PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

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AQUEOUS EXTRACT OF SIDA ACUTA ATTENUATES NICOTINE-INDUCED CEREBELLAR DYSFUNCTION IN ADULT MALE RATS

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ABSTRACT

Sida acuta (SA) has a variety of traditional uses spanning from its fresh root that is chewed for the treatment of dysentery to hot aqueous extract of dried plant orally administered as diuretic. The aqueous extract of the plant has antimicrobial, antimalarial, analgesic and antiplasmodial effects. This study was designed to investigate the neuroprotective effects of the aqueous extract of the leaves of SA in nicotine-induced cereballar dysfunction. Adult male Wistar rats were randomly separated into the following groups: Vehicle (received distilled water), Nicotine-treated (NIC-treated; received 1.0mg of Nicotine per kg of body weight), SA-treated (received 500mg/kg of body weight of aqueous extract of SA) and NIC+ SA-treated (received 1.0mg of Nicotine and 500mg of SA per kg body weight). The treatment lasted for 28 days and the administration was done daily by oral gavage. The body weight change was monitored using standard animal weighing balance; biochemical assay and cerebellar tissue histology were performed as previously described. The results showed increase in body weight gain and disruption of cytoarchitecture of the cerebellum in nicotine-treated group compared with vehicle-treated group. These alterations of cerebellar morphology may be associated with increased oxidative stress. However, concomitant administration of aqueous extract of SA during treatment with nicotine attenuated cerebellar disruption. The result indicated that administration of aqueous extract of the leaves of SA during treatment with nicotine preserves cerebellar function.

Keywords: Cerebellum, Cyto-architecture, Neuro-protective, Nicotine, *Sida acuta*.
Submitted: January 2018; Accepted March 2018

INTRODUCTION:

Nicotine is one of the main components of tobacco or cigarette which have psycho stimulant properties similar to amphetamine [1,2,3]. The effects of nicotine are well documented in humans and animals, both in vivo and in vitro [4,5,6]. These effects include the following; increase in blood pressure,

mobilization of sugar and catecholamines level of blood [4], alteration of antioxidant defense mechanisms [7], contribute to development of lung cancer [8], hepatic toxicity, infertility [9] etcetra. Studies have shown that nicotine abuse induces oxidative stress, apoptosis and inflammation in brain cells [10]. In addition, nicotine-induced neurotoxicity has been reported to be more pronounced in some brain regions like hippocampus, amygdala and cerebellum [11,12]. The deleterious toxic effects of nicotine have been linked to increased production of reactive oxygen species (ROS) [13,14]. ROS damages DNA, proteins, carbohydrates, and lipids and affects enzyme activity and cellular genetic machinery [13]. However, the biological systems possess a number of mechanisms to remove ROS, such as the integrated antioxidant defense systems [13,15] and also, in recent years, herbal/natural compounds with medicinal values have gained a striking attention in providing enhancement to endogenous defense mechanism. Natural flavonoids and their derivatives are being widely considered as supplementary therapeutics against neurodegenerative diseases [6,16].

Sida acuta is an erect, branched small perennial herb or shrub which grows abundantly on cultivated fields, waste areas, roadsides and open land areas in tropical and subtropical regions [17, 18]. It is commonly known as broom grass, broom weed, clockplant, common fan petals and common wire weed. *Sida acuta* has a variety of traditional uses. In Central America, the plant is used to treat asthma, renal inflammation, colds, fever, headache, ulcers and worms [19], in Colombia the plant is known for the treatment of snake bites [20], in West Africa, particularly in Burkina Faso and even Nigeria, the plant is traditionally used in the treatment of malaria, diarrhea and many other diseases [21].. The hot aqueous extract of the dried entire plant is administered orally in India as a febrifuge, diuretic and to prevent vomiting and gastric disorders [22,23].. In Papua New Guinea, the fresh root is chewed for the treatment of dysentery [24].

In addition, several studies have reported the effectiveness of this noble plant as antimalaria, antiulcer, worm expeller, antipyretic, antidiarrhea, antiplasmodial, analgesic and antidepressant [25,26,27]. In Nigeria, S. acuta is one of the plants most commonly used for the treatment of hypertension, erectile dysfunction and hemorrhoids [28,29]. The pharmacological activities of S. acuta have been linked to its components which include: alkaloids such as vasicine, ephedrine and cryptolepine (the main alkaloid in the plant) [18], saponosides, coumarins, steroids, tannins, phenolic compounds (evofolin-A and B),

scopoletin, loliolidand4-ketopinoresinol, polyphenol, sesquiterpene and flavonoids [30].

This current study attempted to investigate the ameliorative effect of S. acuta on nicotine-induced cerebellar dysfunction in adult male rats.

MATERIALS AND METHODS:

Preparation of the extract:

Samples of *S. acuta* were collected locally. The plant was botanically authenticated in the Department of Plant Biology, University of Ilorin, Nigeria. Authentication number UIV 14 was issued and the plant was deposited at the herbarium. The leaves of the plant were air-dried and pounded into powder using pestle and mortar and kept in an air-tight container. 580 g of the sample was percolated in distilled water for 48 hours and stirred intermittently with magnetic stirrer. It was filtered and the filtrate was evaporated in steam bath until substantial water has been removed. It was later dried in the oven at 37°C to concentrated extract.

Animals, Grouping and protocol:

Twenty adult male Wistar rats weighing 180-210g were obtained from the animal house, College of Medicine and Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. The rats were housed in wire mesh cages and maintained in a well ventilated room at 25±2 ◦C, on a 12-h light/12-h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatized for two weeks, the rats were randomly distributed into four groups (n=5); Vehicle (received distilled water), Nicotine-treated group (NIC-treated; received 1.0mg nicotine per kg body weight (b.w)), SA-treated (received 500mg per kg b.w of aqueous extract of SA) and NIC+ SA-treated (received 1.0mg of nicotine plus 500mg/kg b.w of SA). The treatment lasted for 28 days and the administration was done daily by oral gavage. The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals [31] and was approved by the Institutional Review Board of University of Ilorin. Every effort was made to minimize both the number of animals used and their suffering. Initial and final body weights were monitored using animal weighing balance (Olympia SCL66110 model, Kent Scientific Corporation, Torrington, CT06790, USA) and the body weight gain were obtained.

Sample preparation and biochemical analysis:

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p). Blood was collected from the apex of the heart into heparinized bottle and centrifuged at 3000 rpm for 15 minutes using a bench centrifuge and the plasma was stored frozen until it was needed for biochemical assay. Biochemical analysis of plasma malondialdehyde (MDA), which is a marker of lipid peroxidation and superoxide dismutase

(SOD) an antioxidant were performed using assay kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK) [32].

Tissue homogenate:

The cerebella were excised, blotted and weighed. After weighing, 500mg of tissue was carefully removed and homogenized with a glass homogenizer following centrifugation at 3000rpm for 10 minutes. Supernatant was used for the measurement of Glucose-6- phosphate dehydrogenase (G6PDH) and Glutathione peroxidase (GPX) activities by standardized enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK) [33].

Histology:

The cerebellar tissues were fixed in 10% buffered formol saline for histological examination using hematoxylin and eosin (H&E) staining techniques and examined microscopically.

Statistical analysis:

All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS, version 22 of statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p< 0.05$.

RESULTS:

Effect of aqueous extract of SA on body weight in nicotine-treated male rats:

Table 1 depicts the effect of administration of SA and nicotine on body weight. The results showed significant loss in body weight during treatment with nicotine alone when compared with vehicle-treated group. However, concomitant treatment with aqueous extract of SA during treatment with nicotine significantly improved the body weight ($p<0.05$).

Effect of aqueous extract of SA on the histology of cerebellum in nicotine-treated male rats:

H & E stained section of cerebellar cortex of Vehicle- and SA-treated groups showed the well known normal structure. They showed three distinct layers from outside inwards; the molecular layer, the mono layer of Purkinje cells and the closely packed granular cell layer. The Purkinje cells were arranged in one row between the molecular and granular layers. The granular layer was composed of closely packed numerous small granular cells with dark nuclei (Figure 1a, c). Examination of the photomicrograph of a section of cerebellar cortex in NIC-treated rat revealed disruption of purkinje cell layer, vacuolation of molecular layer and dispersed granular cells with evidence of inflammation (Figure 1b).

Examination of the photomicrograph of a section of cerebellar cortex in NIC+SA-treated rat showed mild disruption of purkinje and granular layers and normal molecular layer (Figure 1d).

Effect of aqueous extract of SA on oxidative stress markers in nicotine-treated male rats: Plasma MDA and SOD are potent biomarkers of oxidative stress. Treatment with nicotine significantly induced oxidative stress with significant increased level of plasma MDA and decreased level of SOD (Figure 2) when compared with vehicle-treated group. However, concomitant administration of SA during treatment with nicotine ameliorated oxidative stress with a decreased level of plasma MDA and a significant increased level of SOD when compared with nicotine-treated group (Fig 2).

Table 1: Effects of aqueous extract of *S. acuta* and nicotine on body weight of adult male rats

Groups	Initial body weight (g)	Final body weight (g)	Body weight change (g)
Vehicle-treated	200.1± 11.0	215.1 ± 10.0	15.0 ± 9.0
NIC-treated	195.5 ± 3.5	191.8 ± 3.5	-3.7 ± 8.2*
SA-treated	187.9± 5.7	197.6± 5.3	10.3± 12.6
NIC+SA-treated	190.4±7.4	214.5 ± 11.5	24.5±13.25#

Data are expressed as mean ± S.E.M. n = 5. (*p<0.05 vs. Vehicle; #p<0.05 vs. NIC)

Effect of aqueous extract of SA on some cerebellar endogenous defense enzymes in nicotine-treated male rats: Glucose-6-Phosphate dehydrogenase (G6PDH) and Glutathione peroxidase activities are bioindicators of the cellular defense mechanism against oxidative stress. Exposure to nicotine significantly reduced G6PDH and GPX activities compared with the vehicle. Nevertheless, administration of SA during treatment with nicotine significantly increased G6PDH and GPX activities compared with nicotine-treatment group (Figure 4a, b).

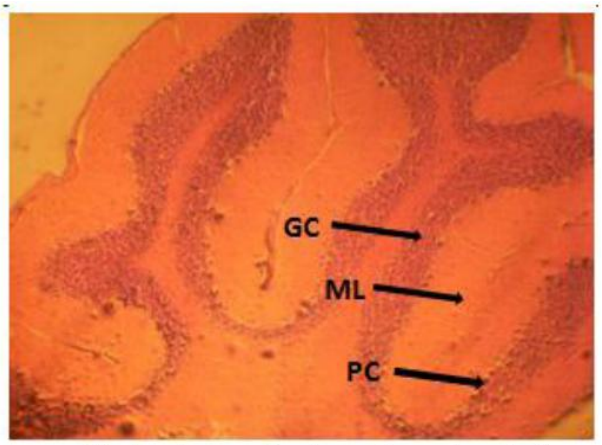

Fig. 1a: Photomicrograph of a section of cerebellum in Vehicle-treated rat

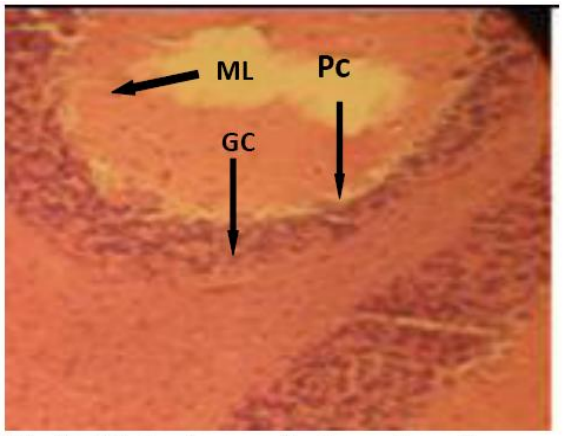

Fig 1b: Photomicrograph of a section of cerebellum in NIC-treated rat

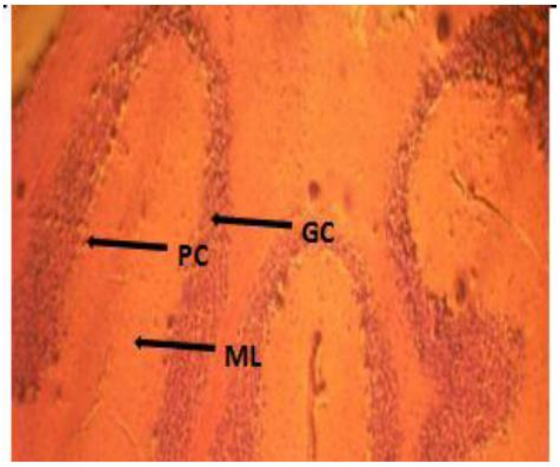

Fig. 1c: Photomicrograph of a section of cerebellum in SA-treated rat

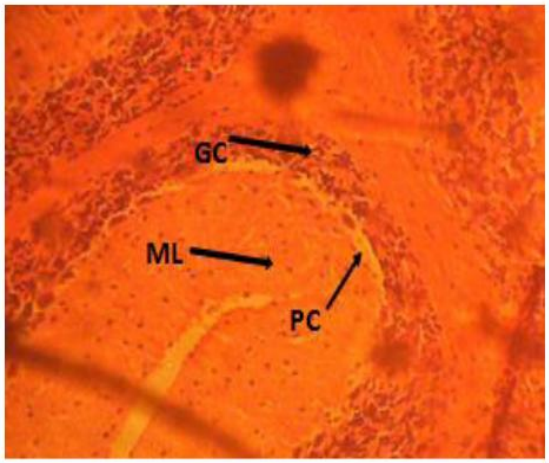

Fig. 1d: Photomicrograph of a section of cerebellum in SA+NIC-treated rat

Vehicle-treated rat, shows normal molecular layer, closely packed granular cell layer and mono layer of purkinje cells, NIC-treated rat, shows disruption of purkinje cell layer, vacuolation of molecular layer and dispersed granular cells with evidence of inflammation, SA-treated rat, shows normal molecular layer, closely packed granular cell layer and mono layer of purkinje cells and NIC+SA-treated rat, shows mild disruption of purkinje and granular layers and normal molecular layer. (H & E paraffin stain; ×200, transverse section).ML (molecular layer); GC (Granular cells); PC (Purkinje cells).

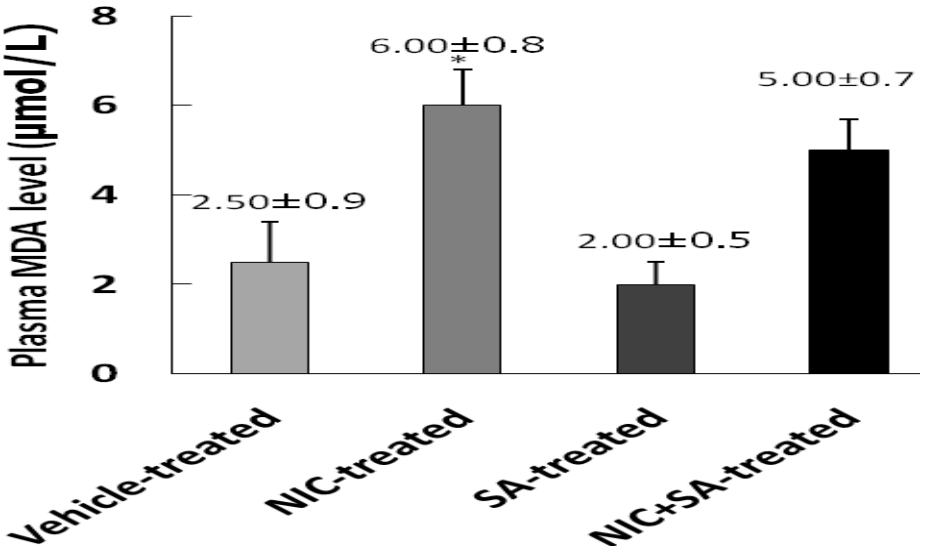

Fig. 2a: Effect of *Sida acuta* and nicotine treatment on plasma MDA concentration of male Wistar rats.

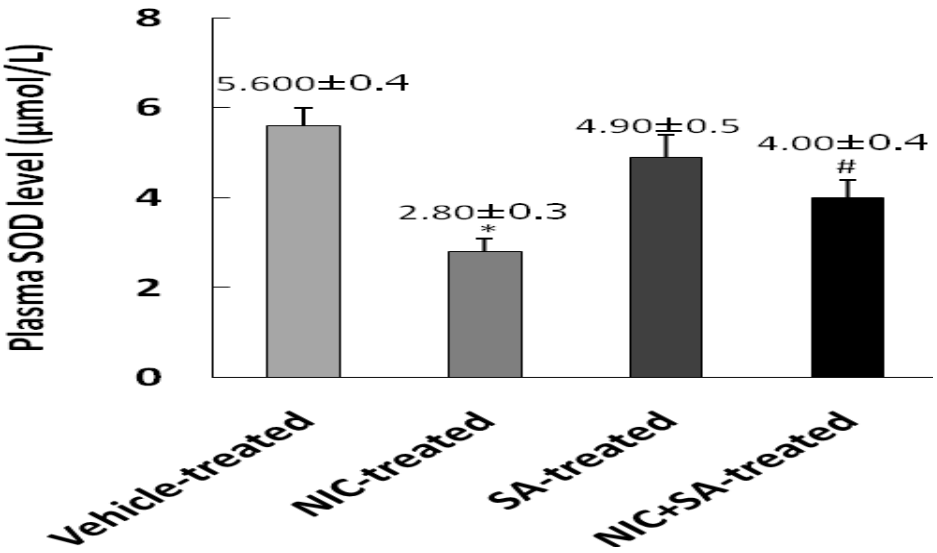

Fig. 2b: Effect of *Sida acuta* and nicotine treatment on plasma SOD concentration of male Wistar rats.

Data are expressed as mean ± S.E.M; n = 5. (* $p<0.05$vs Vehicle, # $p<0.05$vs NIC).

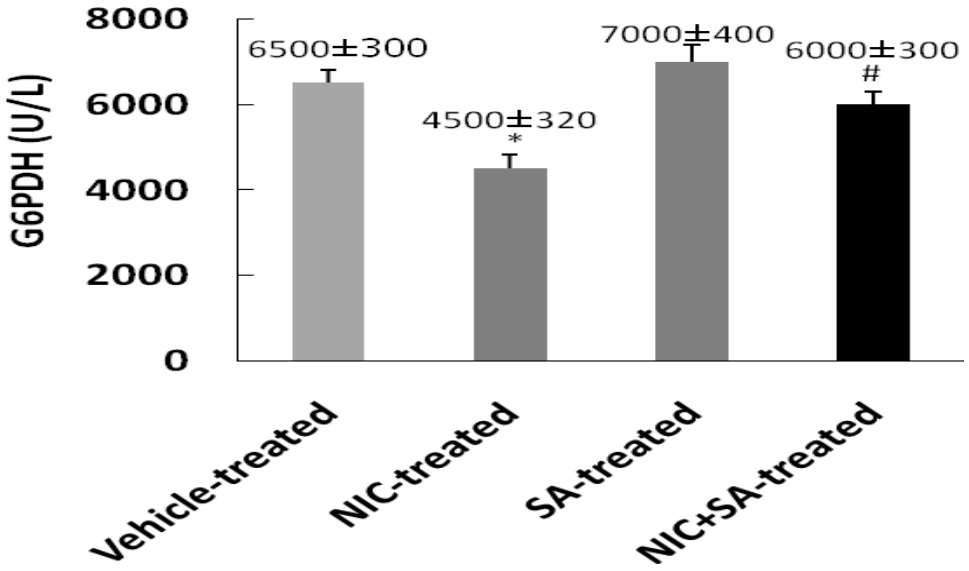

Fig. 3a: Effect of *Sida acuta* and nicotine treatment on cerebellar Glucose-6- Phosphate dehydrogenase activity of male Wistar rats.

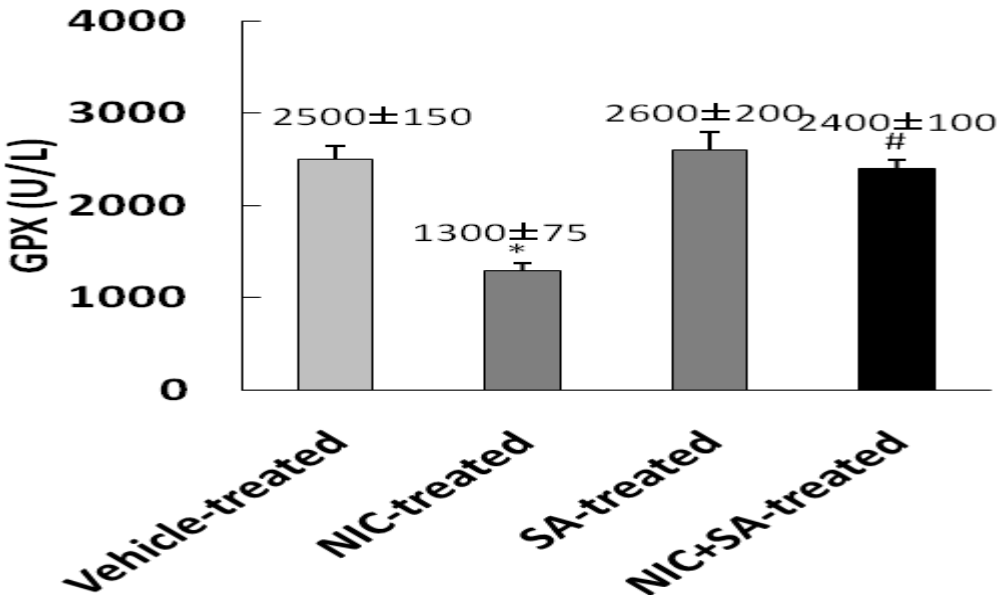

Fig. 3b: Effect of *Sida acuta* and nicotine treatment on Glutathione peroxidase activity of male Wistar rats.

Data are expressed as mean ± S.E.M. n = 5 (* p<0.05vs Vehicle, #p<0.05 vs NIC).

DISCUSSION:

The present study has attempted to investigate the ameliorative effect of aqueous extract of *S. acuta* on nicotine-induced cerebellar dysfunction. Our results indicate that exposure to nicotine led to loss of body weight, disruption of purkinje cell layer, vacuolation of molecular layer and dispersed granular cells with evidence of inflammation in the histology of cerebellar cortex when compared with the vehicle-treated group. These alterations in the cytoarchitecture of cerebellar cortex were associated with increase in plasma MDA level, decrease in plasma SOD level, decreased G6PDH and GPX activities.

However, administration of aqueous extract of *S. acuta* together with nicotine reduces the extent of the loss of body weight, the monolayer arrangement of purkinje cells, molecular and granular layers in the histology of cerebellar cortex.

Nicotine as a psycho-stimulant compound carries a high potential for abuse and addiction [34]. Our present finding that treatment with nicotine causes reduction in body weight is in consonance with previous observation that nicotine intake via smoking or smokeless route caused transient anorexia and increased energy expenditure that reduced body weight in humans as well as in rats [35,36,37]. However, concomitant administration of *S. acuta* and nicotine to rats reduces the extent of loss of body weight, which implies that aqueous extract of *S. acuta* has the capacity to reduce the negative impact of nicotine on normal body weight.

The current pattern of histopathological changes in cerebellar cortex is in line with earlier observation [38]. However, administration of *S. acuta* during treatment with nicotine preserved the distinct structural layers of cerebellar cortex with mild disruption of purkinje and granular layers.

Furthermore, our current results revealed that the altered cerebellar structure and function in nicotine-treated animals was associated with increase in circulating level of MDA and decrease in circulating level of SOD when compared with the vehicle-treated group. Alteration in the circulating levels of MDA and SOD observed in the present study is an indication of oxidative stress, which suggests that nicotine-induced cerebellar degeneration may be mediated by oxidative stress. This finding provides further evidence to previous studies that nicotine abuse induces oxidative stress, apoptosis and inflammation in brain cells [16]. Elevated level of MDA indicates increased lipid peroxidation which damages the cell membrane and causes apoptosis [39, 40. Our present result that treatment with nicotine

led to significant increase in plasma MDA is consistent with earlier studies [16, 40]. The decrease in the circulating level of SOD, G6PDH and GPX activities observed in the current study during treatment with nicotine may indicate an altered cerebellar redox status.

This suggests that the cerebellar tissue disruption observed in the current study may be associated with oxidative stress.

However, concomitant administration of aqueous extract of *S. acuta* during treatment with nicotine seems to reduce nicotine-induced cerebellar toxicity.

CONCLUSION:

The present results suggest that administration of aqueous extract of the leaves of SA during treatment with nicotine preserves cerebellar function which is accompanied with increased SOD, G6PDH and GPX activities.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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