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RESEARCH ARTICLE

Neuroprotective effect of *Bauhinia variegata* Linn. leaf extracts in streptozotocin induced diabetes in Sprague Dawley rats

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Abstract

Background *Bauhinia variegata*, a ayurvedic medicinal plant reported for its valuable effects in various diseases. It has shown significant effects in type 1 and type 2 diabetes.

Objective The present work was designed to study effects of aqueous and alcoholic extract (AE and AlcE) of *Bauhinia variegata Linn.* leaves in diabetic neuropathy.

Methods Male *Sprague Dawley* rats become diabetic using intraperitoneal injection of streptozotocin (STZ) at 55 mg/kg dose. After 6 weeks, animals were divided and were treated with AE and AlcE at a dose of 250, 500, and 1000 mg/kg (*p. o.*) for the next four weeks. Various parameters such as glucose, thermal hyperagesia, mechanical allodynia, MNCV and oxidative stess were assessed at the end of the study.

Results Diabetic animals showed a significant reduction in response time in tail immersion and hot plate test as compared to animals in normal control group. AE and AlcE at 250, 500, and 1000 mg/kg dose significantly increased response time in the tail immersion test. Whereas, AE and AlcE at doses 500 and 1000 mg/kg showed significant improvement and in response time in the hot plate test.

AlcE at 250, 500 and 1000 mg/kg dose increased the nociceptive threshold significantly. AE at doses 500 and 1000 mg/kg showed significant improvement in the nociceptive threshold. The decrease in motor nerve conduction velocity was observed in diabetic control animals, which was significantly improved after AlcE treatment as compared to AE treatment. Treatment with AE and AlcE decreased the lipid peroxidation and increased the antioxidant enzyme activity significantly in the sciatic nerve.

Conclusion The results showed that *Bauhinia variegata* extracts may be considered as a effective option for management of diabetic neuropathy.

Keywords Catalase · MNCV · Sciatic nerve · SOD · Streptozotocin · Von Frey

Introduction

Diabetic neuropathy is one of the important micro-vascular complication of diabetes which affects more than 1/2 of the diabetic population [1]. Diabetic neuropathy is a sensory disorder, which can be characterized by loss of sensation, induction of pain, decrease in nerve conduction velocity, and degeneration of nerve fibers [2].

Progression of diabetic neuropathy involves various complex processes. It includes factors like ischemia or endoneurial hypoxia, increased oxidative stress, reduced myo-inositol, increased polyol efflux, deficiency of various growth factors, and increased formation of advanced glycation end products. All these factors are directly associated with long-term increased blood sugar levels.

Various approaches are being tested and are successfully implemented for the management of diabetic neuropathy. These include the use of aldose reductase inhibitors, antioxidants, selective PKC inhibitors, and neurotrophic factors [3]. The pain associated with diabetic neuropathy directly affects the patient's quality of life and hence the alleviating the neuropathy-associated pain remains an important approach that involves the use of therapeutic agents like

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tricyclic antidepressants, anticonvulsants, and opioids. But these agents are associated with many side effects like drowsiness, blurred vision, tolerance, and respiratory depression [4].

B. variegata Linn. is a medicinal plant mentioned in Ayurveda, a traditional system medicine in India. Leaves of the plant are reported to have various biological activities which include antioxidant [5], anti-hyperlipidemic activity [6] and were found effective in the treatment of type I as well as type II diabetes [7, 8]. The present study was designed to check the effect of *B variegata* Linn. in the management of diabetic neuropathy. The effect of aqueous and alcoholic extract of *B. variegata* Linn. leaves (AE and AlcE) was studied in streptozotocin-induced diabetic neuropathy in rats.

Materials and methods

Chemicals

Streptozotocin for induction of diabetes was purchased from Sigma-Aldrich (St. Louis, MO, USA). A diagnostic kit for the determination of blood glucose was procured from Transasia Biomedicals Ltd, India. Various chemical for determination of anti-oxidant parameters like reduced glutathione, 1, 1, 3, 3-tetra methoxy propane, nitro blue tetrazolium and 2-thiobarbituric acid were also procured from Sigma Chemical Co, St. Louis, MO, USA.

Collection of leaves and preparation of extract

The aqueous and alcoholic extract of *B. variegata* Linn. leaves were prepared as per previously reported method [9].

High performance liquid chromatography (HPTLC) was used for standardization of both the extract [8]. The solvent system used was n-hexane: ethyl acetate: formic acid: acetic acid (7:3:0.1:0.15). Quercetin, a flavonoid known to be present in *B. variegata* Linn. leaves was used as standard.

Experimental animals

Male *Sprague–Dawley* rats (180–200 g) were purchased from Bharat Serum and Vaccines Ltd., Thane, India, and were housed in SVKM's animal facility Mumbai. Animals were acclimatized for 7 days before starting the experiment. Animals were provided with a standard pellet diet (Nutrimix Laboratory Animal Feed, Maharashtra, India) and purified water *ad libitum* throughout the study period. Temperature of 22 ± 2 °C and relative humidity at 75 ± 5% was maintained in the animal facility. The light/dark cycle of 12 h was also maintained. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC- CPCSEA/IAEC/SPTM/P-45/2015).

Diabetes induction and treatment

Diabetes was induced by streptozotocin 55 mg/kg dose using intraperitoneal *(i.p.)* injection [7, 8].

After 72 h, Animals with a plasma glucose level of more than 250 mg/dL were considered as diabetic and were selected for further study.

Six weeks after induction of diabetes, diabetic animals were randomized into 8 groups.

One group was maintained as the diabetic control group which received a vehicle as treatment. The remaining six groups of diabetic animals received AE and AlcE at the dose of 250, 500, and 1000 mg/kg body weight *p.o.* using an oral gavage needle for the next four weeks. Extracts for dosing were freshly prepared in a 1% solution of sodium carboxymethylcellulose (CMC) as the vehicle. One group was assigned with age-matched non-diabetic animals (n = 8), which was maintained as a normal control group. Animals in the normal control group received 1% solution of sodium CMC for a period of four weeks.

Estimation of plasma glucose

At the end of the study, blood was withdrawn from retro-orbital plexus, plasma was separated by centrifugation and glucose level was measured by GOD-POD kit (Transasia Biomedicals, Ltd. India) using manufacture's protocol.

Behavioral parameters

Thermal hyperalgesia

Determination of thermal hyperalgesia were carried out using tail-immersion test and hot-plate test. Tail immersion test was carried out as per the method described previously [10]. In hot-plate test, animals were individually placed on a hot-plate (IITC Life Sciences, USA). The temperature of the plate was adjusted to 55 ± 1 °C. The latency to the first sign of paw licking or jump response was recorded as reaction time [11].

Mechanical hyperalgesia

Mechanical hyperalgesia was assessed by quantifying the withdrawal threshold of the hind paw using a Von Frey anesthesiometer (IITC Life Science, USA). The force causing the withdrawal response was recorded by the anesthesiometer.

Measurement of motor nerve conduction velocity (MNCV)

Motor nerve conduction velocity (MNCV) was determined as per the method described by Bhatt and Veeranjaneyulu [12]. MNCV was determined in the sciatic posterior tibial conducting system by using the data acquisition system (iWorx, USA). The result are expressed as m/s.

Sciatic nerve oxidative stress parameters

Animals were sacrificed and sciatic nerves were isolated. The oxidative stress parameters of the sciatic nerve were carried out as per the method described previously. Briefly, the homogenate was used for the estimation of malondialdehyde (MDA) as a measure of thiobarbituric acid reactive substances (TBARS) [13]. Post-nuclear supernatant (PNS) was prepared for the determination of catalase activity, while post-mitochondrial supernatant (PMS) was used for further estimation of superoxide dismutase (SOD) activity [14, 15].

Statistical analysis

GraphPad Prism ver. 5.00 software was used for statistical analysis. One way ANOVA (analysis of variance) Bonferroni's multiple comparison test were performed to check statistical difference between treatment groups.

$P < 0.05$ was considered as a level of significance.

Results

HPTLC analysis

HPTLC analysis showed AE contains 0.101 ± 0.01% w/w and AlcE contains 0.24 ± 0.05% w/w of quercetin.

Effect of treatment on body weight

Animals in diabetic group showed significant decreased in body weight ($p < 0.001$) when compared with normal animals. Treatment with extracts showed an increase in the body weight of the animals but with no statistical significance (Fig. 1).

Effect of treatment on plasma glucose level

Plasma glucose animals in diabetic control group was significantly ($p < 0.001$) increased when compared with normal control animals. AE at dose 1000 mg/kg and AlcE at dose 500 and 1000 mg/kg significantly reduced the elevated plasma glucose ($p < 0.001$, $p < 0.01$ and $p < 0.001$ respectively) (Fig. 2).

Effect of treatment on behavioral parameters

Thermal hyperalgesia

The nociceptive threshold was significantly lowered in diabetic rats in both the tail-immersion and hot-plate tests as compared to normal control animals (DC- 2.60 ± 0.63 sec and 2.20 ± 0.09 sec and NC-13.11 ± 0.43 sec and 5.54 ± 0.32 sec, P<0.001). AE and AlcE significantly increased the threshold value in tail immersion test at all three dose levels (AE- 5.00 ± 0.57 sec p<0.05, 5.66 ± 0.55 sec p<0.01, 7.66 ± 0.71 sec p<0.001 and AlcE- 5.36 ± 0.32 sec p<0.01, 6.03 ± 0.57 sec p<0.001, 9.33 ± 0.55 sec, p<0.001), whereas AE and AlcE at dose 500 mg/kg and 1000 mg/kg significantly increased the threshold value in hot plate test (AE- 3.15 ± 0.2713 sec p<0.05, 3.85 ± 0.31 sec p<0.001 and AlcE- 3.35 ±0.27 sec p<0.01, 4.56 ± 0.24 sec p<0.001) (Fig 3).

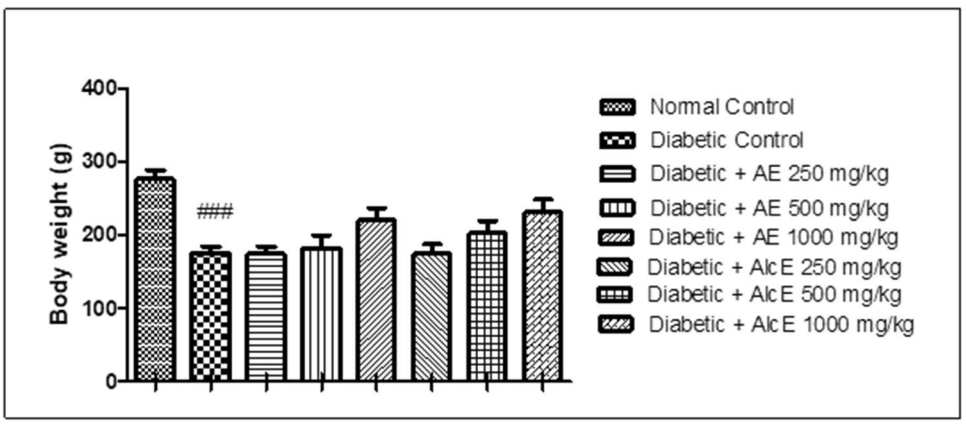

Fig. 1 Effect of AE and AlcE on Body weight. All values are expressed as Mean ± S.E.M. (n = 6). ###p < 0.001, when diabetic control group compared with normal control group

Mechanical allodynia

The tactile withdrawal threshold response to Von Frey filament was also reduced in diabetic rats when compared with the normal animals (DC-24.12 ±1.960 Gr and NC-72.53 ± 2.52 Gr, p<0.001). AE at dose 500 and 1000 mg/kg and AlcE at 250, 500 and 1000 mg/kg significantly increased the tactile withdrawal threshold after treatment for 28 days (AE- 33.84 ± 0.99 Gr, p<0.01 and 53.38 ±1.40 Gr, p<0.001 AlcE- 31.82 ± 1.65 Gr p<0.05, 38.08 ± 1.38 Gr, p<0.01 and 54.99 ± 2.49 Gr, p<0.001) (Fig 4).

Effect of treatment on motor nerve conduction velocity (MNCV)

Significant decrease in MNCV was recorded in diabetic control animals in comparison with that of normal animals (DC-21.71 ± 0.37 m/s and NC- 46.97 ± 2.95 m/s, p<0.001). AE at dose 500 and 1000 mg/kg and AlcE at 250, 500 and 1000 mg/kg significantly improved the MNCV after treatment for 28 days (AE- 31.10 ± 1.025 m/s, p<0.05, 36.98 ± 1.37 m/s, p<0.001 and AlcE- 29.34 ± 1.80 m/s p<0.05, 32.28 ± 1.05 m/s ,p<0.01 and 39.26 ± 1.43 m/s, p<0.001) (Fig 5).

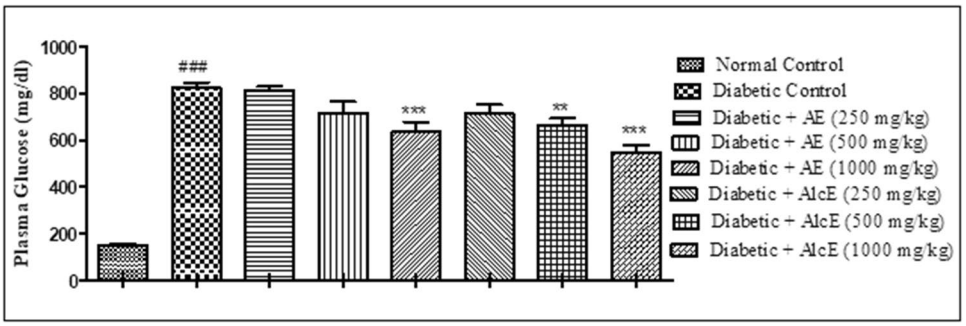

Fig. 2 Effect of AE and AlcE on plasma glucose. All values are expressed as Mean ± S.E.M. (n = 6). **p < 0.01, ***p < 0.001, when treatment group compared with diabetic control group. ### p < 0.001, when diabetic group compared with normal control

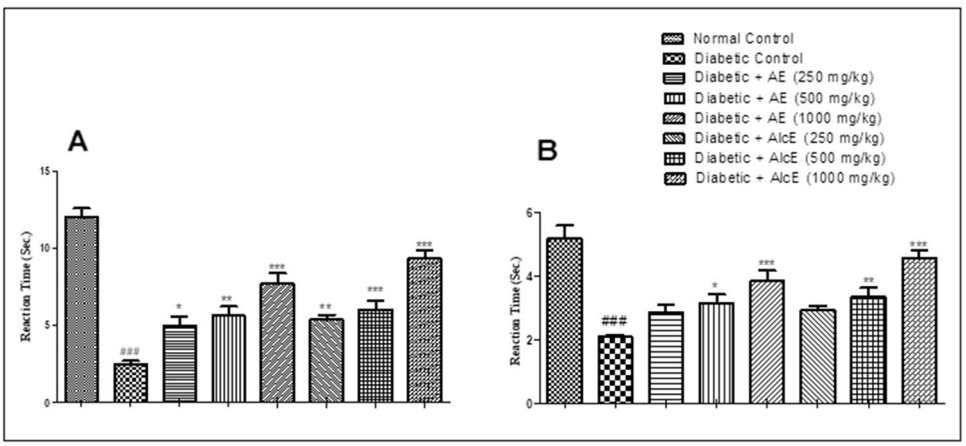

Fig. 3 Effect of AE and AlcE on thermal hyperalgesia (A. Tail Immersion, B. hot plate test). All values are expressed as Mean ± S.E.M. (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, when treatment group compared with diabetic control group. ###p < 0.001, when diabetic group compared with normal control

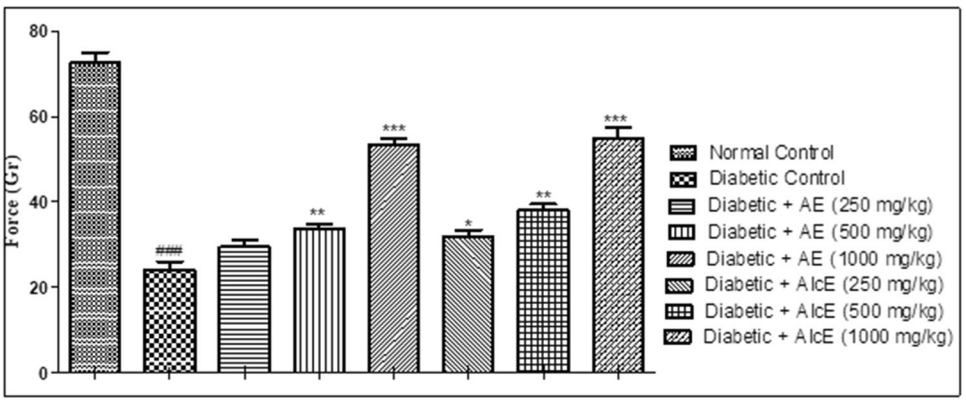

Fig. 4 Effect of AE and AlcE on *mechanical allodynia*. All values are expressed as Mean ± S.E.M. (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, when treatment group compared with diabetic control group. ###p < 0.001, when diabetic group compared with normal control

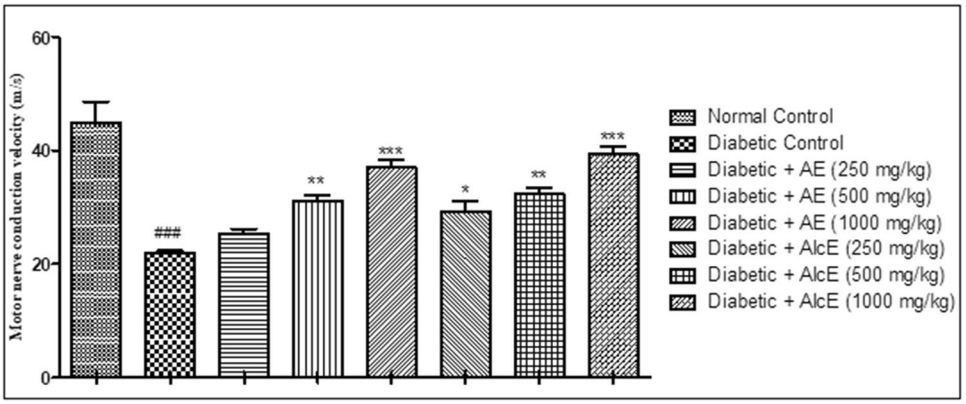

Fig. 5 Effect of AE and AlcE of *B. variegata* on motor nerve conduction velocity. All values are expressed as Mean ± S.E.M. (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001, when treatment group compared with diabetic control group. ###p < 0.001, when diabetic group compared with normal control

Effect of treatment on sciatic nerve oxidative stress parameters

The level of MDA was significantly higher in sciatic nerves of diabetic control animals, while the enzyme activities of SOD and CAT were decreased when compared to that of normal control animals ($p < 0.001$). AE at dose 500 and 1000 mg/kg and AlcE at dose 250, 500 and 1000 mg/kg significantly lower the MDA level (AE- $p < 0.01$, $p < 0.001$ and AlcE—$p < 0.05$, $p < 0.01$ and $p < 0.001$). The activity of SOD and CAT was also found to improve significantly after treatment with AE and AlcE at all three dose levels (Table 1).

Discussion

The leaves of *B. variegata* are a rich source of flavonoids. Various report showed that flavonoids can be effectively used in the management of diabetic neuropathy [16]. Important flavonoids like quercetin and rutin are present in the leaves of the plant. It was found that quercetin [17] and rutin [18] have a protective effect in diabetic neuropathy. Also, *B. variegata* extract was found to be safe up to the dose of 5000 mg/kg in female rats in an acute oral toxicity study. Lowest observed adverse effect level in repeated dose toxicity was found to be more than 1000 mg/kg in repeated dose toxicity study [19].

Table 1 Effect of treatment on sciatic nerve oxidative stress parameters

Group	MDA (nmol/mg protein)	SOD (U/ml)	CAT (Micromoles of H_2O_2 decomposed/min/mg protein)
Normal control	1.40 ± 0.10	1.29 ± 0.10	0.071 ± 0.004
Diabetic control	3.07 ± 0.23###	0.19 ± 0.02###	0.009 ± 0.0023###
Diabetic + AE (250 mg/kg)	2.66 ± 0.09	0.25 ± 0.01	0.017 ± 0.0012
Diabetic + AE (500 mg/kg)	2.30 ± 0.12**	0.40 ± 0.01**	0.030 ± 0.0026**
Diabetic + AE (1000 mg/kg)	1.79 ± 0.06***	0.64 ± 0.01***	0.0456 ± 0.0012***
Diabetic + AlcE (250 mg/kg)	2.41 ± 0.18*	0.33 ± 0.02	0.0250 ± 0.0025*
Diabetic + AlcE (500 mg/kg)	2.21 ± 0.15**	0.50 ± 0.02***	0.0333 ± 0.0029**
Diabetic + AlcE (1000 mg/kg)	1.70 ± 0.05***	0.74 ± 0.01***	0.0513 ± 0.0018***

All values are expressed as Mean ± S.E.M. (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 when compared with diabetic control. ### p < 0.001 when compared to normal control

In the present study, the aqueous and alcoholic extract of leaves of *B. variegata L.* (AE and AlcE) at dose 250, 500 and 1000 mg/kg were evaluated for its effect in the treatment of streptozotocin-induced diabetic neuropathy in rats. Streptozotocin-induced diabetic neuropathy in rats is one of the widely studied models for the evaluation of various drugs in the treatment of diabetic neuropathy.

In the present study, plasma glucose level was significantly higher in diabetic control animals. It was observed that AE and AlcE significantly decreased plasma glucose and improved thermal hyperalgesia, mechanical allodynia, and motor nerve conduction velocity in diabetic animals [12]. AE at high dose and AlcE at mid and high dose significantly reduced the elevated level of plasma glucose. Leaves of BV are rich in flavonoids such as quercetin and rutin which are reported to be a potent inhibitor of sodium-glucose co-transporter and improving peripheral utilization of glucose from the intestine [20].

Oxidative stress damage and endothelial dysfunction due to prolonged hyperglycaemia are important manifestations behind the development of diabetic neuropathy. Assessment of mechanical and thermal stimuli provides an idea about hyperalgesia and allodynia which is an important parameter to understand abnormal sensation and pain associated with diabetic neuropathy [21]. Diabetic animals showed thermal hyperalgesia and mechanical allodynia. Treatment with AE and AlcE inhibited nociceptive pain and reduced thermal hyperalgesia and mechanical allodynia.

Axonal degeneration and myelin breakdown is an important observation in STZ induced diabetes in rats. Prolonged hyperglycaemia activates the polyol pathway that causes deposition of sorbitol in the sciatic nerve which affects Na^+/K^+/ATPase activity and reduced nerve conduction velocity [22]. Diabetic animals showed significant reduction in motor nerve conduction velocity as compared to normal control animals. AE and AlcE treatment significantly improved nerve conduction.

Like many other diseases, it is now well evident that oxidative stress has a key role in the development of diabetic neuropathy [4, 12, 23]. Diabetes-related increase in oxidative stress is associated with increased lipid peroxidation, DNA damage, and cell death. It also leads to nerve tissue damage [24]. Increased oxidative stress in diabetic conditions affects the perception of pain [25, 26]. This also leads to a decrease in motor nerve conduction velocity. In the present study, it was observed that lipid peroxidation was increased in nerve tissues of diabetic animals which were shown by increased levels of malondialdehyde. Decreased activities of endogenous antioxidant enzymes, superoxide dismutase, and catalase were also observed in diabetic animals. Treatment with AE and AlcE decreased the oxidative stress significantly by lowering the lipid peroxidation and improving antioxidant enzyme activity due to the presence of quercetin and rutin in it.

Present study focuses on effect of *Bauhinia variegata* leaf extract in the management of diabetic neuropathy. No specific drugs are available for the treatment of diabetic neuropathy. Available treatment reduces severity of neuropathic pain. So present study did not contain positive control group.

Conclusion

From the results, it can be concluded that AE and AlcE possess a beneficial effect in diabetic neuropathy which is because of its antioxidant potential. AlcE contains a higher amount of quercetin as compared to AE. Besides this, AlcE showed better results starting from a low dose (250 mg/kg) as compared to AE. This confirms that AlcE would be a better treatment option for diabetic neuropathy as compared to AE.

Author's contribution YK and MG designed the experiments. MG and AL conducted the experiments. AL, MG and YK interpreted the results and wrote the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The data that support the findings of this study are available on request from the corresponding author.

Code availability Not applicable.

Declarations

Ethics approval The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC).

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interests The authors declare that they have no conflict of interest.

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