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RESEARCH ARTICLE

Saponin from the fruit of *Solanum anguivi* protects against oxidative damage mediated by Fe^{2+} and sodium nitroprusside in rat brain synaptosome P2 fraction

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Abstract *Solanum anguivi* fruit saponin has antidiabetic property via interference with cellular energy metabolism and inhibition of reactive oxygen species (ROS) generation. In the current study, brain specific in vitro anti-oxidant role of *S. anguivi* saponin was investigated in the P2 synaptosomal fraction of rat brain. Using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay, *S. anguivi* saponin concentration- dependently (10–200 μg/ml) reversed Fe^{2+} and sodium nitroprusside-induced decrease in mitochondrial activity via inhibition of ROS production, ROS-induced oxidation of protein and non-protein thiol-containing molecules and lipid peroxidation as measured by thiobarbituric acid reactive substances levels. Conclusively, *S. anguivi* fruit saponin represents a class of natural compounds with the ability to reverse synaptosomal disruption, loss of mitochondrial integrity and function often associated with the progression of Huntington's disease, Alzheimer disease, Parkinson disease and amyotrophic lateral sclerosis diseases.

Keywords Saponin · *Solanum anguivi* · Oxidative stress · Synaptosomal P2 fraction · Antioxidant

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Introduction

Incomplete reduction of oxidized oxygen fed into the cellular catabolism through mitochondrial oxidative phosphorylation is a key source of reactive oxygen species (ROS) (Apel and Hirt 2004). Upon bonding with cellular macromolecules, ROS negatively affects histological architecture, compromises the integrity of subcellular organelles and alters the 3-dimensional structures of cellular proteins and enzymes with a concomitant loss of enzymatic functions, cellular membrane integrity, altered gene expression and replication regulations. When unresolved, aggressive cell loss due to apoptosis and necrosis occurs. These sequence of events are well documented in the pathogenesis cancer (Feig et al. 1994), diabetes (Inoguchi et al. 2003), inflammation/immune injury, arthritis, coronary diseases, hemorrhagic shock, and neurodegenerative diseases (Rego and Oliveira 2003).

In addition to the enzymatic machineries (catalase, superoxide dismutase, glutathione peroxidase, glutathione peroxidase) (Mates 2000) evolutionarily adapted to scavenge free radicals, some phytochemicals act as complementary antioxidant agents due to their electrophilicity, ability to promote anti-oxidant enzyme gene expression and to positively modulate the actions of anti-oxidant enzymes

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(Malireddy et al. 2012). Although many of these phytochemicals and their plant sources exist in literature, the current study focuses on saponin from *Solanum anguivi* Lam (Solanaceae). Antioxidant properties and Ca^{2+}-induced mitochondrial swelling inhibition (Elekofehinti et al. 2013) have been previously reported for *S. anguivi* saponin. Here, we further investigated its in vitro ROS-quenching properties in synaptosomal and mitochondrial fractions of rat brain.

Materials and methods

Chemicals

Reduced glutathione (GSH), malonaldehydebis-(dimethyl acetal) (MDA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (USA). All other chemicals used in this study were of the highest analytical grade.

Plant material

The fruits of *S. anguivi* were collected from Adekunle Ajasin University, Akungba Akoko horticultural garden. They were identified and authenticated at the herbarium of Plant Science and Forestry Department, University of Ado Ekiti, Nigeria (voucher specimen number UHAE: 286). The fruits were air dried and grounded into a powdery fine texture and stored at room temperature in air tight polythene bag prior to use.

Extraction and isolation of saponins from *S. anguivi* fruit

One hundred grams (100 g) ground sample extracted in 1,000 ml methanol (24 h) and concentrated was partitioned in hexane and water (1:2, v/v, O/N). The aqueous layer was concentrated and partitioned (ethyl acetate/n-butanol, 1:3, v/v). Concentrated butanol fraction was resolved on silica gel TLC plate [Merck, Kieselgel 60F-254, n-butanol: acetic acid: water (60:10:30 v/v/v)] and developed with Lieberman–Burchard reagent followed by column purification (silica gel column, 60–120 mesh), non-saponin components was washed with n-hexane followed by n-butanol: acetic acid: water (1:1:1 v/v/v) elution (Majinda 2012). Pure saponin fractions were pooled after Lieberman–Burchard together and used for further experiments.

Animals

Male Wistar rats, weighing 270–320 g from our own breeding colony (Animal House-holding, UFSM, Brazil) were kept in cages with free access to food and water in a room with controlled temperature (22 ± 3 °C) and in 12 h light/dark cycle. The protocol of this study has been approved by the Brazilian Association for Laboratory animal Science (COBEA) of the Federal University of Santa Maria.

Preparation of rat synaptosome P2 fraction and experimental grouping

Rat brain synaptosome P2 fractions were prepared as described (Dunkley et al. 2008). Synaptosome P2 fraction (2 mg protein) was incubated with or without (control) different concentrations of saponins (10–200 µg/ml) in the presence or absence of the pro-oxidant [i.e., Fe^{2+}(10 µM) and SNP (5 µM)] for 30 min at 25 °C in an incubation medium containing in mM: 10 HEPES buffer, 220 mannitol, 68 sucrose, and 10 KCl, pH 7.0 (total incubation volume = 300 µl). After incubation, cell viability, non-protein thiol (NPSH), total thiol content, lipid peroxidation (TBARS) and ROS production were determined.

Assessment of cell viability or mitochondrial activity

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay was used to monitor mitochondrial activity (Riss et al. 2004). Absorbance values were monitored at 550 nm (SpectraMax, USA) and data were reported as percentage of control.

Assessment of non-protein thiol (NPSH) and total thiol (T-SH) content

Graded concentration (10–200 µg/ml) of saponin was added to synaptosomal fraction in the presence or absence of Fe^{2+}(10 µM)/SNP (5 µM) and 300 µl of 10 % trichloroacetic acid. Following centrifugation (4,000×*g* at 4 °C for 10 min), the protein pellet was used for total thiol determination (T-SH), while the free thiol groups (NPSH) were determined in the clear supernatant as described (Seligman et al. 2005). The results were corrected with protein content and expressed in percentage.

Assessment of lipid peroxidation and ROS

The lipid peroxidation end-products were quantified using TBARS assay (Dawn-Linsley et al. 2005). Measurements were recorded at 532 nm (SpectraMax, USA). Malondialdehyde (0–3 nmol/ml) was used for the standard curve while protein concentration was determined Bradford method(Bradford 1976). ROS production in isolated synaptosomal P2 fraction was measured using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) fluorescence probe.

The formation of the oxidized fluorescent derivative (DCF) was measured in the supernatant as the result of reactive oxygen/nitrogen species (ROS/RNS) generation (Shimadzu RF-5301) with excitation and emission wavelengths of 488 and 525 nm respectively and with slit widths of 1.5 nm.

Statistical analysis

The results are expressed as mean ± S.E.M (standard error of mean) of three to four independent determinations. Statistically significant differences among groups were analyzed by one-way ANOVA followed by the Duncan multiple range test when appropriated. Differences were considered to be statistically significant when $p < 0.05$.

Results

Effect of saponins from *Solanum anguivi* on cell viability

The effect of saponins on mitochondrial activity was investigated and the result showed that saponin did not have any effect on mitochondrial viability as revealed by MTT reduction assay (Fig. 1a). This indicates that saponins from *S. anguivi* do not interfere with the metabolic activity of the mitochondrial. Fe^{2+} and SNP decreased mitochondrial activity as shown in Fig. 1b and c respectively, when compared to control ($p < 0.05$). Co-treatment with saponin significantly reduced Fe^{2+}- and SNP-induced disruption of mitochondrial activity.

Effect of saponins from *Solanum anguivi* on lipid peroxidation in synaptosomal P2 fraction

Saponin did RSnot have any significant effect on synaptosomal membrane lipid peroxidation (Fig. 2a). Both Fe^{2+} and SNP caused a significant increase in TBARS production but Fe^{2+} induced lipid peroxides more effectively than SNP ($p < 0.05$, Fig. 2b, c). Saponins from *S. anguivi* caused a significant decrease ($p < 0.05$) in both Fe^{2+} (Fig. 2b) and SNP (Fig. 2c) stimulated TBARS production but could not bring the values to control level.

Effect of saponins from *Solanum anguivi* on ROS production in synaptosomal fraction

The effect of saponin on ROS production in synaptosomal fraction of rats' brain is presented in Fig. 3a. Saponin (10–200 µg/ml) did not modify the production of ROS when compared to control ($p > 0.05$). Treatment with Fe^{2+} and SNP resulted in significant production of ROS ($p < 0.05$) when compared with control (Fig. 3a, b, $p < 0.05$). However, co-treatment of synaptosomal fraction with saponin (10–200 µg/ml) significantly attenuated the production of ROS in synaptosomal fraction (P2).

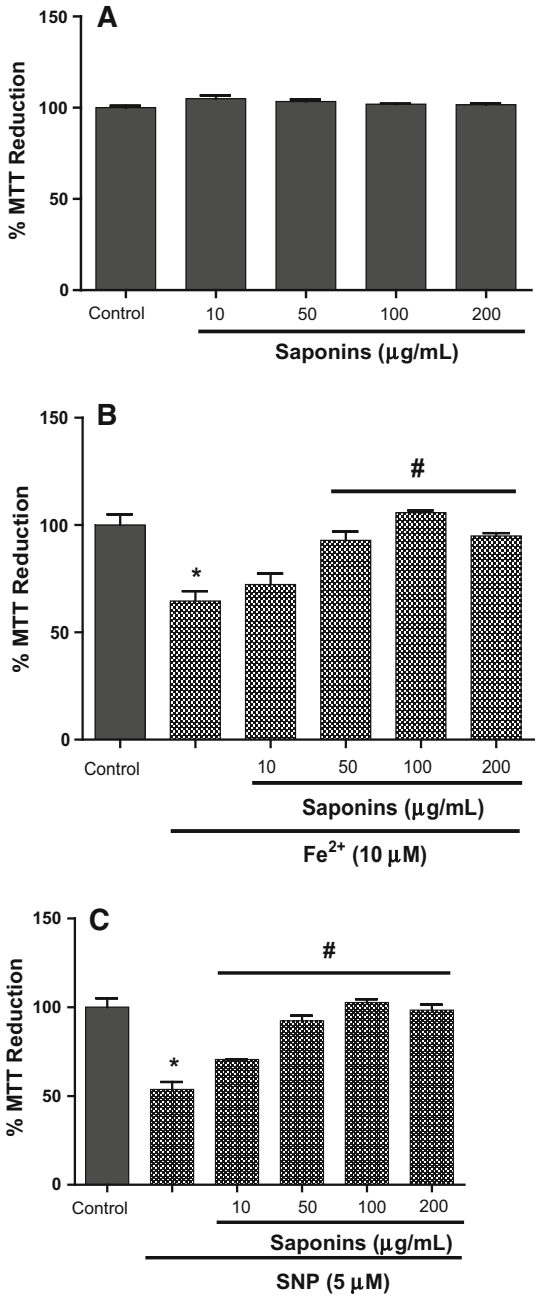

Fig. 1 Effect of saponins from *S. anguivi* fruits on mitochondrial viability in synaptosomal fraction of rats brain (**a**), co-treatment with Fe^{2+} (**b**) or SNP (**c**). *Columns* represent mean ± S.E.M. of three independent experiments. MTT reduction was significantly inhibited by the Fe^{2+} and SNP and co-treatment with saponins (10–200 µg/ml) markedly attenuated this effect ($p < 0.05$). The results are expressed as percentage of control

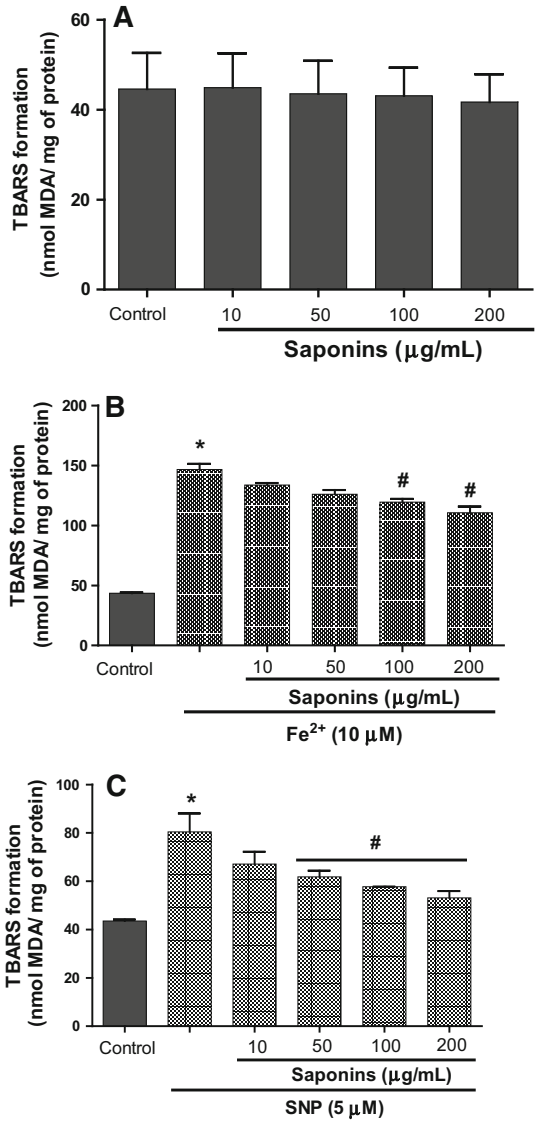

Fig. 2 Effect of saponins from *S. anguivi* fruits on lipid peroxidation in untreated synaptosomal P2 fraction of rats brain (**a**) and co-treatment with Fe^{2+} (**b**) or SNP (**c**). TBARS is expressed as nmol of malondialdehyde per mg of protein. Data are presented as mean ± S.E.M. resulting from three independent experiments.*$p < 0.05$ as compared with control, $^{\#}p < 0.05$ as compared to the Fe^{2+} and SNP-treated synaptosomal fraction

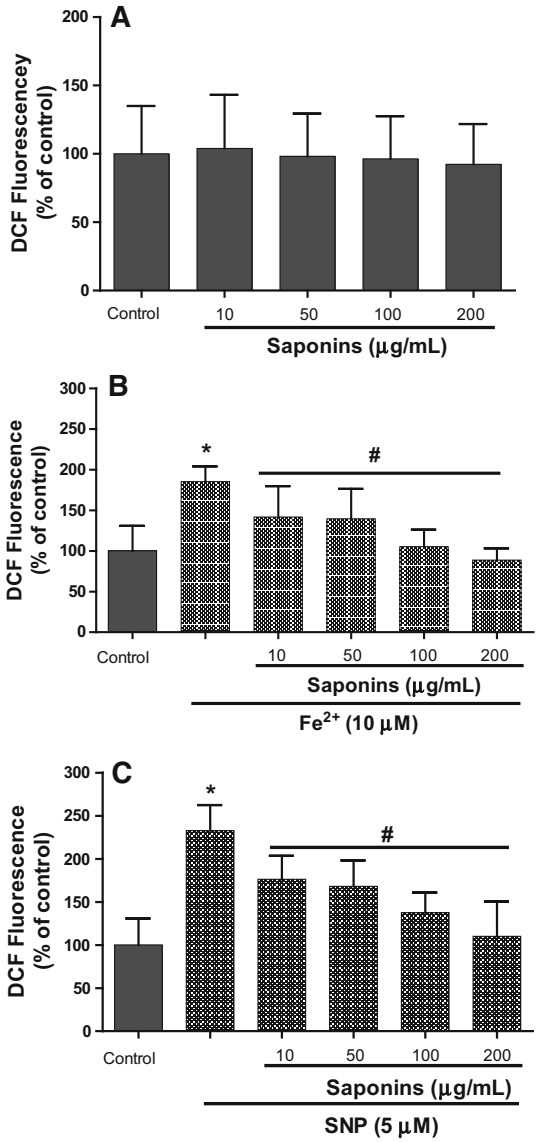

Fig. 3 Effect of saponins from *S. anguivi* fruits on synaptosomal P2 fraction of rats brain (**a**), and co-treatment with Fe^{2+} (**b**) or SNP (**c**). Both Fe^{2+} and SNP induced DCFH oxidation in the incubation medium. *Columns* represent mean ± S.E.M. resulting from three independent experiments and data are expressed as percentage of control (untreated cells). *$p < 0.05$ versus untreated slices (control), $^{\#}p < 0.05$ versus Fe^{2+} and SNP-treated cells

Effect of saponins from *Solanum anguivi* on total-SH and non-protein thiol (NPSH) in synaptosomal P2 fraction

Oxidative stress can be associated with a decrease in total-SH content and NPSH levels in cells. Treatment of synaptosomal fraction with saponin did not cause a reduction in NPSH level (Fig. 4a) and total-SH content (Fig. 5a) in synaptosome. Both Fe^{2+} and SNP caused a significant reduction in NPSH level, which was mitigated by co-treatment of synaptosome with saponins (10–200 µg/ml) (Fig. 4b, c). Saponin was able to restore to control level, the total-SH content that was significantly reduced by Fe^{2+} and SNP (Fig. 5b, c).

Discussion

The iron (Bilgic et al. 2012; Pfefferbaum et al. 2009) and polyunsaturated lipid-rich neuronal environment (Janssen et al. 2014) coupled with low expression of anti-oxidant

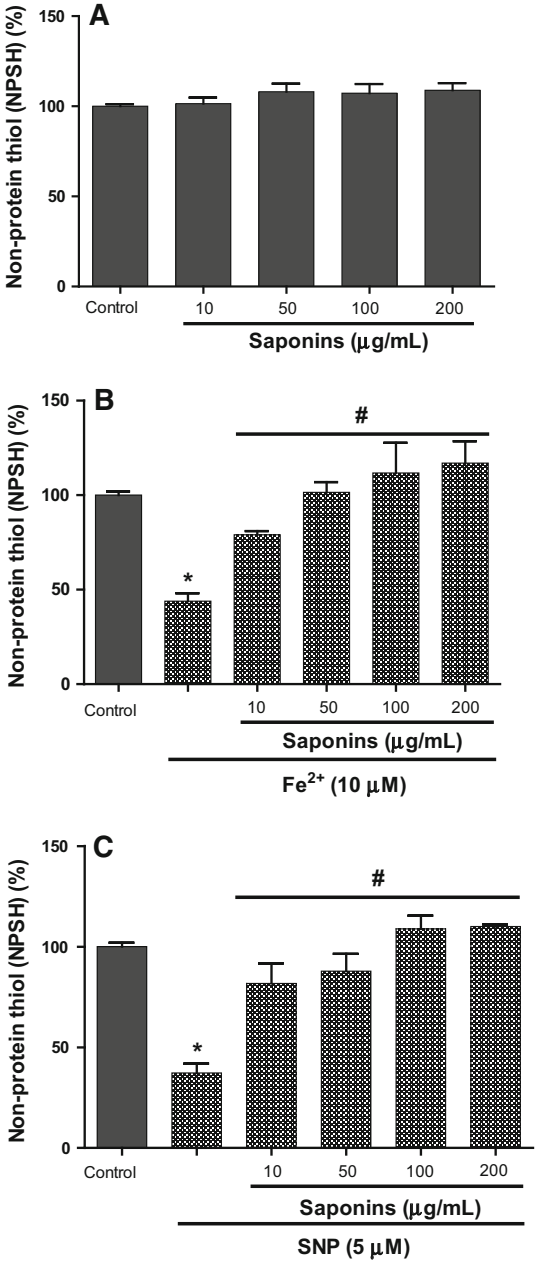

Fig. 4 Effect of saponins from *S. anguivi* fruits on synaptosomal P2 fraction of rats brain (**a**), and co-treatment with Fe^{2+} (**b**) or SNP (**c**) on non-protein thiol (NPSH) content in synaptosome P2 fraction of rats brain. *Columns* represent mean ± S.E.M. resulting from three independent experiments and data are expressed as percentage of control (untreated cells). *$p < 0.05$ as compared with control, $^{\#}p < 0.05$ as compared to the Fe^{2+} and SNP-treated synaptosomal fraction

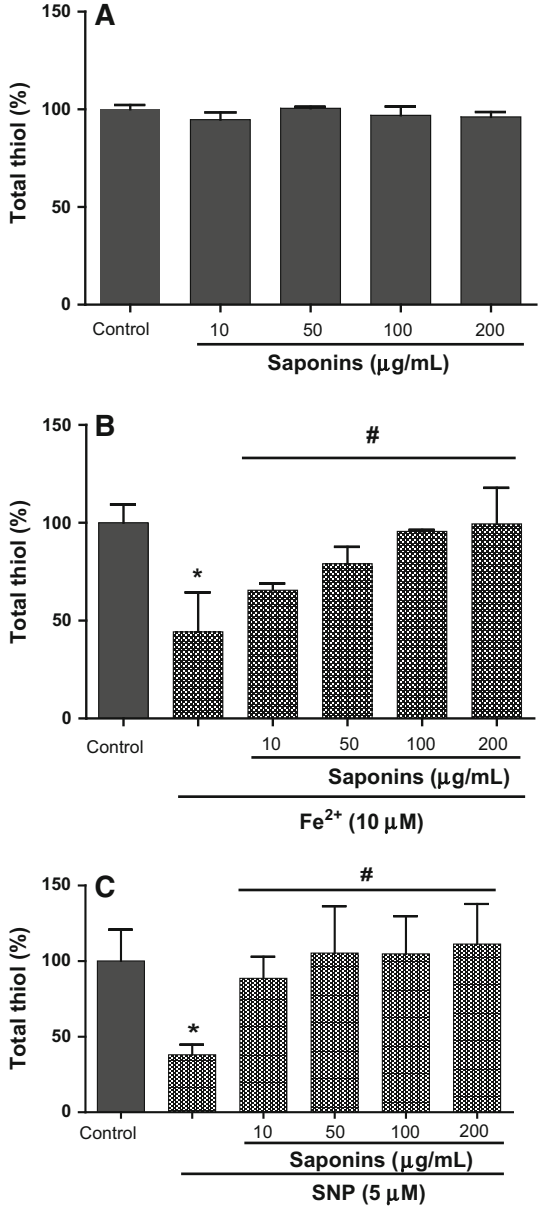

Fig. 5 Effect of saponins from *S. anguivi* fruits on total thiol (SH) content in synaptosomal P2 fraction of rats brain (**a**), and co-treatment with Fe^{2+} (**b**) or SNP (**c**). *Columns* represent mean ± S.E.M. resulting from three independent experiments and data are expressed as percentage of control (untreated cells). *$p < 0.05$ as compared with control, $^{\#}p < 0.05$ as compared to Fe^{2+} and SNP-treated synaptosomal fraction

enzymes account for high susceptibility of the brain cells to free radical-induced oxidative damages. Such damage significantly contributes to the progression of Huntington's, AD, PD and ALS diseases (Rego and Oliveira 2003; Apel and Hirt 2004; Reddy 2009; Cavallucci et al. 2013). Increase in the number of reported cases of these neuropathologies therefore calls for deepened investigation of natural and synthetic anti-oxidant (phyto)-chemicals capable of crossing the blood brain barrier (BBB).

The current study identified *S. anguivi* fruit saponin as one of such naturally occurring compounds with in vitro anti-oxidant properties in rat brain. Since, BBB-crossing is often identified as the limitation for most drugs whose site of action is the brain, this limitation is much reduced in

saponin as earlier report did establish that saponin moderately partition into the brain (Wang et al. 2007).

Another key consideration is the neurotoxicity of phytochemicals, here, *S. anguivi* saponin selectively preserved mitochondrial function in MTT assay while reversing Fe^{2+}- and SNP-induced loss of mitochondrial function. This finding reiterates non-toxicity of saponin which warrants its use as cell-permeabilizing agent thus, allowing organellar functions to be studied in intact cells (Kuznetsov et al. 2008). In addition to maintaining mitochondrial integrity, *S. anguivi* saponin similarly protected synaptosomal fractions from the debilitating effects of ROS produced by Fe^{2+} and SNP. Undoubtedly, this finding has clinical implications in protecting the histological architecture and molecular machineries required to maintain inter-neuronal electrical and chemical communication which becomes compromised following ROS attack on synaptosomes (Magni et al. 2009). It is also worth noting that thiol-containing macromolecules such as synaptosomal plasma membranes of calpain I (Siman et al. 1983), volume-sensitive taurine efflux proteins (Martinez et al. 1994) and non-protein mono/dithiols involved in vesicular GABA release (Robillard et al. 1987) may also benefit from *S. anguivi* saponin due to protein thiol protecting properties.

In conclusion, results of the present study strongly demonstrated that saponin from *S. anguivi* exerted in vitro neuroprotection in rat brain synaptosomal fraction against Fe^{2+}- and SNP-induced toxicity and provides a basis for further investigation of possible clinical applications in Huntington's, AD, PD and ALS diseases.

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Conflict of interest The authors declare no conflict of interest with any person or any organization.

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