Advances in Biological Research 9 (5): 305-317, 2015
ISSN 1992-0067
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DOI: 10.5829/idosi.abr.2015.9.5.95109

Neuroprotective Effect of Jobelyn in the Hippocampus of Alcoholic Rat Is Mediated in Part by Alterations in GFAP and NF Protein Expressions

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Abstract: Alcohol induced neurodegeneration drives the progression of an alcohol use disorder. Unfortunately, in the adults' brain, the hippocampus that could compensate for neurodegeneration with neurogenesis is particularly susceptible to alcohol induced neurodegeneration. The objective of this work was to determine if Jobelyn, a nutraceutical, could protect the hippocampus from neurodegeneration in a binge alcohol rat model of alcohol use disorders. Three groups of Wister rats were used: control, alcohol exposed with no supplement and alcohol exposed with Jobelyn supplementation. Rats were given alcohol thrice a day at 8 hours interval for 4 days. The control rats received isocaloric non alcoholic diet for equivalent days. H&E stains, glia fibrilary acidic protein and neurofilament immunolabellings were obtained for pathological and immunohistochemical studies. Results showed thatafter 4 days of binge treatment, the histological index of neurodegeneration was significantly lower in the Jobelyn treated rats compared with the rats that received only alcohol. GFAP immunohistochemistry showed that Jobelyn prevented astrocyte death in the DG, CA3 and CA1 regions of the hippocampus. Except in the DG, this was not accompanied by a reduction in GFAP expression. NF immunohistochemistry showed that Jobelyn can reduce neuroinflammation in these three regions of the hippocampus. In the CA3 region, Jobelyn prevented a reduction in NF proteins expression in alcohol exposed rats. However, Jobelyn supplementations lead to NF protein overexpression in the CA1 region. Conclusively, this study indicates that moderate activation of astrocytes and NF protein expression are critical for neuronal survival in alcohol toxicity. It is opine that the neuroprotective effect of Jobelyn in the hippocampus of alcoholic rats is mediated in part by modulation GFAP and NF protein expressions in the DG, CA3 and CA1 regions of the hippocampus.

Key words: Alcohol use disorders · Neurodegeneration · Nutraceutical · Protection

INTRODUCTION

It is generally believed that the adult brain can generate at least some new neurons and incorporate them into existing neural circuits [1]. Interneurons, the local circuit neurons are indispensable in any neural circuits [2, 3]. Debatable perhaps, the functions of any particular region of the brain would be truncated if either the hippocampal SGV or SVZ of the lateral ventricles is damaged. Unfortunately, certain interactions of man with his environment in his work and leisure places have not been entirely harmless to his health [4, 5]. Alcohol consumption is a leisure activity that is potentially neurotoxic, but ironically, drinking is part of the human socialization [6, 7]. Binge alcohol drinking or alcohol misuse has been shown to induce neurodegeneration in the dentate gyrus of the hippocampus. Presently, it is unimaginable that alcohol consumption would become old fashioned. Since man is a behavioural drinker, a reasonable option is to mute its deleterious effect in the *milieu interieur* of the brain. We hypothesize that Jobelyn supplementation in binge alcohol drinking may help to safe guard the brain.

Jobelyn® a West African sorghum based nutraceutical is widely distributed and used worldwide [8]. Jobelyn is GRAS-certified by the Food and Drug Agency (FDA) USA [9]. Experimental, epidemiological and human studies indicate several health benefits of Jobelyn [10, 11].

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Jobelyn rapidly increases haemoglobin and PCV levels in animals with trypanosome-induced anaemia [12]. As an anti-oxidant, Jobelyn is effective and efficient against the five predominant reactive species found in the body (singlet oxygen, peroxyl radicals, hydroxyl radicals, peroxynitrite and super oxide anion), making it perhaps one the very best free radical scavengers [8]. Jobelyn alone or in combination with antiretroviral drugs has been shown to increase CD4 counts in HIV patients making it a possible good alternative and/or supplement to antiretroviral drugs in the management of HIV/AIDS [13]. Studies have shown that Jobelyn selectively inhibits COX-2 enzyme unlike the common non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit all COX enzymes [14]; resulting to unwanted side effects such as peptic ulcers—an inflammatory condition in itself [15]. Common non-steroidal anti-inflammatory drugs (NSAIDs) inhibit all COX enzymes [14]. These paradoxes are not consistency with good health. Acting as a selective COX-2 inhibitor, Jobelyn steadily reduce inflammation and pain, without the unwanted side effects common with NSAIDs. It also strengthens the immune system and thereby enhances body's defensive mechanisms [8, 9]. The primary endeavour of this study is to evaluate the potential of Jobelyn supplementation in a rat model of alcohol use disorders (AUDs) with particular reference to alcohol induced neurodengeneration in the rat's hippocampus.

MATERIALS AND METHODS

Animals: Twenty seven adult male Wister rats (250–320 g) were randomly divided into three groups of nine animals per group: control (A), alcohol (B) and Jobelyn (C). Rats were maintained on a 12 hours light–dark cycle and had free access to rodent chow and water. However, rats were fasted overnight before the start of experiment, but the free access to water was maintained throughout the duration of experiment. All protocols followed NIH guidelines on Animal Care and Use.

Induction of Alcohol Use Disorder: Alcohol abuse and alcohol dependence was induced in groups B and C rats by a binge alcohol administration. Briefly, rats were infused intragastrically through an oro-gastric gavage tube. Groups B and C rats were given an initial dose of 5 g/kg ethanol in a solution of 25% (w/v) ethanol in diluted nutritionally complete diet (50% v/v, Vital mike® Ghana), modified after Collins *et al.,* [16]. In addition group C rats received Jobelyn (4mg/kg body weight). These treatments were administered at approximately every 8 h for 4 consecutive days at 6 a.m., 2 p.m. and 10 p.m. Except for the initial 5 g/kg dose, subsequent doses were determined using a six-point intoxication scale [17]. Briefly, the rats were scored according to the following behavioural patterns: normal rat, 0; hypoactive, 1; ataxia, 2; ataxia with dragging abdomen, delayed righting reflex 3; loss of righting reflex, 4; and loss of righting reflex and loss of eye-blink reflex, 5. Each score is associated with a particular dose of alcohol (Table 1). Control animals (group A) received a diet of Vital mike® (50% v/v) made isocaloric with glucose.

Brain Isolation: Rats were anesthetized with a mixture of ketamine (75 mg/kg) and diazepam (2.5 mg/kg) (ip) and were transcardially perfused with 10% phosphate buffer formal saline (0.1M, pH 7.4) solution. After complete perfusion, rats were carefully decapitated and heads were completely immersed in 10% phosphate buffer formalin saline for 48 hours.

Tissue Processing: Brains post fixed *in situ* were exposed and excised from respective cranial cavity. The hippocampi enclosed within surrounding cortical tissue were isolated by a coronal section at approximately Bregma -1 to -6 coordinates and transferred to increasing grades of alcohol for dehydration and then cleared with xylene. They were embedded with paraffin wax overnight, serially sectioned at 5cm thickness with a rotary microtome, mounted on a glass slide and stained with Haematoxylin and Eosin [18, 19].

Histological Study: Semi quantitative scales were employed to access for histological and morphometric differences. The representative sections (n=6 per group) were evaluated and injured or degenerating neurons were identified by any of these three criteria: intensely eosinophilic cytoplasm, loss of Nissl substance and pyknotic nuclei / cell body shrinkage [20]. Using the method of Paterniti *et al.,* [21], damaged neurons within the hippocampus were counted and scored on a six-point scale: 0, no lesion observed; 1, one to five damaged/ degenerating neurons; 2, five to ten damaged/ degenerating neurons; 3, more than ten damaged/ degenerating neurons; 4, small infarction (less than one third of area of interest "AOI"); 5, moderate infarction (one third to one half of the AOI); and 6, large infarction (more than half of the AOI). Counting was done with x10 ocular by x40 objective. 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.

Table 1: Intoxication scale and associated alcohol dose

Intoxication score	Behavioural attributes	Ethanol Dose (g/kg)
0	Normal animal	5
1	Hypoactive, mildly ataxic	4
2	Ataxic, absence of abdominal elevation, delayed righting reflex	3
3	Loss of righting reflex, retain eye blink reflex	2
4	Loss of righting reflex, retain eye blink reflex	1
5	Loss of righting reflex, loss of eye blink reflex	0

Immunohistochemistry: The Avidin Biotin Complex (Avidin biotin immunoperoxidase) method [22] was applied in this work. All primary and secondary antibodies, ABC kit and support reagent were produced by Novocastra, Leica Biosystems Newcastle, UK. Three micrometer (3 ìm) sections were cut from paraffin-embedded tissues and mounted on slides. Sections were repeatedly rinsed in phosphate buffered saline (PBS) and then pretreated with 30% hydrogen peroxidase in methanol (20 – 40 min) to block any possible endogenous peroxidase. For antigen retrieval the sections were boiled in 0.01M citrate buffer (15 min). Non-specific binding was blocked with 1% BSA (bovine serum albumin fraction V) (Novocastra, Leica Biosystems Newcastle, UK) TRIS solution 1 h. Sections were then incubated for 12 hours overnight (2 - 6 °C) with the primary antibodies: mouse monoclonal anti-GFAP (Novocastra, Leica Biosystems Newcastle, UK, 1:100) and anti-NF (Novocastra, Leica Biosystems Newcastle, UK, 1:100). After rising in PBS solution, sections were incubated with biotinylated secondary antibody (Novocastra, Leica Biosystems Newcastle, UK) and horseradish peroxidase-conjugated streptavidin (ABC kit, Novocastra, Leica Biosystems Newcastle, UK), for 1 hour. Sections were then incubated in 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Novolink DAB, Novocastra, Leica Biosystems Newcastle, UK) for 15 minutes for visualization of antigen-antibody reactions. They were then counterstained with hematoxylin, washed with tap water, dehydrated in alcohol, cleared in xylene and mounted in DPX.

IHC Interpretation: Cells (or cell membrane or nuclei depending on the antigenic sites) that were labelled by the above-mentioned antibodies and displayed brown/ yellowish brown reaction products were considered immunopositive. The haematoxylin stained cells without any form of brown colours are scored negative. Non specific binding/brown artifacts on cells and connective tissue are disregarded.

Photomicrography: Microscopic image was transmitted to a LCD monitor by a microscope-camera-monitor composite contrivance as recently described [23]. Digital photomicrographs were captured directly by the attached computer, labelled appropriately and saved for analysis.

Statistical Analysis: Values were expressed as mean ± SEM. A one-way analysis of variance and the Tukey's post hoc test were used to assess the significance of differences between groups. A p value < 0.05 was considered to be significant. Analysis of variance was performed using Graph-Pad Prism 5 (GraphPad Software, San Diego, CA).

RESULTS

Histologic Examination: Histological analysis of the dentate gyrus of the dorsal hippocampus showed marked degenerative changes in the neurons of rats that were fed with alcohol alone (Figure 1B). Neurons were inflamed with basophilic degeneration. The administration of Jobelyn to alcohol fed rats significantly reduced the severity of these degenerative charges (Figures 1C and 1D). There was alteration in the cytoarchitectural arrangement of the neuron in the dentate gyrus of alcohol fed rats. Normally, the neurons were closely packed in the DG as seen in the control (Figure 1A), opposed to the pattern seen in the alcohol group (Figure 1B). These observations were greatly reduced with the administration of Jobelyn to alcohol fed rats (Figure 1C). Additionally, the DG neurons of rats that were given Jobelyn Supplementation showed the granule-like arrangement seen in the control group, but was not observed in the alcohol group. It is plausible that swellings or inflammation of neurons in the alcohol group may have masked the granule-like arrangement. Inflammation or swelling of neurons following alcohol toxicity was earlier reported [24, 25]. The hilar neurons of the alcohol also showed basophilic degeneration as opposed those of other groups, where basophilic degeneration was not evident. In the *regio III cornus ammonis* (CA3 Area) There was evident morphological alteration of neurons after administration of alcohol (Figure 2B). Neurons had generally changed from a pyramidal cyton to spherical, with majority showing some level of basophilic degeneration (Figure 2B). Jobelyn supplementation to

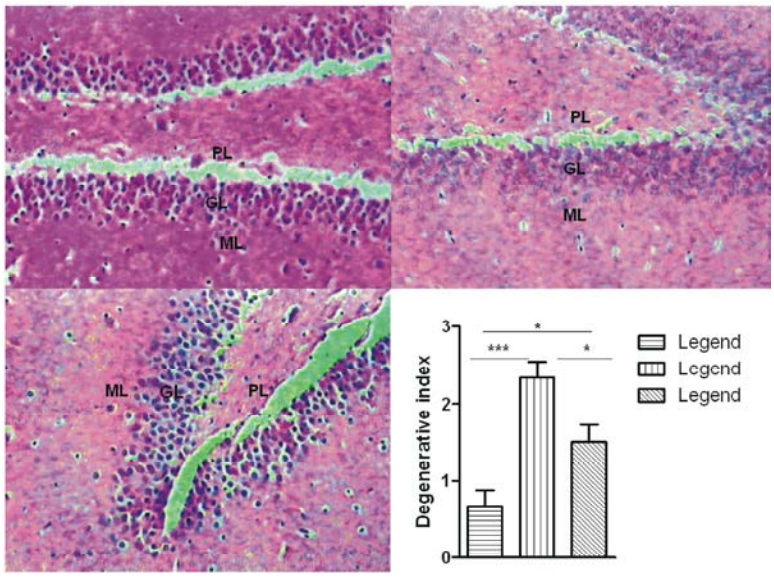

Fig. 1: Jobelyn reduced alcohol induced degeneration in the dentate gyrus. Representative sections (H&E x100) of control animals (A), alcohol treated animals (B) and Jobelyn supplemented animals (C). Observe the three layers of the DG: granular layer (GL), molecular layer (ML) & polymorphic layer (PL). Observe the loss of neurons and the morphological alteration in the cytoarchitecture of the alcohol group (B). Also observe basophilic degeneration and swelling in the alcohol group (B). Observe that the administration of Jobelyn (C) prevented the alterations seen in the alcohol group. Also note the similarities in the cytoarchitecture of groups A & C. DI expressed as mean ± SEM. DI of control differs significantly from other and Jobelyn differs significantly from alcohol. *** $p < 0.001$, * $p < 0.05$.

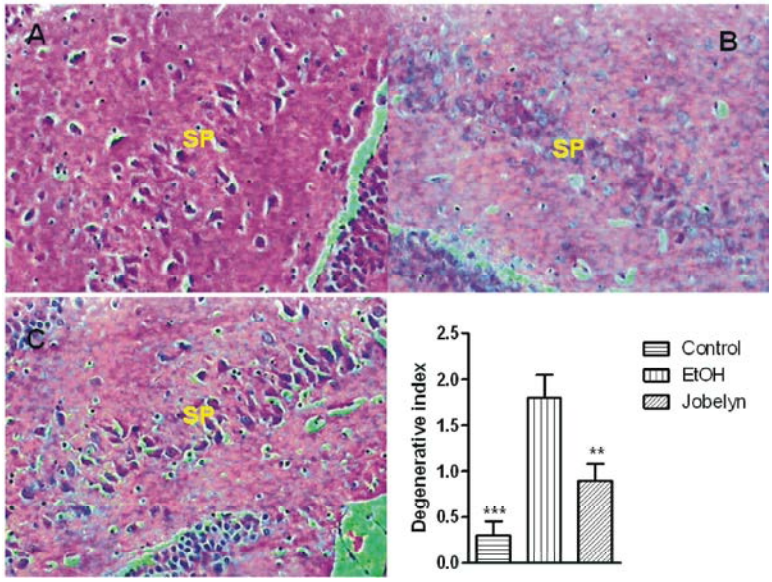

Fig. 2: Jobelyn reduced alcohol induced degeneration in area CA3. Representative sections (H&E x100) of control animals (A), alcohol treated animals (B) and Jobelyn supplemented animals (C). Observe the morphological alteration of neurons after administration of alcohol (B) and preservation of the cytoarchitectural morphology of the pyramidal neurons in stratum pyramidale (SP) in Jobelyn supplementation (C). Also observe basophilic degeneration and swelling of neuron in the alcohol group (B). Observe that the administration of Jobelyn (C) prevented these alterations. DI expressed as mean ± SEM. DI of control and Jobelyn differs significantly from the alcohol group. *** $p < 0.001$, ** $p < 0.01$. The DI of control and Jobelyn were not significantly different.

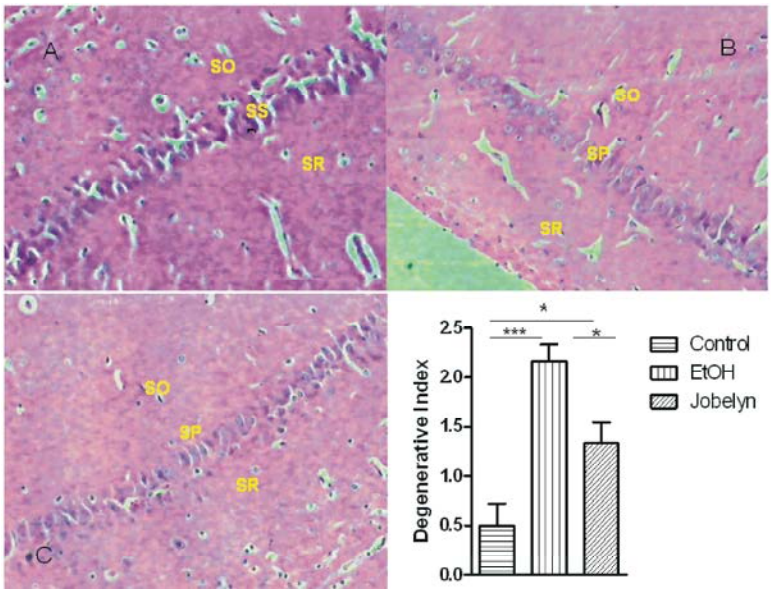

Fig. 3: Jobelyn reduced alcohol induced degeneration in area CA1. Representative sections (H&E x100) of control animal (A), alcohol treated animal (B) and Jobelyn treated animal (C). Note layers SO- stratum oriens, SP- stratum pyramidale, SR- stratum radiatum. Observe the morphological alteration of the neurons of the SP with nuclei condensation in alcohol group (B). Also observe neuronal swellings / basophilic degeneration in alcohol group (B). Observe that the morphology of neurons in the SP of the Jobelyn group (C) were fairly consistent with that of the control (A). Also observe that neuronal swellings / basophilic degeneration were not evident in C. DI expressed as mean ± SEM. DI of control differs significantly from others and Jobelyn significantly from alcohol. *** $p < 0.001$, *$p < 0.05$.

alcohol fed rats preserved the cytoarchitectural morphology of the pyramidal neurons (Figures 2C). The alcohol group also displayed nuclei condensation. This was not obvious in other groups perhaps due to the healthier state of the neurons. With regards to *regio I cornus ammonis* (CA1 Area), our observations were similar. Neurons from the CA1 region of the alcohol group showed signs of basophilic degeneration and swellings (Figure 3B). The administration of Jobelyn (Figure 3C) prevented basophilic degeneration and swellings.

Immunohistochemisrty

Glial Fibrillary Acidic Protein (GFAP)

Dentate Gyrus: In the dentate gyrus (DG), there was increase expression of GFAP (dark brown) in the alcohol group (Figure 4B) compared the control (Figure 4A). There was also a reduction in astrocyte population compared with others groups, probably due to astrocytes death or degeneration (black arrow). The administration of Jobelyn led to a reduction of GFAP expression (Figure 4C) and the prevention of astrocytes death. The GFAP expressing cells appeared healthy in the treatment groups. Focal GFAP-IR was more intense in the "B" group but reduced in the control (4A) and Jobelyn group (4C).

CA3 Area: Astrocytes in the control groups were predominantly in physiological state (Figure 5A). Alcohol caused an increase in GFAP protein expression (Figure 5B) in CA3 area of the hippocampus proper. The administrations of Jobelyn to alcohol fed rat had no change in the GFAP-IR. However the number of astrocytes in the alcohol group (Figure 5B) was greatly reduced. Observe hyperactivity (increased in GFAP-IR) of astrocytes in groups B (Figure 5B) C (Figure 5C); astrocytes showed overexpression of GFAP and were hypertrophic. However, we noted that Jobelyn and prevented astrocytes loss (Figure 5C).

CA1 Area: Astrocytes in the control groups were predominantly in physiological state (Figure 6A). Alcohol caused a reduction in astrocytes population in the CA1 region of the hippocampus (Figure 6B). Though the population of astrocytes was reduced in the alcohol group, they were however hyperactive (Figure 6B).

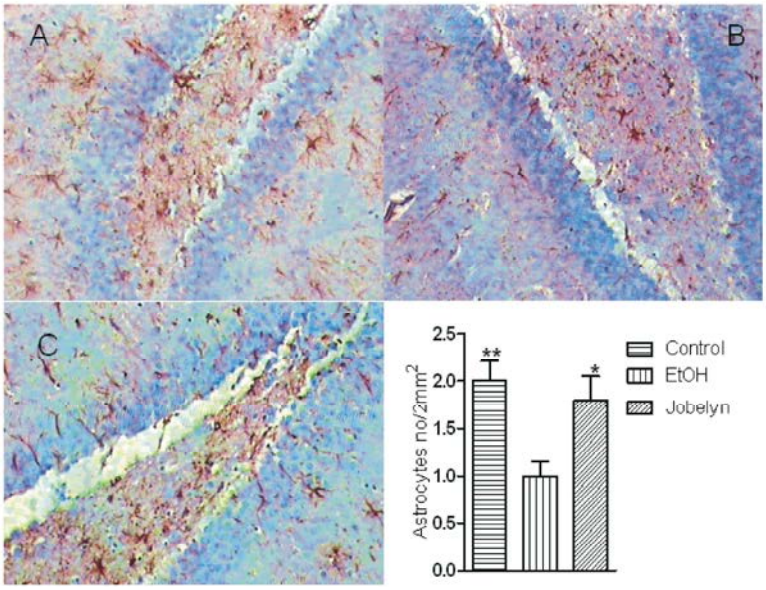

Fig. 4: Jobelyn alterations of GFAP expression in the DG dentate gyrus. Representative photomicrographs (GFAP labelled x100) of the DG of control (A), alcohol (B) and Jobelyn (C) groups. GFAP was overexpressed (dark brown) in the alcohol group. Note that the number of healthy GFAP-expressing cells declined in the alcohol group compared with others. Jobelyn supplemented rats showed a reduction in GFAP expression. Also observe that the GFAP-expressing cells appear healthy in the Jobelyn treated group. Astrocytes count expressed as mean ± SEM differs significantly **p <0.01, *p<0.05, compared to alcohol group.

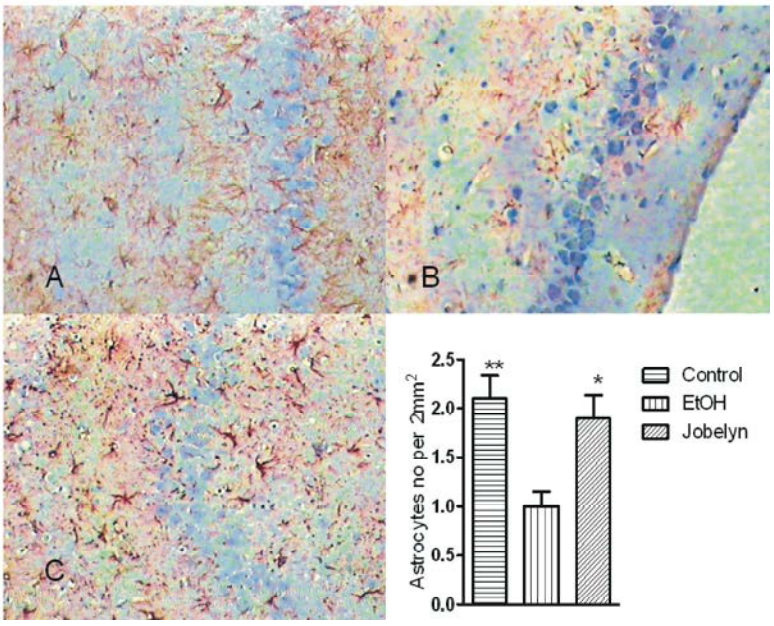

Fig. 5: Jobelyn alterations of GFAP expression in the CA3 area. Representative photomicrographs (GFAP labelled x100) of control (A), alcohol (B) and Jobelyn (C). Observe the normal expression in the control (A). Observe hyperactivity (overexpression) of astrocytes in groups B and C. Also observe a reduction in astrocytes number in "B". Observe that Jobelyn (C) prevented a reduction in astrocyte population. Astrocytes count expressed as mean ± SEM differs significantly **p <0.01, *p<0.05, compared to alcohol group.

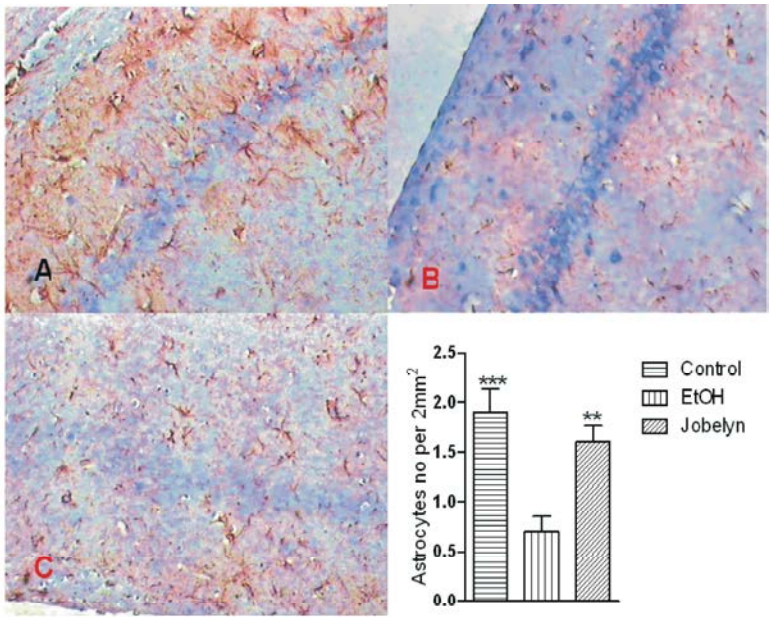

Fig. 6: Jobelyn alterations of GFAP expression in the CA1 area. Representative photomicrographs (GFAP labelling x100) of control (A), alcohol (B) and Jobelyn group (C) groups. Note that the astrocytes in groups B and C were overexpresssed. Astrocytes count expressed as mean ± SEM differs significantly ***p <0.001, **p<0.01, compared to alcohol group.

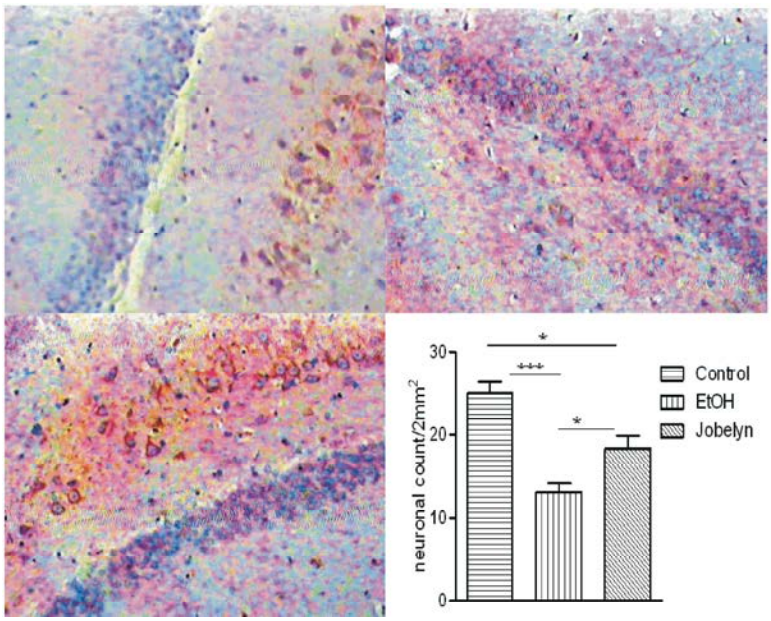

Fig. 7: Jobelyn alterations of NF expression in the dentate gyrus. Representative photomicrographs (NF labelling x 200) of the dentate gyrus of the control animal (A), alcohol treated animal (B) and Jobelyn treated animal (C) groups. Note the granulation and closely packed neurons in the DG (A) as opposed to the loosely packed neurons in the alcohol (B). Individual neurons were delineated in group B, suggestive of a reduction in neuronal population. In the Jobelyn (C) treated rats distortion were minimal, individual neurons were not overtly delineated suggestive of densely packed neurons. Neuronal count expressed as mean ± SEM differs sign b/w control and others and b/w the Jobelyn and alcohol. *** p <0.001, * p<0.05.

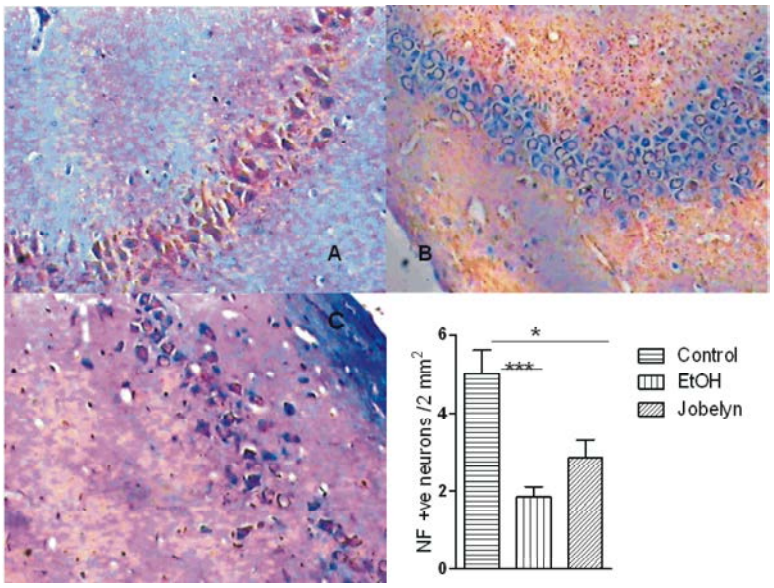

Fig. 8: Jobelyn alterations of NF expression in the CA3 area. Representative photomicrographs (NF labelled x200) expression of NF proteins in control (A), alcohol (B) and Jobelyn + alcohol (C) groups. Note that vast numbers of neuronal cell bodies were positive for NF-IR (brown coloured) in groups A and C. Observe that NF protein expression was greatly reduced in the alcohol group (B). NF positive neuronal count expressed as mean ± SEM differs sign b/w control and others. *** $p < 0.001$, * $p < 0.05$. Jobelyn vs alcohol was NS ($p > 0.05$).

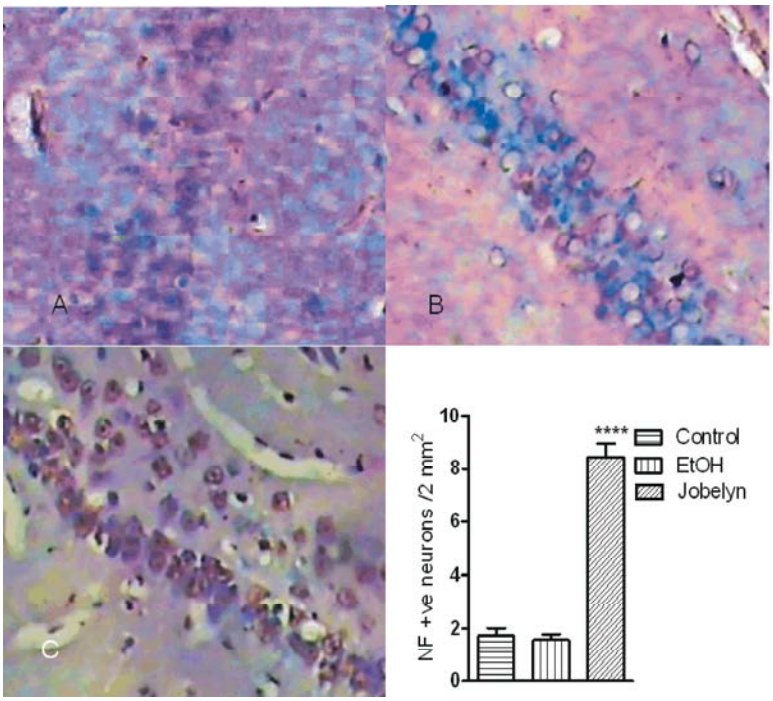

Fig. 9: Jobelyn alterations of NF expression in the CA1 area. Representative photomicrographs (NF labelled x200) of control (A), alcohol (B) and Jobelyn (C) groups. NF-IR was poor in the perikarya of the control animals. In the alcohol group (B) a few cells showed strong NF-IR (dark brown). Note that NF proteins were overexpressed (brown colouration) in almost all the neurons in (C). NF positive neuronal count expressed as mean ± SEM differs sign b/w Jobelyn and others. *** $p < 0.001$. Control vs alcohol was NS ($p > 0.05$).

We observed that the administration of Jobelyn did not change the alteration induced by alcohol, but it however prevented astrocytes death (Figure 6C).

Figure xx. A representative photomicrograph (GFAP labell x200) showing the expression

Neurofilament (NF)

Dentate Gyrus: Data indicated that neurons are generally positive NF immunolabelling. However, the granular cytoarchitectural pattern of the GL of dentate gyrus was not evident in the group that received alcohol only - there was loss of granulation (Figure 7B). In groups A and C, the granular cytoarchitectural pattern was preserved (Figure 7A & C). Hippocampal neurons were arranged compactly and orderly in the control group. Hippocampal neurons were reduced in number and arranged in a loose fashion in the alcohol group. The level of reduction in hippocampal neurons was lower in groups C compared with the alcohol group. Note the relative thickness and cell population of the GLs. GL was thinnest in the alcohol group (Figure 7C) and also less compacted.

CA3 Area: We observed that the expression of NF protein in the *regio* III *cornus ammonis* (CA3) of the hippocampus proper was different from that of the DG. Alcohol toxicity leads to a reduction in NF protein expression (Figure 8B). The administration of Jobelyn to alcohol exposed rat produced no alteration in NF protein expression (Figure 8C) as evident from comparison with the control group (Figure 8A).

CA1 Area: In the *regio* I *cornus ammonis* (CA1), we observed that NF-IR was poor in the perikarya of the control group (Figure 9A). In the alcohol group (Figure 9B) a few cells showed strong NF-IR (dark brown). However, to our surprise, we discovered that almost all the neurons in Jobelyn group showed NF protein overexpression (Figure 9C).

DISCUSSIONS

This study demonstrated that Jobelyn supplementation to alcoholic rats reduces the level of alcohol induced neurodegeneration and also prevented cytoarchitectural alterations in the dentate gyrus and hippocampus proper. There were significant reductions in the number of degenerating neurons in the DG, CA3 and CA1 areas of the dorsal hippocampus in Jobelyn supplemented rats. It was earlier reported that certain medicinal botanicals could reduce the level of neurodegeneration in this region of the brain [26, 27]. Our result also indicated that Jobelyn reduced the extent of neuroinflammation in these areas of the hippocampus. The anti- inflammatory properties of Jobelyn have been previously reported [8]. Certain studies have shown that agents that can inhibit or reduce neuroinflammation following assaults may ameliorate or minimize neurodegeneration [25, 28]. Jobelyn also maintained the cyto architectural structure of the stratum pyramidale layer of the CA1and CA3 areas of the hippocampus proper. Alterations in the neuronal morphology in these areas have been associated with neurodegeneration [2, 29]. Immunuhistochemical evidence from this present study is suggestive that modulation of GFAP and NF proteins expressions may be part of mechanism through which Jobelyn protect or safe guard the hippocampal neurons from alcohol toxicity.

GFAP overexpression or downregulation is pathologic in the CNS depending on location and circumstance [30, 31]. GFAP immunolabelling was over expressed in the DG of the alcohol group compared with the control. There was also a decline in the number of healthy GFAP-expressing cells in the alcohol group compared with others probably due to astrocytes death or degeneration. In our study, these alterations were greatly reduced in the Jobelyn group. The role of astrocyte in CNS myelination, development and maintenance has been stressed [32]. Astrocyte also plays critical roles in neuroprotection as glutamate transporters essential for clearance of glutamate from the CNS extracellular space [31], enzymatic antioxidant defence [33], production of neuroprotective growth factor and neurotrophic molecules [33, 34]. However, it also expresses nitric oxide synthase which can result in the production of potentially neurotoxic levels of nitric oxide [35, 36]. Astrocytes also mediate the release or production of numerous pro-inflammatory and anti-inflammatory cytokines [37]. The essential point here is that the activities of astrocyte are closely regulated by intrinsic factors within the CNS since the consequences of alteration in GFAP expression are very unpredictable. Our results suggest that the administration of Jobelyn seem capable of preventing wide deviation from this dynamic equilibrium in the dentate gyrus and CA3 and CA1 areas.

In the CA3 area, the administration of Jobelyn to alcohol exposed rats led to an increasein astrocyte number compared with the rats exposed only to alcohol. The number of astrocytes in the alcohol group was significantly reduced. Suggestive that Jobelyn may play a role in preventing the death of astrocytes in this region.

Table 2: Jobelyn modulations of GFAP and NF expressions in the neurons of the DG, CA3 and CA1 regions of the hippocampus in Binge alcohol exposure

	IHC	A	B	C
DG	Anti-GFAP	Positive (normal IR) & normal astrocyte population	Overexpression & decreased astrocyte population	Reduction in normal IR & astrocyte population is normal
	Anti-NF	Negligible	Negligible	Negligible
CA3	Anti-GFAP	Positive (normal IR and normal astrocyte population)	Overexpression & decreased astrocyte population	Overexpression & normal astrocyte population
	Anti-NF	Positive	Reduced expression	Prevented reduction
CA1	Anti-GFAP	Positive (normal IR); normal astrocyte population	Overexpression & decreased astrocyte population	Overexpression & normal astrocyte population
	Anti-NF	Barely expressed	Mildly expressed	Overexpression (in > 4/5 of cell pop)

It is our view that a moderate GFAP protein expression in the CA3 region of the hippocampus is critical for astrocyte survival. In the *regio 1 cornus ammonis* (CA1 region), there was also an increase in the number of reactive astrocyte in the Jobelyn group. On the other hand, in the alcohol group, though the numbers of astrocytes were significantly reduced, they however exhibited strong immunoreaction for GFAP. Suggestive that alcohol exposure may have deprived the CA1 region of the hippocampus of the usual physiological neuroprotective function of the astrocytes [31]. As evident from this present study, the administration of Jobelyn to alcohol exposed rats restored or encouraged the normal GFAP protein expression in the CA1 area and hence guaranteed the neuronal protective and maintenance function of the astrocytes as previously reported [31, 32]. We therefore opine that a moderate activation of astrocytes in the hippocampal CA1 area of alcoholic rats by the administration of Jobelyn is critical for neuronal survival and maintenance.

Cytoarchitectural alteration has been linked with degenerative conditions in the CNS [2, 29, 38]. The granular cyto architectural pattern of the GCL was not evident in the alcohol group. This alteration has previously been associated with degenerative changes in the hippocampus [2, 39]. Jobelyn supplementation prevented wide deviation from the compact arrangement of neurons in the GCL of dentate gyrus and maintained the neuronal cyton of CA1 and CA3 areas. Derailment from the compact arrangement of neurons in the dentate gyrus is associated with neuronal assaults [40, 41]. However, we observed no remarkable NF-IR in the DG of control and the other experimental groups. Contrarily, there was remarkable NF-IR in the CA1 and CA3 areas of the hippocampus. Jobelyn caused an overexpression in NF protein in almost all neurons in the CA1 region. However, in the CA3 region, Jobelyn caused a mild NF protein expression in a few selected neurons. This dichotomy between the dentate gyrus and hippocampus proper in regards to NF-IR may be partly due to the fact that the cells of area CA1 and CA3 are larger than those of the granular layer [42] and their possession of the characteristic spiny thorns on the apical dendrites [43], which has a high density of neurofilaments. It is also plausible that this dichotomy may connote functional differences between these neurons [44]. Type-specific dendrite morphology is characteristic of neurons and has significant functional implications in determining what signals a neuron receives and how these signals are integrated [45]. Though it is difficult to accurately postulate if the alteration / regulation in NF expression would be beneficial or detrimental considering that each neuron must secure its dendritic field; manage signals that converge onto its dendritic field and aptly flexible for adjustment in development and in response to experience. It is of note that there was no significant difference between the NF-IR of the DG of control and Jobelyn groups. More significantly, this result has demonstrated that the overexpression seen in the CA1 area on administration of Jobelyn to otherwise alcoholic rat was plausibly a neuroprotective mechanism.

CONCLUSION

This study indicated that Jobelyn supplementation improved the cytohistological outcome in the hippocampus of alcohol misuse rats. Part of the possible mechanisms responsible for this effect includes moderate activation of astrocytes in the DG, CA3 and CA1 areas of the hippocampus. Also included in this protective repertoire is plausibly NF protein overexpression in the CA1. We found that these alterations are critical for neuronal survival and maintenance in the hippocampus. Unfortunately, there is no report that supported or contradicted our view. Though we may not be very precise on the extent that the alterations of these

intracellular proteins has on the neuroprotective potentials of Jobelyn, it is however evident that they attenuated neurodegeneration in the hippocampus of alcoholic rats. Jobelyn could be a potential option for AUDs management in humans.

Conflict of Interest: The authors declare that there is no conflict of interest

ACKNOWLEDGMENT

We acknowledge Health Forever Products, Lagos, Nigeria, for the gift sample of Jobelyn supplement capsule.

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