

Streptozotocin (STZ) was purchased from SRL Pvt. Ltd, India. Standard kit for estimation of total serum Alanine Transaminase (ALT), Creatinine (CRE) and Triglyceride (TGL) were purchase from Coral system, Goa. Estiamtion of blood glucose level was done with Glucometer (ACCU-CHEK active). All other the chemicals used in the study were of analytical grade.

2.4 Experimental Animals

Adult male Swiss albino mice (25-35g) were procured from stock animal facility of the Dept. of Zoology, Rajiv Gandhi University. The animals were kept in polypropylene cages at a temperature of 24±2°C with 12h light/dark cycle and fed with standard diet and water ad libitum [15].

2.5 Induction of Experimental Diabetes

Diabetes were induced in overnight fasted mice by single intraperitoneal (i.p) injection of freshly prepared STZ (100mg/kg) dissolved in 0.1 M citrate buffer (pH = 4.5). To avoid hypoglycemic shock 10% sucrose solution were given immediately to the mice. After 72 h mice with marked hyperglycemia (fasting blood glucose level greater than 200mg/dl) were selected for the experimental study [16, 17]. Fasting blood glucose level were monitored using blood glucose test strips with elegance glucometer (ACCU-CHEK active).

2.6 Effect of Plant Extract on Oral Starch Tolerance in Normal Mice

This test was conducted to evaluate the starch tolerance activity after administration of ethanol extract of *Saccharum spontaneum* (SS) on non-diabetic mice. The overnight fasted non-diabetic mice were divided into four experimental groups of 6 animals (n=6) each. The normal untreated mice (Group I) received distilled water. Group II were administered with acarbose (10mg/kg, P.O) which serve as positive control. Group III and Group IV were administered with 100mg/kg and 1000 mg/kg of ethanol extract of SS. After thirty minutes of respective treatment, the mice were administered orally with starch (3g/kg). The blood glucose levels from each group were determined at 0, 30, 60, 120, 150 minutes interval after starch treatment via tail puncture [18]. The peak blood glucose (PBG) and area under the curve (AUC) for each group were determined. The maximum blood glucose concentration was taken as PBG and AUC was calculated using the following equation:

$$\textbf{AUC (mmol/L)} = \frac{BG_0+BG_{30}\times 0.5}{2} + \frac{BG_{30}+BG_{60}\times 0.5}{2} + \frac{BG_{60}+BG_{120}\times 1}{2} + \frac{BG_{120}+BG_{150}\times 0.5}{2}$$

Where, BG represents the blood glucose concentration at the intervals of 0, 30, 60, 120 and 150 minutes.

2.7 Effect of Plant Extract on oral Starch Tolerance in Diabetic Mice

This test was conducted to evaluate the starch tolerance activity after administration of ethanol extract of SS on diabetic mice. The normal untreated mice (Group I) received distilled water, while the diabetic mice were divided into four experimental groups (n=6). Group II serve as diabetic control without any treatment. Group III were administered with acarbose (10mg/kg, P.O), Group IV and Group V were administered with 100mg/kg and 1000 mg/kg of ethanol extract of SS. Thirty minutes after treatment, the mice were administered orally with starch (3g/kg). The blood glucose levels from each group were determined at 0, 30, 60, 120, 150 minutes interval after starch treatment via tail puncture [18]. The peak blood glucose (PBG) and area under the curve (AUC) for each group were determined similarly as described above.

2.8 Effect of Long Term/Continuous Plant Extracts Treatment on Diabetic Mice

The long term (21 days) experiment was conducted to examine the efficacy of plant extract on diabetic mice. The overnight fasted male mice were divided into five groups (n=6). Normal untreated mice were provided with distilled water only (Group I) .STZ induced mice with confirmed diabetes were further divided into four sub groups. Group II serve as the diabetic control, Group III received standard drug acarbose (10mg/kg/day, P.O), Group IV and group V were treated with 100 mg/ kg and 1000mg/ kg of ethanol extract of SS (P.O) for consecutive 21 days respectively. During the study period, animals in all groups had free access to normal diet and water. Changes in

body weight were noted and blood glucose levels from each group were estimated on 0, 7th, 14th and 21st day.

2.9 Biochemical Parameters

All the biochemical analysis were carried out using commercial kits following the manufacturer standard protocols in a semi-auto analyzer. Serum alanine transaminase (ALT) was estimated using Reitman and Frankel method [19], serum Creatinine level was measured using Jaffe's reaction method [20] and triglycerides (TGL) was estimated using Foster and Dunn method [21].

2.10 Statistical Analysis

All generated data were expressed as mean ± standard error of mean (SEM) and variation among the groups were subjected to one way ANOVA followed by Tukey's multiple comparison tests using Graph pad Prism 5.0 software. The level of significance was determined at $P<0.05$.

3. RESULTS

3.1 Effect of SS on Oral Starch Tolerance Test in Non-Diabetic Mice

The effect of ethanol extract of SS on starch tolerance test in overnight fasted non-diabetic mice is presented in Table 1 and Fig. 1. In this study, the peak blood glucose (PBG) and area under curve (AUC) were estimated at different interval of time from 0 min to 150 min. The administration of both doses of SS caused 4.15% and 7.55% reduction in PBG after 30 minutes of oral starch load respectively. A 4.51% and 8.71% reduction in AUC was also observed 100mg/kg and 1000mg/kg SS treated group. However, the differences were found not to be significant when compared with the diabetic control. The standard drug, acarbose however exhibited a significant reduction in PBG level (19.38%) and AUC (21.51%).

Table 1. Effect of ethanol extract of *Saccharum spontaneum* (SS_{EtOH}) on peak blood glucose (PBG) and area under the curve (AUC) after starch loading in non-diabetic mice. Data are represented as Mean ± SEM (n=6).p<0.05 vs. control. NC: Negative control, AC: Acarbose, SS: Saccharum spontaneum

Group	PBG(mg/dl)	% Reduction of PBG	AUC(mg/dl)	%Reduction of AUC
I: Normal control	139.28 ± 5.67		355.55 ± 7.93	
II: Acarbose	113.57 ± 8.33	19.38	282.21 ± 19.17	21.51
III: SS_{EtOH}(100 mg/Kg)	135.03 ± 2.21	4.15	343.33 ± 4.91	4.51
IV: SS_{EtOH}(1000 mg/Kg)	130.23 ± 3.24	7.55	328.25 ± 3.560	8.71

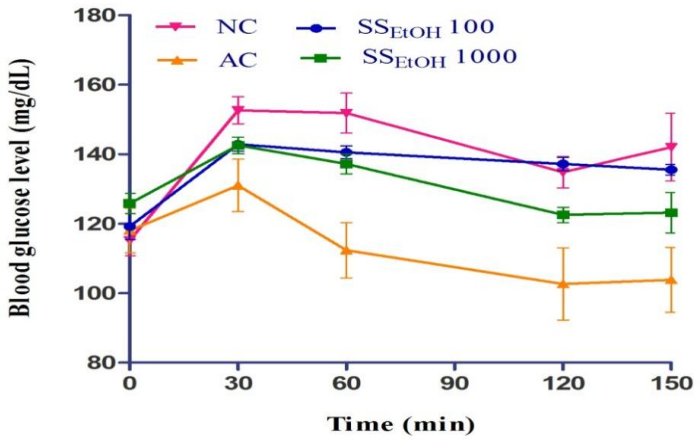


Fig. 1. Effect of ethanol extract of SS on Starch tolerance test after oral starch administration in non-diabetic mice. Values are represented as Mean ± SEM (n=6). NC: Negative control, AC: Acarbose, SS: Saccharumspontaneum

3.2 Effect of SS on Oral Starch Tolerance Test in Diabetic Mice

In diabetic mice, the administration of SS during starch tolerance test caused reduction in PBG and AUC level after 30 minutes of oral starch load. The blood glucose level was found to be significantly higher in diabetic control group ($P<0.05$) when compared with the normal control. A 4.06% and 10.75% reduction in PBG were observed in diabetic control treated with 100 & 1000 mg/kg of SS respectively. Similarly a reduction of 5.04% and 11.48% in AUC were observed in groups treated with 100 and 1000 mg/Kg of SS [Table 2 and Fig. 2]. However, the differences in PBG and AUC were not significantly different in SS treated group when compared with diabetic control group. Significant reduction in PBG (21.99%) and AUC (23.79%) was observed in acarbose treated group.

3.3 Effect of Long Term Treatment with SS on Body Weight, Blood Glucose Level and Other Biochemical Parameters

The effect of ethanol extract of SS on body weight of STZ induced diabetic model is presented in Table 4. The body weight of normal untreated control group remains constant throughout the study, whereas in diabetic control slight decrease in body weight was observed when compared to the normal control. The diabetic group treated with SS and AC showed slight increase in the body weight. However, no significance difference was observed in the body weight of different groups of animals till the end of 21 days treatment.

The effect of ethanol extract of SS on blood glucose level in STZ induced diabetic model is presented in Table 3. The blood glucose levels of diabetic untreated group continued to increase gradually till the end of the study period and were found to be significantly higher than the normal group ($P<0.05$). The administrations of SS extract have no significant effect in the blood glucose level of diabetic mice during the initial 0-7 day period of study. However, on 14^{th} and 21^{st} day of high dose SS treatment (1000 mg/Kg), the blood glucose level was significantly reduced when compared with the diabetic control group. Similarly, the administration of standard drug acarbose also caused significant reduction in blood glucose level in diabetic mice.

The effect of ethanol extract of *SS* on various biochemical parameters such as ALT (hepatic markers), creatinine (renal marker) and TG (lipid profile) were assessed in all control and experimented group [Table 5]. The Serum ALT, creatinine and TG activity were significantly ($P<0.05$) elevated in the diabetic control group when compared with normal control group. However, daily administration of SS and acarbose significantly improved the level of Creatinine and TG level in diabetic treated groups, while no improvement was observed in the level of ALT.

Table 2. Effect of SS_{EtOH} on peak blood glucose (PBG) and area under the curve (AUC) after starch loading in diabetic mice. Data are represented as Mean ± SEM (n=6)

Group	PBG(mmol/L)	% Reduction of PBG	AUC (mmol/L)	% Reduction of PBG
I: Normal control	128.17 ± 8.79		328.54 ± 20.64	
II:Diabetic control	328.26± 29.58		832.50± 71.86	
III: Acarbose	256.06± 11.88	21.99*	634.41 ± 29.00	23.79*
IV: SS_{EtOH} (100 mg/Kg)	314.93± 19.60	4.06	790.50 ± 46.89	5.04
V: SS_{EtOH} (1000 mg/Kg)	292.97± 17.04	10.75	736.96 ± 41.39	11.48

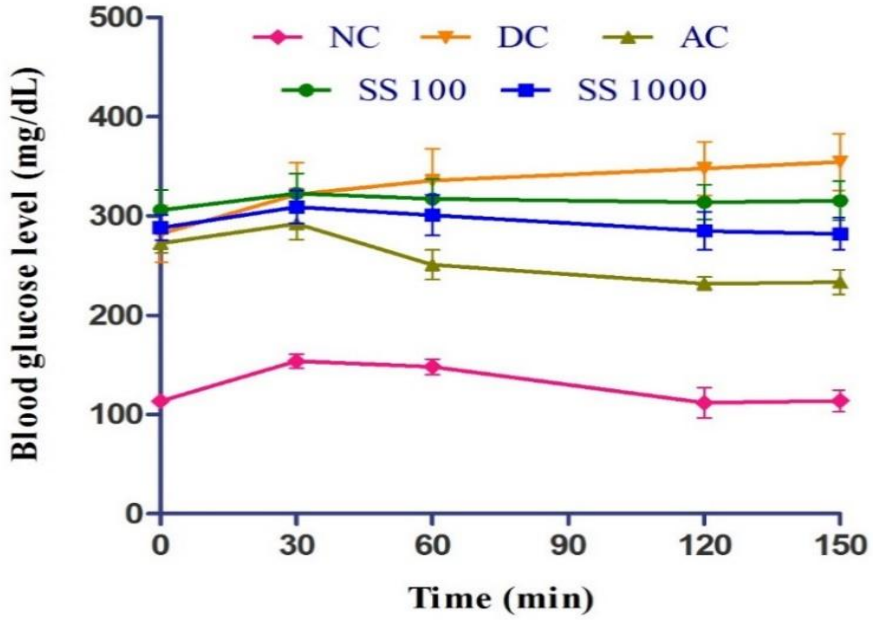


Fig. 2. Effect of ethanol extract on Starch tolerance test after oral starch administration in non-diabetic mice. Values are represented as Mean ± SEM (n=6). NC: Negative control, AC: Acarbose, SS: Saccharumspontaneum

Table 3. Effect of SS on body weight of control and experimental group. NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharum spontaneum. Data are represented as Mean ± SEM (n=6). * p<0.05 significance between normal control and diabetic control

Group	Body weight (g) at different days during the experiment period			
	Day 0	Day 7	Day 14	Day 21
I: Normal control	33.96 ± 2.86	34.75 ± 2.391	34.96 ± 2.347	34.91 ± 2.505
II:Diabetic control	31.47± 0.532	30.86 ± 0.468	29.89 ± 0.468	28.72 ± 0.360*
III: Acarbose	27.31± 1.084	27.65±1.201	28.17 ± 1.023	29.04±0.955
IV: SS_{EtOH} (100 mg/Kg)	30.32± 1.609	30.15 ±1.331	29.99 ± 1.001	29.96±1.041
V: SS_{EtOH} (1000 mg/Kg)	27.82± 1.259	27.80 ±1.616	27.94 ± 1.651	28.36±1.561

Table 4. Effect of SS on blood glucose level of control and experimental group. NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharum spontaneum. Data are represented as Mean ± SEM (n=6); ns - not significance; ###p<0.001 when compared to the normal control; *p<0.05, ***p<0.001 when compared to the diabetic control

Group	Blood glucose level (mg/dl) at different days during the experiment period			
	Day 0	Day 7	Day 14	Day 21
I: Normal control	118 ± 4.81	108.33 ± 3.38	117.33 ± 8.66	110.00 ± 6.42
II:Diabetic control	353.33 ± 7.53###	362.33 ± 10.11###	379.00 ± 4.52###	402.66 ± 3.92###
III: Acarbose	351.75 ± 10.17	347.25 ± 24.08ns	276.75 ± 7.46***	229.75 ± 5.15***
IV: SS_{EtOH} (100 mg/Kg)	374.50 ± 5.95	370.5 ± 7.57ns	357.75 ± 7.00ns	346.5 ± 7.19***
V: SS_{EtOH} (1000 mg/Kg)	379.50 ± 7.77	365.25 ± 11.25ns	342.75 ± 10.53*	309.75 ± 14.95***

Table 5. Effect of SS on biochemical parameters.NC: Negative Control, AC: Acarbose, DC: Diabetic Control, SS: Saccharum spontaneum. Data are represented as Mean ± SEM (n=6).ns represent no significance difference; $^{###}$p<0.001 when compared to the normal control, **p<0.01, ***p<0.001 when compared to the diabetic control

Group	Triglyceride (mg/dl)	Creatinine (mg/dl)	ALT (U/L)
I: Normal control	115.427±7.404	0.308±.012	29.818±0.314
II:Diabetic control	182.527±10.018$^{###}$	0.621±0.076$^{###}$	40.053±0.880$^{\#}$
III: Acarbose	115.427±5.902***	0.412±0.063***	33.969±0.262^{ns}
IV: SS_{EtOH} (100 mg/Kg)	129.491±7.100**	0.390±0.041***	38.787±5.157^{ns}
V: SS_{EtOH} (1000 mg/Kg)	124.163±10.328***	0.369±0.046***	34.141±4.782^{ns}

4. DISCUSSION

Diabetes is one of the most prevalent metabolic disorders which have become a greater health concern all over the world. The role of medicinal plant as an alternative source in treatment of diabetes is gaining more popularity [22]. In the present study, the ethanol extract of young shoot of *Saccharum spontaneum* was selected for the evaluation of antidiabetic activity on the STZ induced diabetes mice. Initially the ethanolic extract was screened for acute hyperglycemic effect using oral starch tolerance test [23]. The result obtained showed that the young shoot extract of SS suppressed the elevated blood glucose level in both non-diabetic and STZ induced diabetic mice. However, the reduction level of PBG and AUC in groups treated with AC was more pronounces when compared to the SS treated group.

STZ, an antibiotics produced by *Streptomyces achromogenes* is known to specifically destroy pancreas by reducing the size and number of functionally active b-cells, the cell which produce insulin to regulate blood glucose level [24, 25]. Hence, STZ is widely used as a diabetogenic agent to induce diabetes in experimental animals [26, 27]. The long term 21 day treatment with SS in the STZ-induced diabetic mice showed a significant reduction in their blood glucose levels at the end of the treatment, when compared with the diabetic control. STZ-induced diabetes was also characterized by severe weight loss and this reduction might be due to the degeneration of muscle and loss of tissue protein which is the major contributor to body weight [28]. A significant loss in the bodyweight was observed in diabetic group which was slightly improved in the group treated with SS and AC.

Diabetes mellitus is usually associated with lipid abnormalities and increase the risk for coronary heart disease [29]. A variation in the metabolic and regulatory mechanism due to insulin resistance or deficiency is accountable for lipids accumulation [30]. The characteristic features of dyslipidemia in diabetes are hypercholesterolemia and hypertriglyceridemia [31]. STZ-induced diabetes also developed hyperlipidemia [32]. The present study showed a marked increase in the total triglyceride level in diabetic control group. The reduced ratio of TGL shows a potential improvement in insulin sensitivity while increased TGL ratio is associated with insulin resistant in diabetes [33]. Our study showed a signification reduction in TGL level after the 21 day treatment with SS. The elevation of transaminase such as ALT indicates liver dysfunction resulting from hepatic damage which may increase diabetes complication such as gluconeogenesis and ketogenesis [34, 35]. The repeated administration of SS results in significant reduction of ALT when compared to the diabetic control group. Diabetes may also lead to impaired kidney function and serum level of creatinine can be a useful marker for renal damage [36]. The SS extract attenuated the elevated level of creatinine observed among diabetic control group.

5. CONCLUSION

This study highlights the potential of ethanol extract of *Saccharum spontaneum* in preventing hyperglycemia. The prolong treatment with extract also showed improvement in bodyweight and other biochemical parameters associated with diabetes. This study provides a scientific validation of the safe use of young shoot of *Saccharum spontaneum* by traditional healers in the treatment of diabetes. Hence *Saccharum spontaneum* can be a prospective source of antidiabetic agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the animal experiment was reviewed and approved by the Institutional Animal Ethical Committee, vide letter no. IAEC/RGU/17/12, dated 02/06/2017.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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