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JOBELYN ATTENUATES NEURONAL DEGENERATION IN CEREBELLAR CORTEX OF ADOLESCENT RAT EXPOSED TO BINGE ALCOHOL

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ABSTRACT
Cerebellar neurodegeneration is associated with chronic alcohol abuse. There is no clinically potent drug for alcohol-induced neurodegeneration. Though, it was hypothesised that multimodal neuroprotective agent presented outstanding therapeutic potentials in the management of CNS degeneration, however, the knowledge of what could constitute an effective and safe multimodal agent is not clear-cut. However, medicinal botanicals are naturally multimodal in their mechanism of action. The objective of this study is to assess the effect of Jobelyn supplement in a binge alcohol-induced neurodegeneration of the cerebellar cortex in adolescent rats. Two groups of adolescent rats were binge fed with either alcohol (25% w/v) or alcohol (25% w/v) and Jobelyn supplement in diluted nutritionally complete diet, 8 hourly for four consecutive days. The control animals received isocaloric non-alcoholic diet for equivalent days. The cerebellar cortices of animals in each group were evaluated histopathologically for degenerative changes. The extent of oxidative damage in the cerebellar cortex was assessed by tissue malondialdehyde (MDA), and advanced oxidation protein products (AOPP), concentrations. Results indicated that repetitive ethanol intoxication caused neuronal degeneration and oxidative damage in the cerebellar cortex. Jobelyn supplementation in alcohol-exposed rat significantly lowered the extent of neurodegeneration and oxidative damage. It was opined that the neuroprotective action of Jobelyn in alcohol-induced cerebellar neurodegeneration in adolescent rats is possibly through reductions of oxidative stress and neuronal degeneration.

KEYWORDS: Alcoholism, cerebellum, medicinal plant, nutraceuticals, neuroprotection.

INTRODUCTION
The consequences of alcohol use disorder (AUD) are more devastating in the adolescent brain than in the adult brain.[1,2,3] Studies show that adolescents that began drinking at age 13 years or lower have a three times more risk of developing an AUD than those who started at age 18 years.[4] About 70% of teens, 17 years and above has been involved in heavy drinking.[5] The rate of alcohol use increases sharply between the ages of 12 to 21 years, and adolescents frequently adopt a binge-like drinking pattern.[6,7] Binge alcohol consumption is a behavioral pattern that results in CNS degeneration.[3] Several reports suggested that binge drinking initiates the downward spiral towards developing an AUD.[8,9,10] In man, the adolescence is largely a period of good physical health and it is associated with risky behavior, delinquency, issues of independence and identity. Mental health problems may develop or first become apparent during adolescence.[11,12] The adolescent and adult brains react differently to alcohol in several ways.[3,13,14] Adolescents are more resistant to the intoxicating effect of alcohol and hence consume greater amounts than adults,[15] but paradoxically, the adolescent brain is more susceptibility to alcohol neurotoxicity.[1,2,3] Though the adolescent's brain as a whole is affected in alcohol toxicity,[16] certain regions are particularly susceptibility to alcohol-driven neurodegeneration: hippocampus and cerebellum,[3] prefrontal cortex and hippocampus,[2] and the corticolimbic area.[17] Cumulatively, these suggested a window of vulnerability to AUD in adolescents. It is unrealistic to expect that the adolescent would desist from alcohol consumption; however, the identification of agents that can halt or reduce the neurodegenerative effect of alcohol in individuals would arguably be feasible. Jobelyn® is a nutraceutical that is certified by Food and Drug Administration (FDA) of the USA.[18] Animal models of certain CNS conditions showed that Jobelyn acts as antipsychotic,[19] antidepressant,[20] and anti-apoptotic[21] agents. Jobelyn was also effective in ameliorating stress-induced cognitive deficit,[22] and in the preventing neurodegeneration in alcohol use disorder.[21] Consequently, the aim of this study was to investigate the effect of Jobelyn in the cerebellar cortex

of adolescent male Sprague Dawley rats exposed to binge alcohol consumption.

MATERIALS AND METHODS

Binge alcohol treatment

All procedures in this study are in accordance with the Guidelines for the Care and Use of Laboratory Animals (NRC, 2010). Rats procured and accommodated in the animal unit of the department of Pharmacology, Faculty of Basic Medical Sciences, Niger Delta University, Bayelsa State, Nigeria. They were maintained under 12/12 hour light-dark cycle, at 25 ± 5 °C and were allowed free access to rat chow and water, except during alcohol treatment when the chow was withdraw.

Eighteen adolescent male Sprague Dawley rats (6 – 8 weeks, 100g mean weight) were randomly divided into 3 groups of six rats each: group A (control), group B (ethanol exposed only), and group C (ethanol exposed plus Jobelyn supplement). The protocol for alcohol-induced neurodegeneration has been previously described.[23] Briefly, groups, B and C rats were gavaged with 5 g/kg ethanol (25% w/v in a nutritionally complete diet (50% v/v, Vanilla Ensure). In addition to the ethanol treatment, group C rats received Jobelyn (7mg/kg body weight) as a supplement. The control rats were gavaged with 5 g/kg of a nutritionally complete diet (50% v/v) Vanilla Ensure. All treatments were repeated every 8 hours for 4 days. After the first ethanol dose of 5 g/kg in day-1, subsequent doses were based on the intoxication state of the rat as assessed with a six point behavioral scale: 0-normal rat (5 g/kg), 1-hypoactive (4 g/kg), 2-ataxia (3 g/kg), 3-delayed righting reflex and ataxia with dragging abdomen (2 g/kg), 4-loss of righting reflex (1 g/kg) and 5-loss of eye blink reflex (0 g/kg).[24]

Brain isolation and tissue processing

Rats were anesthetized with a mixture of ketamine (75 mg/kg) and diazepam (2.5 mg/kg) (ip) and humanely decapitated. Heads with brains in-situ in respective cranial cavities were completely immersed in 10% formalin saline for 48 hours. Post-fixed brains were exposed and excised from the cranial cavities and the cerebellar cortex isolated for histological examination. Brain tissues were embedded in paraffin wax overnight, serially sectioned at 5cm thickness with a rotary microtome, mounted on a glass slide and stained with Haematoxylin and Eosin.[25] Additionally, cerebellar cortex (n = 4) per group were carefully and quickly excised for biochemical analyses.

Semi-quantitative evaluation of histological sections

Randomly selected sections (n = 5 per group) were evaluated by light microscopy for tissue damage and neurodegeneration (shrinkage of the neuron, hyperchromasia, and nuclear pyknosis).[26] A Purkinje cells-based semi-quantitative scale[27] was used to assess the extent of neurodegeneration in the cerebellum: no degenerating Purkinje neuron = 0; 1/2 degenerating Purkinje neurons = 1; 3/4 degenerating Purkinje neurons = 2; 5/6 degenerating Purkinje neurons = 3; more than 7 degenerating Purkinje neurons = 4; 6 or more degenerating Purkinje neurons and neuropil vacuolation = 5. Counting was done on a 1mm × 4mm grid graticulate at 400X magnification. Grid was orientated to enclose the Purkinje layer of the folium of randomly selected portions of the hemisphere. The scores from all the sections from each group were averaged to give a final score for the particular group. Values were expressed as mean ± SEM.

Quantitative biochemical assessment of cerebellar damage

To biochemically assess the extent of tissue damage associated with or without the treatment regime; the level of advanced oxidation protein products (AOPP) and malondialdehyde (MDA), which are biomarkers of oxidative stress in proteins and lipids respectively were determined in the sample tissues.

Assay of AOPP levels

Spectrophotometric determination of AOPP levels was performed by Witko's method.[28] Samples of cerebellar cortex 200µl were diluted 1/5 in 20 mM Phosphate buffered saline (PBS) pH 7.4. 10 µl of 1.16 M potassium iodide was added to each tube, followed by 20 µl of 10% acetic acid. The absorbance of the reaction mixture was read immediately at 340 nm, against a blank, containing 1000 µL of PBS, 10 µL of potassium iodide and 20 µL of acetic acid. Chloramine T solution (0-100 µmol/L) was used as calibrator. The chloramine T absorbance at 340 nm is linear within a range of 0-100 µmol/L, and AOPP concentrations were expressed as µmol/L of chloramine T equivalents.

Determination of malondialdehyde (MDA)

The assay for cerebellar cortex lipid peroxidation (LP) was done by the method of Wright et al.[29] with some modifications. The reaction mixture in a total volume of 3.0 ml contained 0.4 ml aliquot of brain homogenate was mixed with 1.6 ml 0.15 M Tris-KCl buffer pH 7.4to which 0.5 ml of TCA (10%), and 0.5 ml TBA (0.75%) were added. All the test tubes were placed in a boiling water bath for a period of 45 min. The tubes were transferred to ice bath and then centrifuged at 3000xg for 10 min. The amount of MDA formed in each of the brain samples were assessed by measuring the optical density of the supernatant at 532 nm. The results were expressed as the µmol MDA formed/gram of tissue by using a molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1}.

Statistical analysis

The one-way analysis of variance and the Tukey's post-hoc analysis were used to assess for intergroup differences (GraphPad Prism 5, San Diego, USA). Values were expressed as mean ± SEM. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Qualitative histological evaluation of cerebellar changes

Light microscopy examination of H&E stained representative sections of various groups showed the three layers of the cerebellar cortex were distinguishable by characteristic neurons. The molecular layer (ML) is poorly cellular, and second layer contained Purkinje neurons (PL) with prominent nuclei. The third layer is the granular layer (GL) that contained numerous cells of various sizes and shapes. There was little or no neurodegeneration in the control group (fig. 1 A). The cerebellar cortex of the alcohol-fed rats showed degenerating neurons (black arrows) and sparse vacuolation in some areas. Degenerative neurons were more evident in the rats that were exposed to alcohol alone than in those that received Jobelyn supplements. The administration of Jobelyn reduced the level of degenerative changes seen in alcohol fed rats.

Semi-quantitative histological evaluation of cerebral changes

The degree of neurodegenerative changes in the cerebellar cortex was assessed as previously described.[27] There were significantly more degenerating Purkinje neurons in the alcohol group and in the control group (Table 1). The control group was significantly different from the alcohol group ($p < 0.001$), but not the Jobelyn group ($p > 0.05$). However, the level of neurodegeneration was significantly reduced in the Jobelyn group ($p < 0.05$, fig 1D) compared to the alcohol group, which suggests that Jobelyn supplementation attenuated alcohol-induced neurodegeneration in the cerebellar cortex.

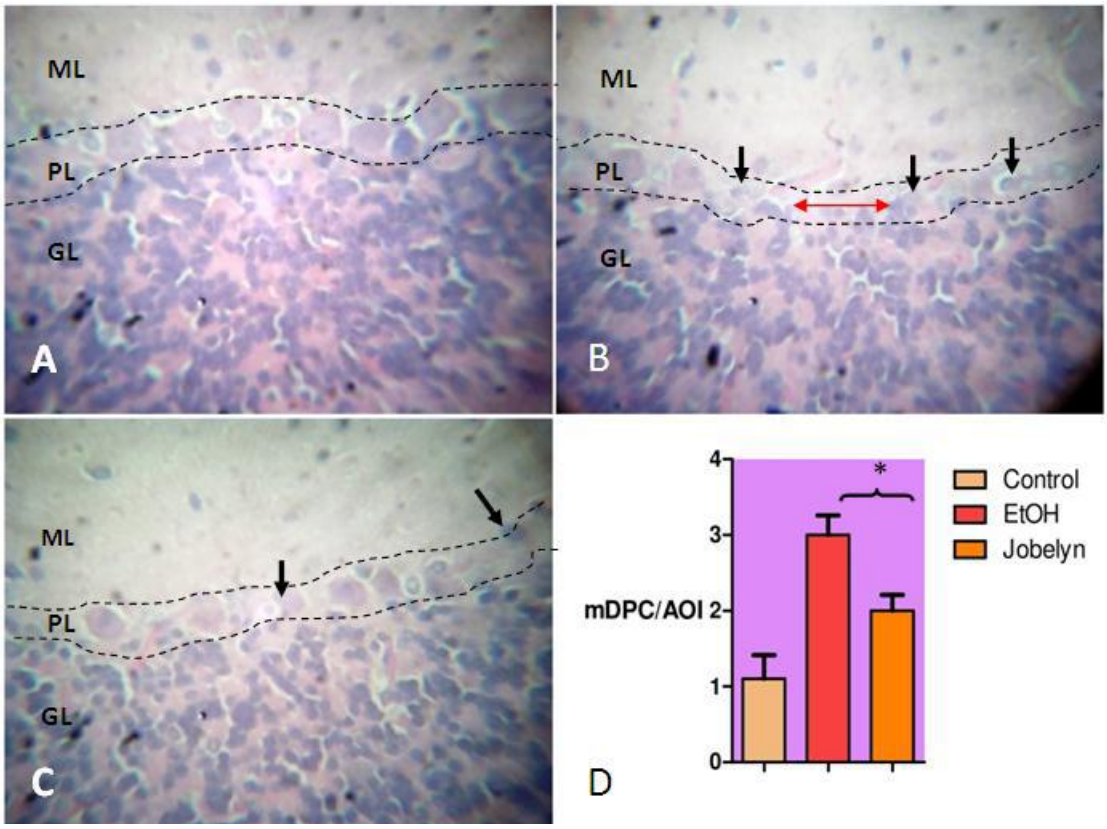

Figure 1: Effects of Jobelyn® on alcohol-induced cytoarchitectural alteration in cerebellar cortex (x400 mag). There was little or no histological alteration in control group (A), but the alcohol group (B) showed loss of delineation (red arrow) between Punkinje layer and granular layer, probably due to degeneration of Purkinje neurons. Delineation were generally mild or absent in the Jobelyn® treated rats (C). The alcohol group (B) showed degenerating Purkinje neurons (black arrow), vacuolation or spaces within the overlying molecular layer suggesting concurrent swelling/degeneration of Purkinje neurons. Degenerating Purkinje neurons were seen in Groups B and C (black arrow). Purkinje neurons in the alcohol group (B) were generally shrunken. Neuropil vacuolation were more in the alcohol group compared to the Jobelyn group. Jobelyn supplementations lower the degree of cerebellar degeneration (D). Representative photomicrographs high power (H&E x400).

AOPP and MDA estimations

The mean AOPP and MDA level were highest in animal exposed to ethanol without Jobelyn supplement. Mean AOPP and MDA level were also lowest in the control (Table 1). The administration of Jobelyn to alcohol-exposed rats significantly lowered the mean level of cerebellar AOPP ($p < 0.05$) and MDA ($p < 0.01$)

compared to the alcohol-exposed rats without Jobelyn supplement ($p < 0.05$ and $p < 0.01$) respectively.

Table 1: Effect of Jobelyn biochemical and histological index of neurodegeneration.

Groups	LPO (µmol MDA /gram tissue x10^{-5})	AOPP (µmol/L x10^{-5})	mDPC/AOI
Control	6.1 ± 1.5	39 ± 6.1	1.1 ± 0.31
EtOH	17.0 ± 2.0**	190 ± 38.0*	3.0 ± 0.26*
Jobelyn	5.7 ± 1.1	73 ± 5.9	2.0 ± 0.21

mDPC/AOI: mean degenerating index of Purkinje cells per area of interest. ** p <0.01, * p < 0.05

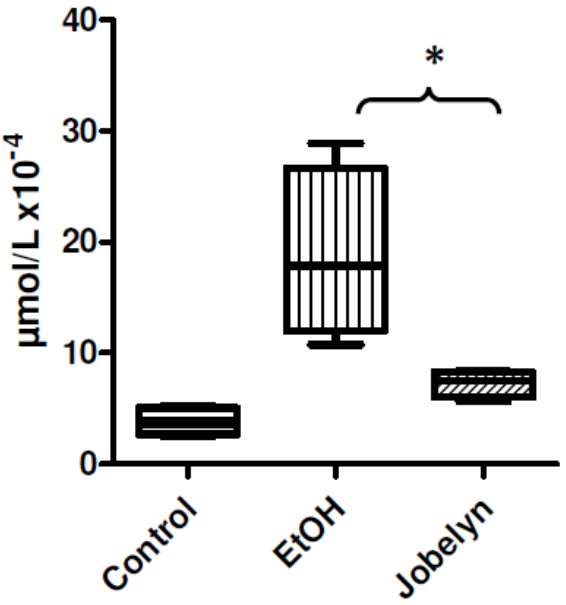

Figure 2: Jobelyn significantly reduced AOPP level in cerebellar cortex of alcohol-exposed rats.

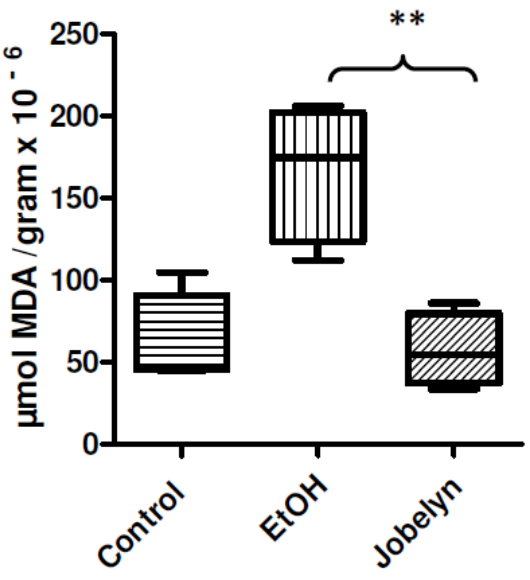

Figure 3: Jobelyn significantly reduced LP level in cerebellar cortex of alcohol-exposed rats.

DISCUSSION

The present study examined the neuroprotective potentials of oral administration of Jobelyn in a rat model of binge ethanol-induced cerebellar neurodegeneration. Jobelyn showed a substantial neuroprotective effect in cerebellar neurodegeneration. Certain plant products have also shown significant neuroprotective potentials of the cerebellar cortex.[27,30,31] This present study showed reduced tissue destruction and oxidative stress in rats treated with Jobelyn. Additionally, Jobelyn generally reduced the severity of neurodegeneration and distortions of the cerebellar cytoarchitecture in alcohol-triggered neurodegeneration in the adolescent rats. Our study also suggested that Jobelyn significantly reduced the level of alcohol-induced degeneration of the Purkinje neurons in the adolescent rats.

Research has shown that the Purkinje neurons are the most susceptible part of the cerebellar cortex to toxic substances[32,33] and that adolescent brain is especially venerable.[34] Although studies have highlighted the neuroprotective potentials of Jobelyn in adult mammalian brain,[21,22] however, the present study is perhaps the first documented account of this effect in the adolescent brain. Free radical-mediated brain damage is common in the aetiology of most neurodegenerative conditions.[35,36] To determine whether the neuroprotective effect of Jobelyn linked to its antioxidative property in the adult brain[21,27] is presence in the adolescent brain, we estimated the level of AOPP and MDA cerebellar cortex. Our study confirmed that ROS and the likes are elevated during alcohol toxicity. However, there were significant reductions in the levels of protein oxidation and lipid peroxidation in adolescent rats exposed to binge alcohol plus Jobelyn supplementation compared with rats exposed only to alcohol. Consequently, we opined that the protective effect of Jobelyn in cerebellar degeneration is plausibly associated to its anti-oxidative properties.

In neuropathological conditions, free radicals are overproduced and these overwhelm the endogenous antioxidant defenses, leading to oxidative stress, which subsequently induce cellular or subcellular membrane damage resulting in organelle or organ dysfunction. In combating the menace of free radical assault in the CNS, it is important to identify potential sources of exogenous antioxidant to the neuropil of the cerebella cortex to compensate for the endogenous depletion of antioxidant system. This study suggests Jobelyn is capable of providing such exogenous source of antioxidant.

CONCLUSION

The results of this study suggested that Jobelyn attenuates alcohol-induced cerebellar neurodegeneration

by alleviating oxidative stress and minimizing cytoarchitectural alteration. Histological and biochemical examinations all revealed improved profile in rats treated with Jobelyn. This provides more insight that clinically effective therapies could be possible with a multimodal therapeutic strategy targeting different pathological pathways involved in alcohol-induced brain damage. This study has therefore repurposed Jobelyn as a good supplementary agent in the management of alcohol-induced cerebellar neurodegeneration.

DISCLOSURE STATEMENT

The authors declare that they have no competing financial interests.

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