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MYRICETIN REVERSES ETHANOL-INDUCED ANXIETY AND DEPRESSION-LIKE BEHAVIOURS THROUGH PRESERVATION OF PARVALBUMIN-POSITIVE GABAERGIC NEURONS AND NISSL SUBSTANCE IN THE AMYGDALA OF WISTAR RATS

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ABSTRACT

High doses of ethanol stimulate neurotoxicity in limbic structures like the amygdala, causing behaviour disorders and structural disorganisation of neurons. The research examined the neuroprotective mechanism of myricetin (natural flavonoid) against anxiety-like (ethanol-induced) and depression-like (caused by ethanol) behaviours in adult male Wistar rats, with particular references to the maintenance of parvalbumin-positive (PV+) GABAergic neurons and Nissl substance in the amygdala. We carried out a randomised selection and grouping of 60 rats into six different groups (n=10): control, ethanol (5g/kg), myricetin (150mg/kg), myricetin (300mg/kg), ethanol + myricetin (150mg/kg) and ethanol + myricetin (300mg/kg). The treatment was orally administered for 21 days. Assessments used in behaviour were the Elevated Plus Maze (EPM) and the Tail Suspension Test (TST). Using histological and immunohistochemical methods, the Nissl substance and PV+ neurons of the amygdala were assessed. There was a significant increase in anxiety-like and depression-like behaviour and decreased Nissl substance and PV+ expression induced by ethanol. The myricetin (especially in the 300mg/kg dose) reversed these changes significantly, ameliorating the behavioural indices and preserving neurons. These data indicate that myricetin can alleviate ethanol-induced dysfunction of the amygdala in terms of emotion and neuronal integrity by maintaining inhibitory neuronal networks. This confirms the treatment value of myricetin on ethanol-associated emotional disorders.

KEYWORDS

Ethanol, Myricetin, Nissl bodies, Parvalbumins and Hippocampus.

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INTRODUCTION

Ethanol, as a psychoactive and neurotoxic compound, is one of the most popular recreation and social use substances worldwide, although its high or frequent use is closely linked to neuronal impairment, behavioural disturbance and mood

swings[1,2]. Ethanol usage continues to be among the leading contributing factors to ill-health and premature deaths worldwide and especially in low- and middle-income countries since unregulated ethanol products are easier to access[3-5]. In neurobiology, ethanol has profound influences on limbic regions, including the amygdala, which makes up an important part of emotional control, the processing of fear and socialisation[6,7]. In the amygdalae, chronic ethanol induces neurological damage and synaptic breakdown, which finally culminates in behavioural deficits resembling tension and melancholy[8,9].

Specifically, the effect of ethanol on PV+ GABAergic interneurons, or more simply the fast-spiking inhibitory neurons involved in the regulation and upholding of synaptic inhibition, is of concern. Their vulnerable nature to toxicity owes itself to the high metabolic demands of these neurons[10-12]. Toxic injury by ethanol defects both the structural and functional integrity of PV+ interneurons that cause emotional disinhibition and anxiety-like behaviours[13,9]. Such hypersensitivity is further compounded by ethanol-related decreases in Nissl substances vital in protein synthesis and survival of the neurons[14-16]. The animal models of behaviour have demonstrated that ethanol exposure induced higher anxiety- and depressive-like behaviour, which is determined by increasing the open-arm activity in the Elevated Plus Maze and immobility in the Tail Suspension Test[17,6].

Myricetin is a subcategory of flavonoids known as flavonols and is commonly present in fruits, vegetables, and medication used medicinally and has attracted attention because of its strong neuroprotective effect[18,19]. It can easily pass through the blood-brain barrier and can also modulate apoptotic effectors (e.g., Bax/Bcl-2) and increase resilience of neuronal cells[20,21]. Markedly, myricetin proved to be capable of not only saving PV+ interneurons and Nissl substance but also enhancing the outcome of behaviour in ethanol and methamphetamine-related neurotoxicity models[22,23].

Although this suggests the potential role of myricetin in amygdala-protective effects against ethanol-induced damage and its behavioural effects, little data is available that assesses the amygdala-protective effects of myricetin (especially by targeting the inhibitory GABAergic circuitry of the amygdala) against neuronal damage caused by ethanol consumption and the behavioural outcomes. This study, hence, seeks to eliminate this knowledge gap by determining whether myricetin may reverse the anxiety- and depression-related behaviours mediated by ethanol reported in Wistar rats and whether this occurs due to maintenance of the PV+ GABAergic neurons and Nissl substance in the amygdala. The understanding of these mechanisms is relevant in establishing specific therapeutic approaches to the ethanol-induced neuropsychiatric disorders and recognition of safe natural agents that can be used to provide neuroprotection.

METHODS

The purpose of this experimental research was to determine the neuroprotective potential of myricetin in modulating the effect of a toxic dose of ethanol on PV+ GABAergic neuron activities, preserving neuronal integrity and alleviating emotional behavioural outcomes in adult Wistar rats. Ethical approval of the animal study was given by the Nnamdi Azikiwe University, Nigeria, Animal Research Ethics Committee under the approval number of NAU/AREC/2025/0060 and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals[24].

Animals

Sixty adult male Wistar rats aged nine weeks (63 days old postnatal age) were purchased at Dabels farm in Awka, Anambra State, Nigeria and Perspex rearing cages of six groups were utilised to accommodate the animals in a specific pathogen-free environment, a one-week acclimatisation stage and subsequent experiments. Cross ventilation was through a wire gauze top in each cage. The animals were maintained in the Animal House, College of Health Sciences, Nnamdi Azikiwe University, in a controlled room temperature of 25-28°C, relative humidity of approximately 60-80 per cent and a photoperiodicity of 12 h of the day / 12 h of the night. Both ethyl alcohol and myricetin were administered orally at room temperature (pH 9.2) via

24-gauge oral straight gavage needles once a day for 21 days. The animals were given water and standard rat chow on an *ad libitum* basis. The animals were treated as listed in Table No.1 according to the "National Institutes of Health Guide for the Care and Use of Laboratory Animals"[24].

Animal Grouping and Administration

The sixty (60) animals were randomly divided into six groups of ten animals each. The groups were identified as A, B, C, D, E, and F (Table No.1). On the first day of treatment, the rats were 71 postnatal days old, weighing 140 ± 10g and on the last day of treatment, the rats were 91 postnatal days old, weighing 180 ± 20g. This final weight was taken 24 hours after the last administration.

To avoid osmotic pressure shock, the substance was then given orally in a volume not more than 10mL/kg to various groups of the rats as follows: A (vehicle-isocaloric amount of glucose), B (5g/kg, 50% w/v per oral of ethanol) C (150mg/kg/day per oral of myricetin), D (300 mg/kg/day per oral of myricetin), E (5g/kg, 50% w/v per oral of ethanol + 150mg/kg/day per oral of myricetin) and F (5g/kg, 50% w/v per oral of ethanol + 300mg/kg/day per oral of myricetin), at room temperature with a pH level of 9.2. In order to mitigate the influence of timing on drug metabolism in experimental animals, the administrations were done from 14:00 to 18:00 with 2 hours apart between the administration of ethanol and myricetin daily. The vehicle used was an isocaloric amount of glucose to match the caloric value of the administered ethanol. This solution eliminated differences in energy intake between experimental groups, which in essence made it possible to isolate the pharmacological and toxicological effects of ethanol and their caloric contribution. Glucose was selected as being metabolically neutral as regards neurotoxicity in physiological doses, whereas ethanol also has a metabolic and neurotoxic effect. Such an approach that relies on an isocaloric vehicle is also congruent with conventional applications in an experimental neurotoxicology study[25-28].

Neurobehavioural Studies

Anxiety-like and depression-like behaviours of the Wistar rats were assessed using the Morris Water Maze test on the treatment days 20-21. Rats were in the behavioural testing room for 2 hours to allow for appropriate acclimatisation. The behavioural tests were conducted in the behavioural testing room, between 09:00 and 14:00 during the light phase of the light/dark cycle.

Elevated plus maze (Assessment of Anxiety)

On the 20th day of treatment, the elevated plus maze test was used to determine the anxiety behaviour of rats. The elevated plus pattern was constructed of plywood and is composed of two opposite open and closed arms (50 x 10 x 40cm) raised 50cm above the ground (Morales-Delgado *et al*, 2018). The animals were set at the centre half of the EPM to acclimatise to the place within 1 min. Then, as habituated to the EPM, the observer (who did not know the treatment groups) measured the behavioural activities in 5 min. Entry was termed as a movement into one of the arms with all four paws. The recorded measurements were the number of open arm entries, the number of closed arm entries, the number of head dips and the time spent in open arms[33,34]. Tucker and McCabe[35] noted that the entry of the open arms and the time spent in the open arms were the main outcomes that measured the anxiolytic or anxiogenic activity, and they were substantially recognised as the leading indicators of anxiety-like behaviour. An evaluation of locomotor activity was determined by measuring the frequency of entry into the enclosed arms, which are more reliable generally in the measurement of movement[35]. The measure of this locomotive activity guarantees that modifications in open-arm exploration are as a result of anxiolytic/anxiogenic consequence, as opposed to altered locomotive activity[35]. This test period was carried out with the application of 70% ethanol and super-hypochlorous water to clean the maze between every test session to eliminate residual odour bias.

Tail Suspension Test

The tail suspension test was used to analyse depression-like behaviour on the 21st day of treatment, as earlier reported by Can *et al*[36]. The test apparatus that was used was white acrylic walls (20 x 40 x 60cm), one of which was open where the test animals were videotaped. The apparatus centre received 250 lx of light. Two animals were tested

simultaneously, and they were isolated with the help of the opaque barrier in the middle of the device. The animals were hanged by the tail 60cm above the ground with adhesive tape < 1cm away from the tip of the tail. A video camera was used to record 6 min of behavioural activities of the animals. Analysis of the behaviour was done later to obtain the cumulative time the animals were immobile. The combined time that every animal was immobile was noted in seconds. The immobile period was defined in this test as the time that the animals ceased struggling for 1 s or more. Manual data collection and analysis were carried out.

Collection of Samples

The animals were anaesthetised with 80mg/kg of ketamine and 10mg/kg of xylazine injected intramuscularly 24 hours after the last treatment after behavioural studies. To access the heart location in the animal, the animals were dissected by making a longitudinal midline incision on the trunk to expose the animal heart, and the animal heart was perfused with 20mL of heparin saline and 20mL of 10 per cent neutral buffer formalin via the right ventricles of the animal heart[37]. Once the cardiac perfusion was done, the animal brains were harvested rapidly by making an occipitofrontal incision, and this cut opens the cranial vault. All the animals had their brain tissues fixed in 10 per cent neutral buffered formalin and were used in histological examinations. Routine paraffin wax embedding and coronal sections were performed on all histological tissues at the same bregma level.

Histological Procedures

Tissue Processing

Autolytic and putrefactive activities were prevented by fixing the brain tissues using 10 per cent neutral buffered formalin in the fixation protocol of 48 h as specified by Al-Sabaawy *et al*[38] and Udodi *et al*[39]. It was dehydrated in ascending concentrations of ethanol (70, 80, 90 and 100 per cent, respectively) and cleared to eliminate alcohol in xylene. Tissues were impregnated with paraffin wax at 60 degrees Celsius and cooled at 20 degrees Celsius and embedded in blocks. Thin sections (5µm) using a microtome were obtained.

Cresyl Violet Staining

The staining with cresyl violet revealed Nissl substance in the basolateral, which implies that the neurons have rough endoplasmic reticulum and ribosome RNA[40]. Samples were deparaffinised in xylene, then rehydrated by gradual immersion in the alcohol solutions, followed by staining in 0.1 to 0.5% cresyl violet acetate (pH 3.5-4.0), which were stained within 7 minutes[41,42]. There was differentiation to eliminate the excess stain in an acetic acid, and the neuronal cell bodies were expressed. The dehydration by acetone and clearing with xylene, as well as mounting on a resin, were performed on the slides. Neuronal nuclei and Nissl bodies were stained violet, and destruction or depletion are the signs of neuronal damage or neuron degeneration[43].

Parvalbumin Immunohistochemistry

The analysis of the structure of the inhibitory circuit was conducted using immunohistochemistry of the calcium-binding protein in GABAergic interneurons parvalbumin[44,45]. The deparaffinised sections were rehydrated, and heat-induced epitope retrieval (HIER) was performed in citrate buffer (pH 6.0) in order to enhance accessibility of antigens[46]. Non-specific binding was prevented with 5 per cent goat serum and 0.1 per cent Triton X-100. Anti-parvalbumin primary antibody (1:200) has been incubated overnight at 4 degrees C and secondary antibodies conjugated to HRP (2 hours) on sections. Diaminobenzidine (DAB) was used to perform the detection and sections were counterstained, mounted and studied under the light microscope. The number of PV+ neurons was counted using ImageJ. The low PV expression can be associated with neurodevelopmental or neurotoxic damages[37,44,45].

Image Analysis and Cell Count

A digital brightfield microscope was used to photograph the sections. Image analysis was performed using the ImageJ program (NIH, USA): to do this, non-overlapping micrographs were made at x200 magnification and then uploaded into the program. The number of positive stained cells was determined by counting the positive Nissl bodies under the ImageJ program Cell Counter to evaluate positive stained cells[40,43] and parvalbumin-expressing

protein immunoreactivities were segmented and measured with Immuno Ratio, which is used to isolate and quantify the expression of parvalbumin proteins (positive immunoreactivity) by digital colour deconvolution[44,45].

Statistical Analysis

Data were expressed as mean ± SEM. The statistical analysis was done using the SPSS (Version 23). The data were analysed using one-way analysis of variance (ANOVA) and the post hoc Tukey test. Data were statistically significant at *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$ when the experimental groups were compared to the control group (group A) and also statistically significant at $^{\#,}$ $p<0.05$; $^{\#\#}$, $p<0.01$; and $^{\#\#\#}$, $p<0.001$ when the experimental groups were compared to the ethanol group (group B).

RESULTS AND DISCUSSION

These findings are organised into three sections: 1) behavioural performance, 2) expressions of Nissl bodies and 3) immunoreactivity level of parvalbumins. All sections are connected with specific research questions and introduced in the form of figures to show the essential findings.

Myricetin Reversed Ethanol-Induced Anxiety-Like Behaviour in the Elevated Plus Maze

The ethanol group (Group B) showed severe levels of anxiety-like behaviour with a decrease in the number of entries to the open arm (Figure No.1), the duration of time spent in the open arms (Figure No.2) and the number of head dips (Figure No.3) when compared to controls. Myricetin (Groups C and D) did not have an anxiogenic effect when given alone. Remarkably, coadministration of ethanol and 300mg/kg myricetin (Group F) was able to produce significant effects on the frequency of open arm entries, time spent in open arms and head dips, in contrast to Group B. This implies complete reversal of ethanol-induced anxiety. The non-significant difference in the assessment of the frequency of close arm entry in Figure No.4 indicates that the explorative activities of the animals in the open arm were anxiolytic/anxiogenic and not altered locomotion.

Figure No.1 shows the results of the open arm entries; the comparison focuses on ethanol alterations and myricetin interventions in treated groups. In the EPM test, Group B (mean= 2.0) presents a statistically significant decrease (p= 0.000***) in the number of open arm entries when compared to the control Group A. This reflects elevated anxiety behaviours due to ethanol toxicity. The intervention treatment with myricetin in groups C, D, E, and F regulated the number of open arm entries to a statistically non-significant level (p= 0.983, 0.983, 0.199 and 0.999, respectively) when compared to group A and a statistically significant level in groups C, D and F (p= $0.002^{\#\#}$, $0.000^{\#\#\#}$, and $0.001^{\#\#\#}$, respectively) when compared to group B.

The mean time spent in the open arm of the EPM test (Figure No.2) showed that Group B was the most anxious, as revealed by the lowest time spent in the open arm (14.35 ± 2.41, p = 0.000***) when compared to the control group (Group A). Groups C and D showed no difference in the time spent in the open arms (30.17 ± 1.62 and 32.90 ± 1.46, respectively) and were thus likely to have a normal anxiety level, comparable to group A. Groups E and F were less anxious, represented in the increased time spent in open arms (21.99 ± 2.16 and 28.28 ± 1.48, respectively), which is comparable to group A. When compared to group B, groups C, D, and F were statistically significant at p= $0.000^{\#\#\#}$, $0.000^{\#\#\#}$ and $0.000^{\#\#\#}$, respectively. This indicates an obvious departure from the ethanol-induced toxicity.

Figure No.3 shows the results of the numbers of head dips in the open arm. In the EPM test, Group B presents a statistically significant decrease (1.83 ± 0.48, p = 0.016**) in the number of head dips when compared to the control Group A. The result in group B reflects elevated anxiety behaviours due to ethanol toxicity. The intervention treatment with myricetin in groups C, D, E, and F regulated the number of head dips to a statistically non-significant level (p= 1.000, 1.000, 0.964, and 1.000, respectively) when compared to group A. Groups C, D, and F were statistically significant at p= $0.012^{\#\#}$, $0.008^{\#\#}$, and $0.023^{\#}$, respectively, when compared to group B.

The number of close arm entries (Figure No.4) represents a measure of general locomotor activity and the results did not differ significantly between groups ($p < 0.05$). The statistically non-significant data obtained in the treatment groups when compared to the control group indicates that the observed differences in open-arm exploration were not due to general movement impairments but rather anxiety-related behavioural differences.

Myricetin Reduced Depression-like Activities in the Tail Suspension Test

The rats treated with ethanol (Group B) exhibited reduced immobility time, which acts as an indicator of increased depression-like behaviour. The immobility times in Groups E and F in myricetin treatment returned to normal control values, although they remained slightly higher in Group F (300mg/kg) when compared to the control (Figure No.5). This indicates an antidepressant-like effect of myricetin.

Figure No.5 shows the results of the immobility time in the tail suspension test. The comparison focuses on ethanol alterations and myricetin interventions in the depression-like behaviour of treated groups compared to the control group. In the tail suspension test, group B presents a statistically significant decrease (110.77 ± 10.89, $p = 0.000$***) in the immobility time when compared to the control group A. This reflects increased depression-like behaviours due to ethanol toxicity. The intervention treatment with myricetin in groups C, D, E and F regulated the depression-like behaviour to a statistically non-significant level ($p= 0.999, 0.889, 0.805$, and 1.000, respectively) when compared to group A and a statistically significant level ($p= 0.000^{###}$, $0.000^{###}$, $0.000^{###}$ and $0.000^{###}$, respectively) when compared to group B.

Myricetin Preserved Nissl Substance in the Amygdala

The histologic examination showed that ethanol significantly reduced the quantity of Nissl substance in the basolateral amygdala, which is evidenced by chromatolysis and diminished staining. The groups receiving myricetin treatment showed a dose-dependent increase in Nissl substance intensity, with the 300mg/kg myricetin-treated group (Group F) showing a quantity of myricetin comparable to that of the control group (Figure No.6). This indicates that myricetin preserved neuronal integrity.

Figure No.6 shows the results of the analysis of the Nissl substance in the basolateral amygdala region of the brain. The comparison focuses on ethanol alterations and myricetin interventions in the level of Nissl substance of treated groups compared to the control group A and ethanol group B. In this assessment, Group B presents a statistically significant decrease (58.67 ± 6.49, $p = 0.010$**) in the number of cells expressing Nissl substance when compared to the control Group A. This reflects neuronal injury as a result of ethanol toxicity. The intervention treatment with myricetin in groups C, D, E and F enhanced the number of cells expressing Nissl substance to a statistically non-significant level ($p= 0.993, 0.070, 0.766$ and 1.000, respectively) when compared to the control group A and a statistically significant level in groups C, D and F ($p= 0.004^{##}$, $0.000^{###}$, and $0.014^{##}$, respectively) when compared to the ethanol group B, which indicates an obvious departure from the toxicity of ethanol and restoration to the normal control level.

Myricetin Protected PV+ GABAergic Neurons from Ethanol-Induced Loss

Significant loss of the PV+ GABAergic interneurons in the basolateral amygdala indicated an alteration in inhibitory signalling, which was caused by ethanol. The treatment with myricetin (300 mg/kg; Group F), in turn, enhanced the density of PV+ neurons (Figure No.7), which means that circuits of inhibition were preserved, protecting emotional regulation.

Figure No.7 shows the results of the analysis of parvalbumin in the basolateral region of the amygdala in the brain. The comparison focuses on ethanol dysregulation and myricetin amelioration in the expression of parvalbumin in treated groups compared to the control group A and ethanol group B. In this assessment, Group B presents a statistically significant decrease (40.33 ± 4.10, $p = 0.002$**) in the expression of parvalbumin when compared to the control Group A. This reflects neuronal injury as a result of ethanol toxicity. The ameliorative treatment with myricetin in groups C, D, E and F enhanced the expression of parvalbumin to a statistically non-significant level except for

group D (p= 0.991, 0.001**, 0.124, and 0.998, respectively) when compared to the control group A and a statistically significant level except for group E (p= 0.001$^{###}$, 0.000$^{###}$, 0.206, and 0.004$^{##}$, respectively) when compared to the ethanol group B, which indicates an obvious departure from the toxicity of ethanol and restoration to the normal control level.

Discussion

This study investigated the neuroprotective effects of myricetin on ethanol-induced anxiety and depression-like behaviours and structural neuronal damage in the amygdala of adult male Wistar rats. The results of our study indicate a dose-dependent reversal of emotional deficit, biochemical alterations and histological changes in the amygdala following myricetin treatment. These findings offer persuasive disclosure of the therapeutic role of myricetin in reducing neuropsychiatric imbalances caused by ethanol.

Exposure to ethanol resulted in anxiety- and depression-like behaviours, evidenced by reduced open arm activity in the Elevated Plus Maze and decreased immobility time in the Tail Suspension Test. These results are in line with previous research demonstrating that chronic ethanol consumption affects emotion-regulating networks, mainly in the amygdala, increasing anxiety and depression-like behaviour[6,9,17]. The possible anxiogenic actions of ethanol can be linked to a GABAergic signalling deficiency, disturbed glutamatergic transmission, and neuroplasticity dysregulation[2,7].

Treatment with myricetin produced a reversal of these behaviours, especially at the dose of 300mg/kg, with the restoration of open arm exploration and prolonging the period of immobility. These findings are consistent with evidence revealing that myricetin has an anxiolytic and an antidepressant-like effect in other models of neurotoxicity and inflammation[21-23]. The mechanism of action of myricetin manifesting its benefits in terms of behaviour could include modulation of the monoaminergic systems, improvement of the GABAergic tone and a reduction in the pro-inflammatory cytokines[18,20].

Exposure to ethanol in this current study resulted in loss of Nissl substance in the basolateral amygdala, signifying the chromatolysis of neurons and inhibited protein synthesis. Degradation of the Nissl substance indicates impairment of rough endoplasmic reticulum and leads to the dysfunction and death of neurons[14]. The same histopathological alterations were found in the hippocampus and the cortex of animals treated with ethanol as reported by Tsermpini *et al*[16]. The co-treatment with myricetin resulted in enhanced staining of Nissl substance, indicating the preservation of the protein synthesis machinery and the structural strength of the neurons. This corroborated previous results in which myricetin enhanced Nissl body distribution and inhibited neuron degeneration in methamphetamine and other toxicity[22,23].

Most importantly in our study, ethanol treatment affected large volumes of PV+ GABAergic interneurons in the amygdala. The PV+ cells play a critical role in sustaining the level of inhibition in the basolateral amygdala[10,12]. Damage to such neurons results in the loss of balance between excitation and inhibition and results in emotional dysregulation and anxiety-like behaviour[8]. Our results replicate other research that showed PV+ interneurons are sensitive to ethanol toxicity[9,11]. Interestingly, the myricetin administration (300mg/kg) completely recovered the density of PV+ cells, pointing to its beneficial effects in maintaining an inhibitory neural network. This neuroprotection may be attributed to both its effect on Nissl bodies to reverse chromatolysis and modulation of intracellular signalling pathways that prevent apoptosis in PV+ neurons[20,21].

The results in my research have established myricetin as a neuroprotective agent that may be used in the treatment of ethanol-induced affective and structural brain diseases. The fact that it can protect key inhibitory populations of neurons corroborates with more recent approaches that treat neuropsychiatric disorders through GABAergic mechanisms[2,7]. Notably, as opposed to a wide range of synthetic neuroprotectants, myricetin is a natural chemical with an excellent safety context and therefore, provides a prospective nutraceutical concept.

Table No.1: Animal groupings and dosage of administration

S.No	Treatment group	Dosage	Reference
A	Control- Vehicle	Isocaloric amount of glucose	29
B	Ethanol	5g/kg, 50% w/v per oral	29,30
C	MYR Low dose	150mg/kg/day per oral	31
D	MYR High dose	300mg/kg/day per oral	32
E	Ethanol + MYR Low dose	5g/kg, 50% w/v per oral + 150mg/kg/day per oral	29,31
F	Ethanol + MYR High dose	5g/kg, 50% w/v per oral + 300mg/kg/day per oral	29,32

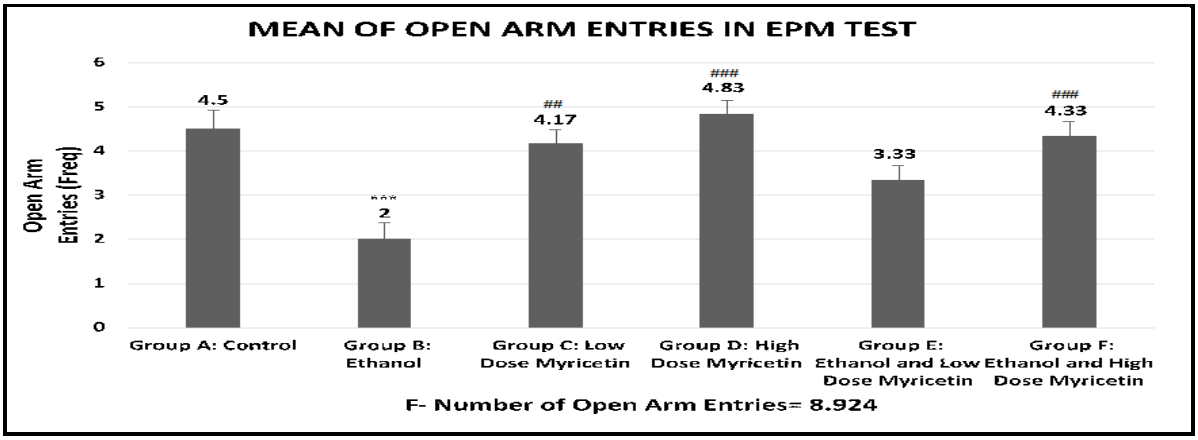

Figure No.1: The mean of the number of open arm entries in the treated animal groups A- F. Group A received an isocaloric amount of glucose; Group B received 5g/kg, 50% w/v per oral of ethanol; Group C received 150mg/kg/day per oral of myricetin; Group D received 300mg/kg/day per oral of myricetin and Groups E and F received both 5g/kg, 50% w/v per oral of ethanol and different doses of myricetin of 150 and 300 mg/kg/day per oral, respectively. Data represent mean ± SEM. ***, p<0.001 when the group is significantly different from control group A, and ##, p < 0.01, ###, p < 0.001 when the group is significantly different from ethanol group B

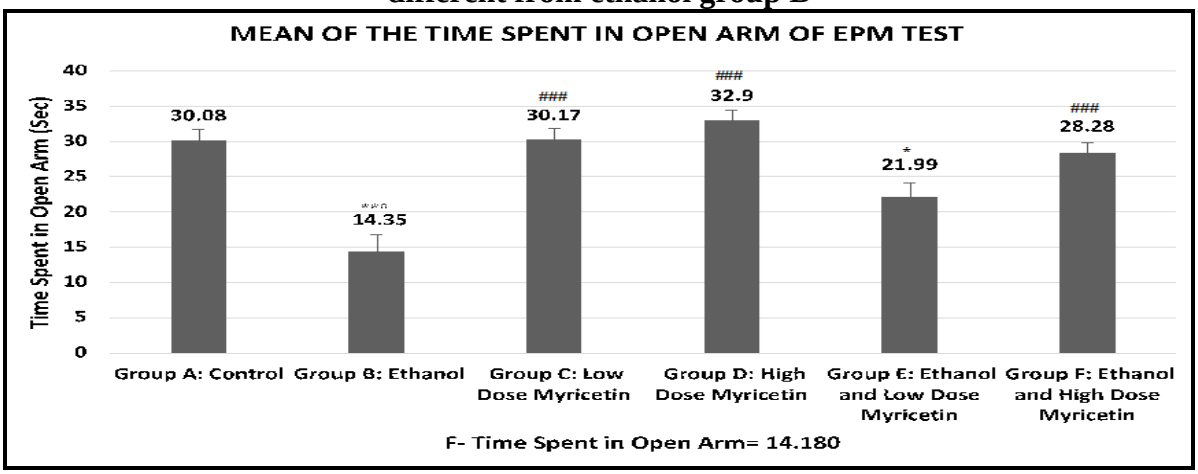

Figure No.2: The mean of the time spent in the open arm in EPM test in the treated animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. *, p<0.05 and ***, p<0.001 when the group is significantly different from control group A and ###, p < 0.001 when the group is significantly different from ethanol group B

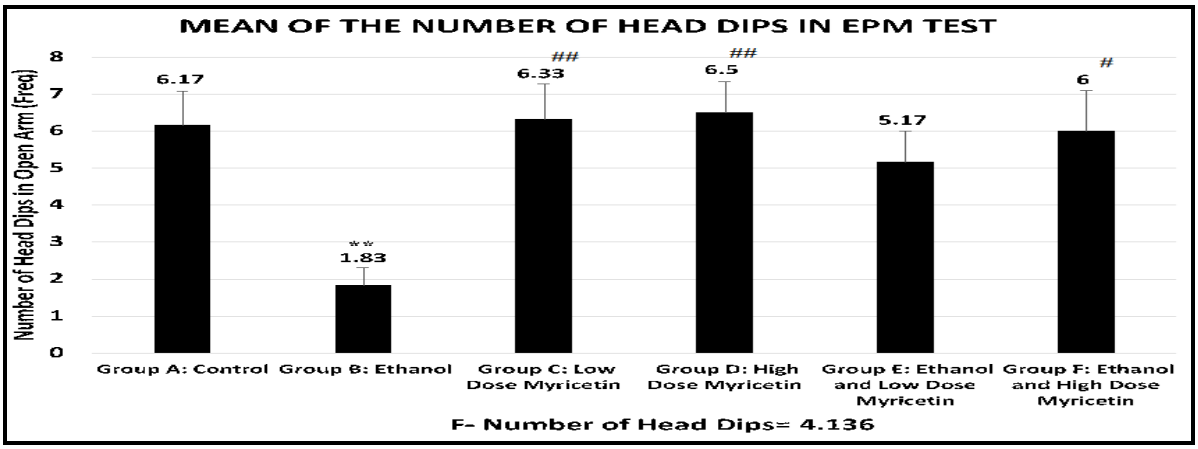

Figure No.3: The mean of the number of head dips in the open arm of EPM test in the treated animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. **, p<0.01 when the group is significantly different from control group A, and #, p < 0.05, ##, p < 0.01 when the group is significantly different from ethanol group B

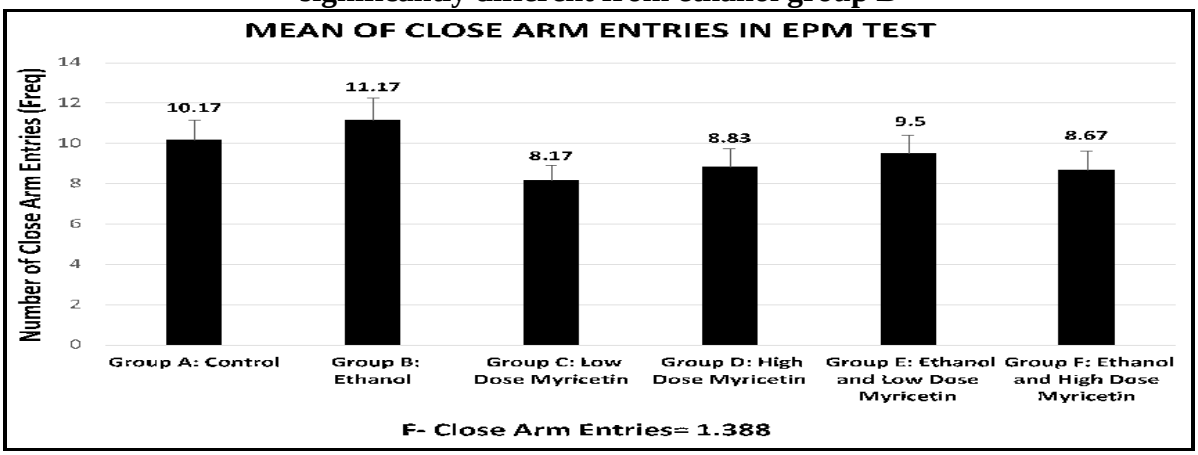

Figure No.4: The mean of the number of close arm entries in EPM test in the treated animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. *p < 0.05 compared to Group A

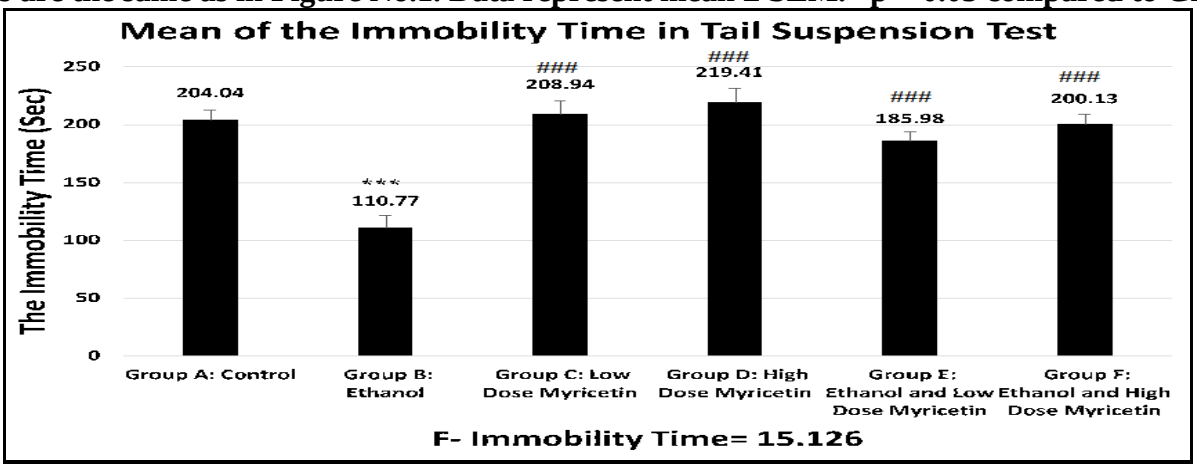

Figure No.5: The mean of the immobility time in tail suspension test for treated and control animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. ***, p<0.001 when the group is significantly different from control group A and ###p < 0.001 when the group is significantly different from ethanol group B

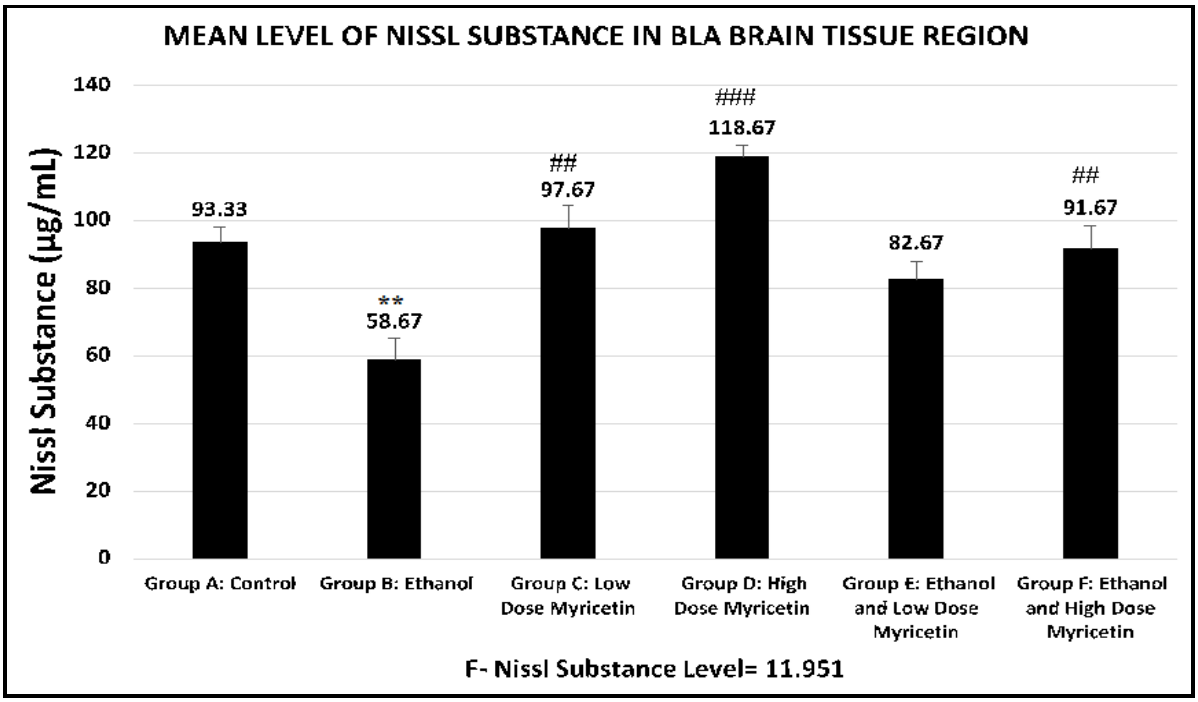

Figure No.6: The mean level of Nissl substance in BLA brain tissue region for treated and control animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. **, p<0.01 when the group is significantly different from control group A and ##, p < 0.01, ###, p < 0.001 when the group is significantly different from ethanol group B

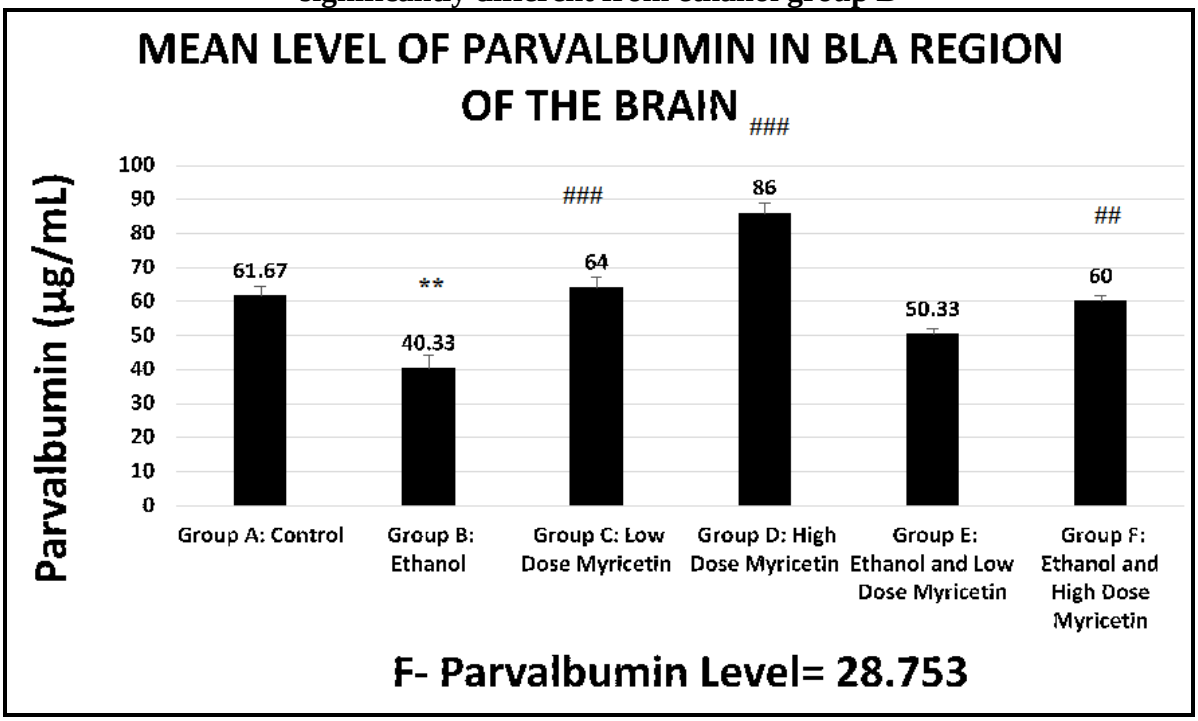

Figure No.7: The mean level of parvalbumin in the BLA region of amygdala in brain tissue of treated and control animal groups A-F. Groups are the same as in Figure No.1. Data represent mean ± SEM. **, p<0.01 when the group is significantly different from control group A and ##p < 0.01, ###p < 0.001 when the group is significantly different from ethanol group B

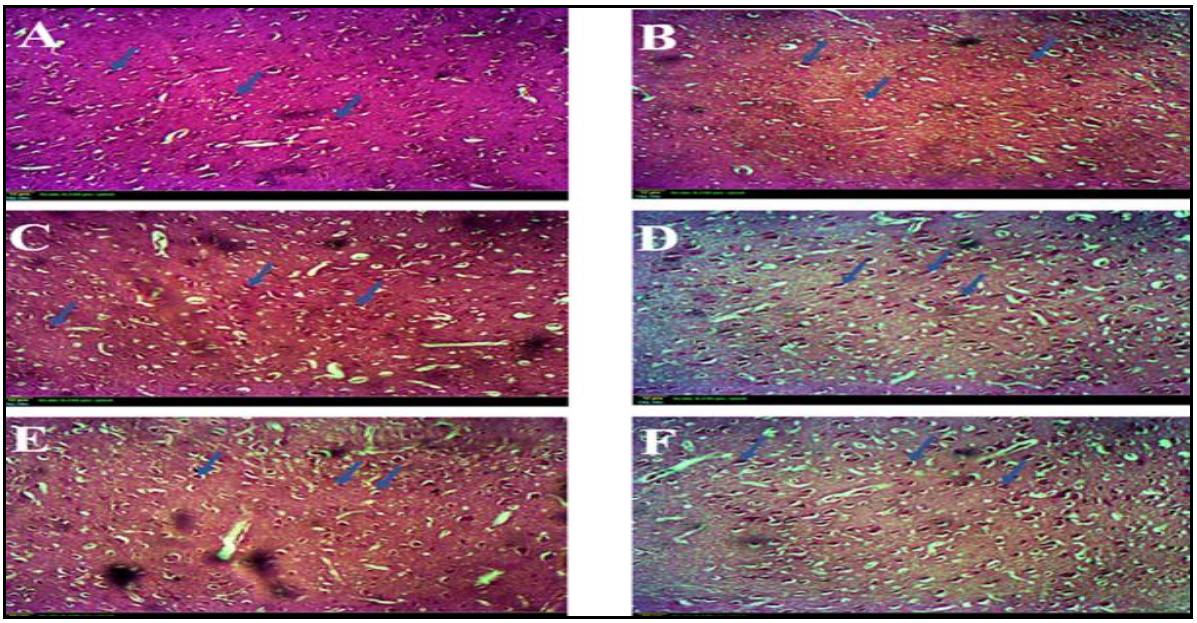

Plate No.1: The photomicrographs of BLA region of the brain in treated rats across the study groups A-F. The ethanol treatment of the rats significantly decreased the expressions of Nissl substance in group B when compared to group A, while the treatment of the rats with myricetin in groups C, D, F significantly increased the expression of Nissl substance when compared to group B. Stained with CSV (X200) and 12µm scale bar

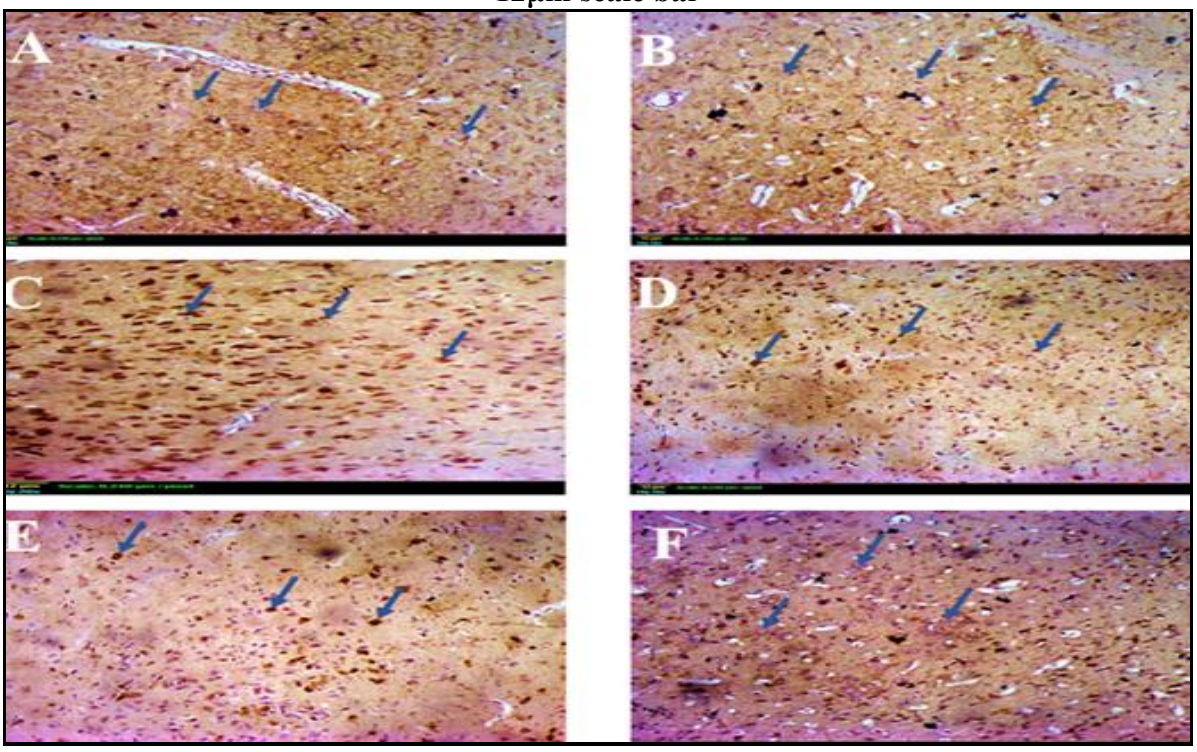

Plate No.2: The photomicrographs of BLA region of the brain in treated rats across the study groups A-F. The ethanol treatment of the rats significantly decreased the expressions of PV+ GABAergic neuron in group B when compared to group A, while the treatment of the rats with myricetin in groups C, D, F significantly increased the expression of Nissl substance when compared to group B. Stained with PV (X200) and 12µm scale bar

CONCLUSION

It has been shown in this study that myricetin reduces ethanol-induced anxiety and depression-like behaviours in adult male Wistar rats, possibly due to its strong antioxidant and preservation of important neuronal structures in the amygdala. In particular, myricetin rescued Nissl bodies and preserved parvalbumin-positive GABAergic interneurons, which play a significant role in sustaining emotional control. These results indicate that myricetin provides neuroprotection against ethanol's behavioural and cellular effects in terms of maintenance of inhibitory neuronal networks in the limbic system.

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CONFLICTS OF INTEREST

No conflict of interest.

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