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Original Article

Is Aqueous Extract of Soybeans Neurotoxic? Preliminary Evidence from Histological Evaluation of the Cerebellum and Cerebrum of Wistar Rats

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Abstract

BACKGROUND AND AIM: Reports indicate that there is a worldwide surge in plant-based eating patterns as typified by the increasing number of vegetarians and vegans. However, several medicinal and nutritional plants, seeds, and fruits can induce neurotoxicity. Accordingly, this study investigated the histological effects of aqueous Soybeans seed extract (ASSE) on the cerebellum and cerebrum of adult Wistar rats.
METHODOLOGY: Following purchase and acclimatization, twenty-four rats were randomly divided into four groups of six rats per group as follows: Group A (control) received 1 ml distilled water daily, group B received 250 mg/kg body weight of ASSE daily, group C received 500 mg/kg body weight of ASSE daily and group D received 1000 mg/kg body weight of ASSE daily. All administration, via an oral gavage, lasted for twenty-eight days. After sacrifice, the cerebellum and cerebrum were separated, weighed, and processed for histological assessment.
RESULTS: Results showed no significant difference (P>0.05) in the body, brain, cerebellar, relative cerebellar, cerebral, and relative cerebral weights of rats treated with all doses of ASSE compared to control. Also, no significant difference (P>0.05) was observed in the cerebellum-brain and cerebrum-brain ratios of treated rats in comparison to control. Histological findings revealed normal and intact histology of the cerebellum and cerebrum layers in treated rats when compared to control.
CONCLUSION: Taken together, these findings provide preliminary histological evidence demonstrating that ASSE was safe for the experimental rats and had no adverse effect on the cerebellum and cerebrum.

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INTRODUCTION

The brain is the most complex of all systems biologically consisting of billions of neurons (Stiles and Jernigan, 2010). Along with the spinal cord, it makes up the central nervous system and consists of three major parts namely: cerebrum, brainstem, and cerebellum. The cerebrum is divided into two (right and left) hemispheres by a longitudinal fissure and constitutes the largest portion of the brain (Raichle, 2010). Signals from the right side of the body are controlled by the left cerebral hemisphere whereas signals from the left side of the body are controlled by the right hemisphere (Bui and Das, 2024). Each hemisphere is made up of the frontal, temporal, parietal, and occipital lobes; however, the frontal lobe plays a role in cognitive functions such as memory, learning, problem-solving, and voluntary activities (Raichle, 2010). The cerebellum is a large brain mass located in the posterior cranial fossa; it is posterior to the fourth ventricle, pons, and medulla oblongata, and inferior to the posterior part of the cerebrum. The basic functions of the cerebellum are the maintenance of equilibrium, impact regulation of muscle tone, coordination of somatic motor activities, and cognitive functions

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(Enogieru and Momodu, 2021a). Disorders of the brain and its parts have been reported to affect the normal functioning of the body and there is an amplified demand for natural supplements or dietary sources that can improve brain health and function (Naureen *et al.*, 2022).

Globally, it is estimated that over 80% of humans in the world still practice traditional methods such as the use of plants as a major tool of health care (Lee *et al.*, 2019). Natural products obtained from plants for the treatment of diseases have proven that nature stands as a golden mark for achieving drug discovery (Koparde *et al.*, 2019). Medicinal plants are reported to be therapeutic agents for the treatment of diseases and have been employed in the treatment of cognitive and motor disorders (Enogieru and Momodu, 2021b; Enogieru *et al.*, 2018). *Glycine max*, also known as soybean or soja beans, is a member of the subfamily *Faboideae* and is one of the most prevalently grown and used oil seeds. Its usage ranges from human to animal food, and industrial products (Gaonkar and Rosentrater, 2019). The reported pharmacological properties of soybeans include antioxidant, antidiabetic, antihypercholesterolemic, antihyperlipidemic, antiobesity, antihypertensive, anticancer, antimutagenic, hepatoprotective, antiviral, anti-inflammatory, immunomodulatory, and wound healing effects (Alghamdi *et al.*, 2018). Studies have shown that soybeans contain important phytochemical constituents such as alkaloids, flavonoids, phenols, saponins, eugenols, and terpenoids (Lisanti and Arwin, 2019). These phytochemicals have been reported to have pharmacological benefits against neurological and neurodegenerative disorders in several in-vitro and in-vivo studies (Enogieru *et al.*, 2021; Khan *et al.*, 2021). Despite the diverse therapeutic potentials of soybeans, there is limited information on its activity on the histoarchitecture of the brain, and accordingly, this study was designed to investigate the possible alterations in the cerebrum and cerebellum of Wistar rats following treatment with aqueous soybeans seed extract.

MATERIALS AND METHODS

Plant Extract

Soybean seeds, purchased at a local market in Benin City, were identified and assigned herbarium number UBH-G470 in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The extraction was carried out according to a previously described method (Enogieru and Omoruyi, 2022), with slight modifications. Briefly, the seeds were washed, air-dried for about 4 hours, and pulverized to powder form. Extraction of the powdered soybeans was done thrice in distilled water at room temperature on a shaker for 48 hours. Thereafter, the extract was filtered using a Whatman filter paper No 42 (125 mm) and the filtrate obtained was lyophilized using a freeze dryer (LGJ-10, SearchEquipment, UK). The resultant dried powdered extract was reconstituted with distilled water to give the desired concentrations used in this study.

Research Design

A total of twenty-four Wistar rats weighing between 160g - 220g were used for this study. They were randomly assigned into four groups (A-D) of six rats each following two weeks of acclimatization. Group A received 1 ml distilled water daily; Group B received 250 mg/kg body weight of aqueous soybeans seed extract (ASSE) daily; Group C received 500 mg/kg body weight of ASSE daily; Group D received 1000 mg/kg body weight of ASSE daily. All administration, via an oral gavage, lasted for twenty-eight days.

Determination of Brain weights

Rats were sacrificed by cervical dislocation and the brains were accessed through a longitudinal cranial incision. The brains were weighed and separated into the cerebellum and cerebrum. To moderate the specific body weight differences, the relative brain weight was expressed as a percentage of the final body weight at sacrifice, as previously reported (Enogieru *et al.*, 2015). The cerebellum and cerebrum brain ratio was expressed as the cerebellum and cerebrum over brain weight, respectively, at sacrifice.

Histological Assessment

The cerebrum and cerebellum were harvested and fixed in 10 % buffered formal saline for 48 hours and processed using the Hematoxylin and Eosin staining technique, as previously described (Drury and Wallington, 1980).

Statistical Analysis

All values are presented as mean ± standard error of the mean for all groups. Significance differences ($P<0.05$) were determined using a one-way analysis of variance (ANOVA) and Tukey's *post hoc* test using Graph Pad Prism Software, Version 9.

RESULTS

Effect of Treatment on Weights

Table 1 shows the weight findings of rats across experimental groups. There was no significant difference ($P>0.05$) in the weight parameters following treatment with 250 mg/kg, 500 mg/kg, and 1000 mg/kg when compared to the control group.

Effect of Treatment on Histology of the Cerebrum and Cerebellum

Figure 1A-D shows the representative cerebellum of rats across experimental groups. Here, the normal layers of the cerebellar cortex can be seen. It begins with the outer molecular layer containing basket and stellate cells. Also observed are the Purkinje cell bodies in the Purkinje cell layer and the granule cells in the granular cell layer. Still deeper is the white matter of the cerebellum containing nerve fibers, neuroglial cells, and small blood vessels. The Pia matter can also be observed in Figure 1C.

Figure 2A-D shows the representative cerebral cortex of rats across experimental groups. Here, the normal layers I-V of the prefrontal cortex can be seen. Firstly, the outer molecular layer containing sparse neurons and glia can be observed. This is closely followed by the outer granular layer containing small pyramidal and stellate neurons. The outer pyramidal layer containing moderate-sized pyramidal neurons can be observed before the inner granular layer which houses densely packed stellate neurons. Also, the inner pyramidal layer containing large pyramidal neurons can be observed. However, the multiform cell layer containing a mixture of small pyramidal and stellate neurons cannot be fully observed.

Table 1: Weights across experimental groups

Parameters	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Initial Body Weight (g)	193.0 ± 14.24	190.4 ± 6.59	191.6 ± 3.34	190.2 ± 18.65
Final Body Weight (g)	210.4 ± 11.78	204.6 ± 7.11	208.0 ± 4.39	206.8 ± 18.27
Weight Change (g)	17.40 ± 2.69	14.20 ± 1.66	16.40 ± 1.36	16.60 ± 3.63
Whole Brain Weight (g)	1.52 ± 0.04	1.48 ± 0.04	1.52 ± 0.04	1.54 ± 0.04
Relative Brain Weight (%)	0.729 ± 0.03	0.725 ± 0.01	0.731 ± 0.01	0.764 ± 0.06
Cerebral Weight (g)	1.16 ± 0.04	1.16 ± 0.04	1.18 ± 0.02	1.18 ± 0.02
Cerebrum-Brain Ratio (g)	0.762 ± 0.02	0.786 ± 0.01	0.778 ± 0.01	0.768 ± 0.01
Relative Cerebral Weight (%)	0.555 ± 0.02	0.568 ± 0.01	0.568 ± 0.01	0.588 ± 0.05
Cerebellar Weight (g)	0.36 ± 0.03	0.32 ± 0.02	0.34 ± 0.03	0.36 ± 0.03
Cerebellum-Brain Ratio (g)	0.238 ± 0.02	0.214 ± 0.01	0.222 ± 0.01	0.232 ± 0.01
Relative Cerebellar Weight (%)	0.17 ± 0.02	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.01

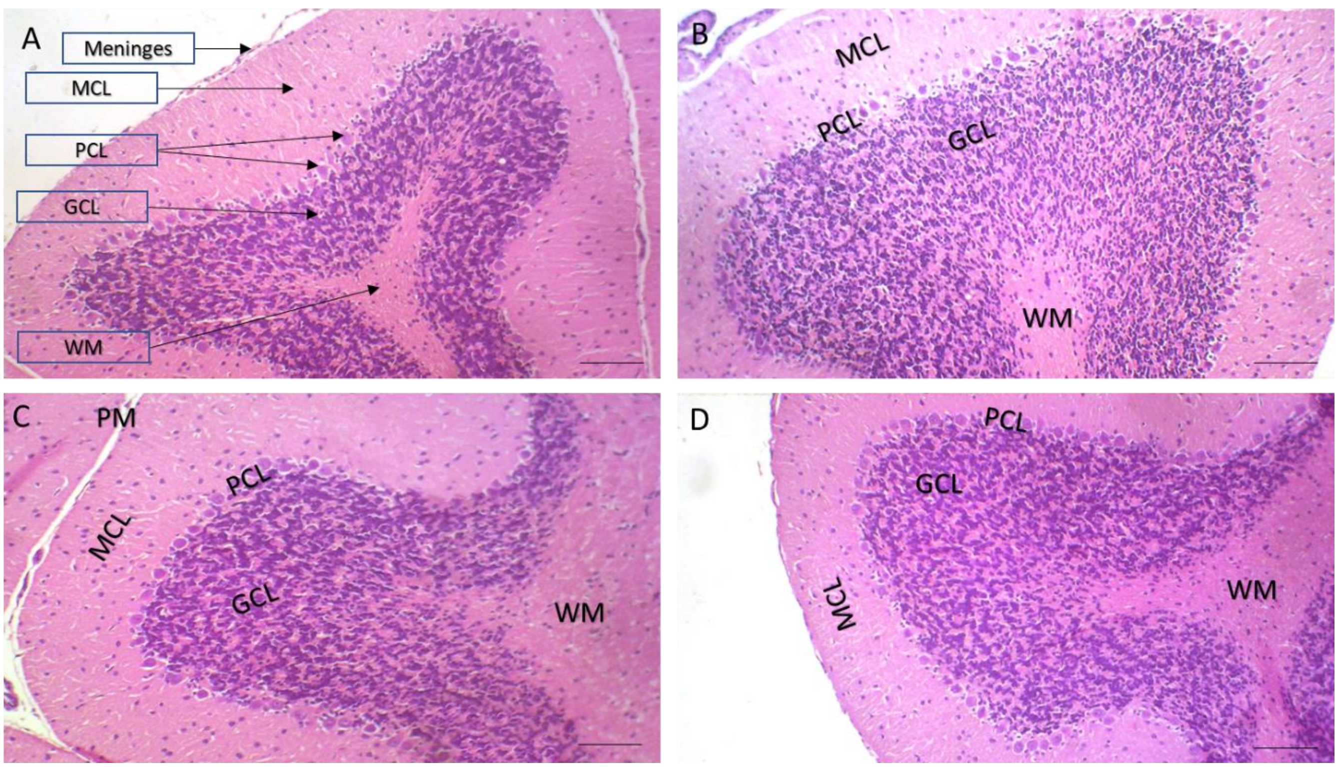

Figure 1: Representative photomicrograph of the cerebellum across experimental groups. (A) Control (B) Group treated with 250 mg/kg ASSE (C) Group treated with 500 mg/kg ASSE (D) Group treated with 1000 mg/kg ASSE. Outer molecular layer (MCL); Purkinje cell layer (PCL); Granular cell layer (GCL); White matter (WM); Pia matter (PM). H&E x 100; Scale bar 100 µm.

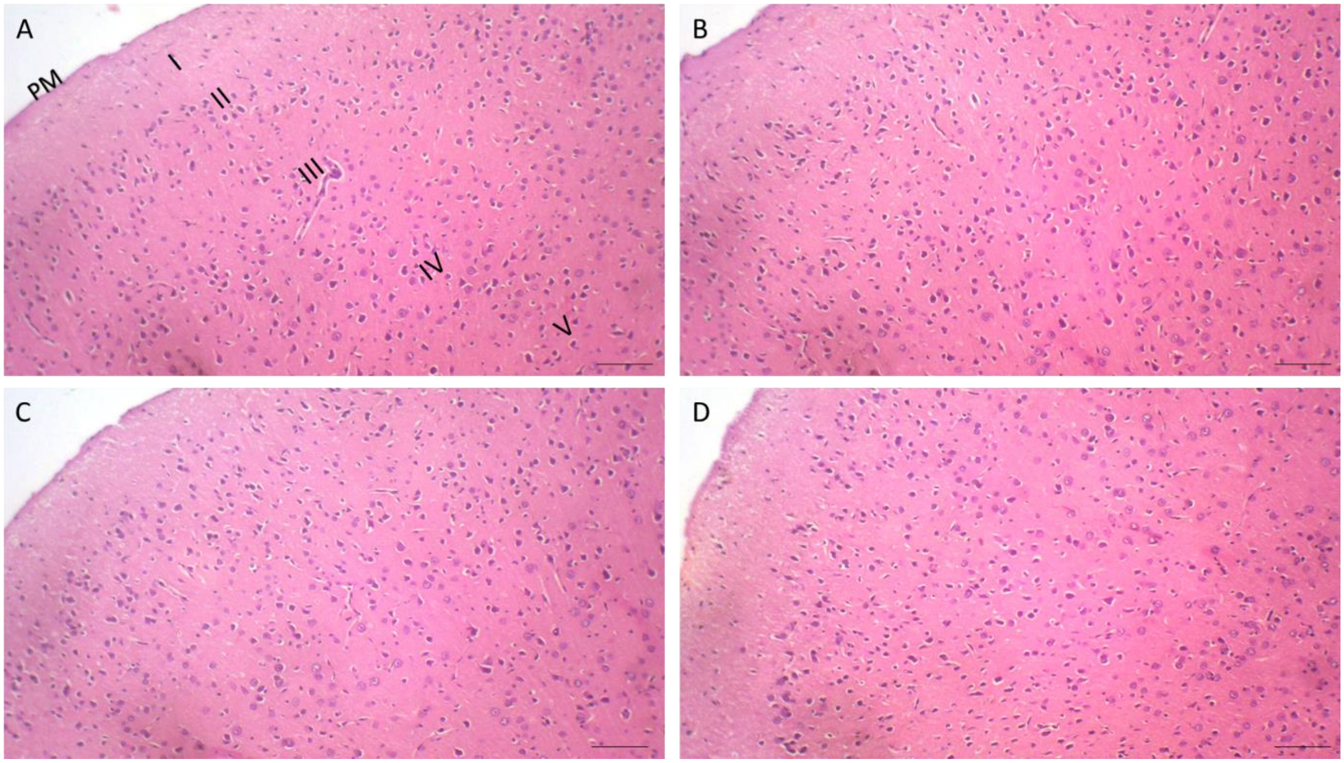

Figure 2: Representative photomicrograph of the cerebral cortex across experimental groups. (A) Control (B) Group treated with 250 mg/kg ASSE (C) Group treated with 500 mg/kg ASSE (D) Group treated with 1000 mg/kg ASSE. Outer molecular layer (I); Outer granular layer (II); Outer pyramidal layer (III); Inner granular layer (IV); Inner pyramidal layer (V). H&E 100x; Scale bar 100 µm.

DISCUSSION

Soybeans is one of the richest and cheapest sources of protein for humans and animal feed (Riaz, 2006). It is one of the few plant-based sources that contains all the essential amino acids needed by the body in building and repairing tissues (Michelfelder, 2009). In recent years, there has been an increased interest in the neuroprotective potential of Soybeans in neurodegenerative disorders (Ahmad *et al.*, 2014). Although the pharmacological properties of Soybeans are well known, some medicinal and nutritional plants, seeds, and fruits are reported to induce neurotoxic syndromes, some of which are irreversible. Accordingly, this study was designed to provide histological evidence on the effects of Soybeans on the cerebellum and cerebrum of Wistar rats.

Findings from this study showed a decrease in the body weight change of rats treated with ASSE when compared to the control rats, although not significant. Several studies have shown the useful effect of soybeans on weight reduction and this effect is partly attributed to its high protein content (Velasquez and Bhathena, 2007). High-protein diets are known to be effective in unregulated food consumption in weight maintenance and reduction (Leidy *et al.*, 2015). The finding from this study agrees with previous reports demonstrating no statistically significant differences in mean body weights, body weight gains, or feed consumption in Soybean treated rats when compared with the control groups (Qian *et al.*, 2018). In a systematic review and meta-analysis of randomized controlled clinical trials on the effect of Soy and Soy Isoflavones on obesity-related anthropometric measures, the authors reported that soy and isoflavones may have different impacts on weight status (Akhlaghi *et al.*, 2017). Also, no significant difference was observed in the brain, cerebral, and cerebellar weights of rats across experimental groups. Organ weights are generally acknowledged in the assessment of toxicities, including plants (Akhigbe, 2014). The body–organ weight ratio reveals the weight of the organs concerning the body weight and reports indicate that toxicity can be revealed by a reduction in the body and organ weight following exposure to substances (Akhigbe, 2014). Consequently, weight reduction has been linked to inflammation, impairment in cellular metabolism and growth as well as lipid peroxidation (Abd El-Kader and Al-Dahr, 2016). From the findings in this study, the non-significant changes in brain, cerebral, and cerebellar weights indicate normal metabolism, absence of lipid peroxidation, and non-induction of inflammation in the rats following treatment with ASSE. To the best of our knowledge, this is the first study demonstrating the effects of ASSE on cerebral and cerebellar weights in Wistar rats.

The effect of substances can be evaluated through the utilization of several techniques and tools, however, the only technique that provides graphic images of the internal milieu of the cells and tissues of an organism are observations made using light microscopy in a field known as histology (Peters *et al.*, 2005). The structure and composition of cells and tissues demonstrate the overall functioning of the organism. The histological findings from this study showed normal microstructure of the cerebellum displaying the Molecular, Purkinje, and Granular cell layers when compared with the control. Similarly, the cerebral cortex of ASSE-treated rats displayed regular outlines of the outer molecular, outer granular layer, outer pyramidal layer, inner granular layer, and inner pyramidal layers. There were no differences observed in the architecture of the cerebellum and cerebrum across experimental groups, thus suggesting that ASSE, at all doses, did not induce adverse microstructural alterations or affect its function following administration in the experimental rats. Consequently, it could be hypothesized that ASSE may help protect regions of the brain against toxic substances, as previously reported (Martin Molinero *et al.*, 2023; Vafaee *et al.*, 2014). The findings on normal cerebellar morphology following ASSE administration agree with a previous study demonstrating that no alterations were observed in the cerebellum of rats administered with soybeans (Martin Molinero *et al.*, 2023). To the best of our knowledge, this is the first study demonstrating the effects of ASSE on the histology of the cerebral cortex in Wistar rats.

CONCLUSION

Taken together, findings from this study suggest that aqueous Soybeans seed extract did not alter the microstructure of the cerebellum and cerebrum in Wistar rats, and can be considered safe for further exploration as a therapeutic agent against neurological and neurodegenerative disorders.

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Conflict of Interest: None declared

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