Research Article

www.ijrap.net

MORPHOMETRIC ANALYSIS OF DIOSMETIN AND GLABRIDIN REINFORCES THE HIPPOCAMPAL INTEGRITY IN ETHANOL INDUCED NEURONAL DAMAGE MODEL RATS

Sasi Kumar A [1]*, Suba Malani S [1], Ramaswamy C [2]

[1]Assistant Professor, Department of Physiology, Tagore Medical College & Hospital, Rathinamangalam, Chennai, Tamil Nadu and Research Scholar, Saveetha University, Chennai, India

[2]Professor, Department of Physiology, Saveetha University, Chennai, India

Received on: 20/04/17 Accepted on: 09/06/17

***Corresponding author**
E-mail: simmamsasi@tagoremch.com

DOI: 10.7897/2277-4343.083201

ABSTRACT

Memory loss and learning disabilities are major problems in human mainly caused by disturbances in hippocampus. Many biological factors are producing inflammation in human brain such as aging, physical, chemical exposure, irradiation and oxidative stress. Extensive release of neuroinflammatory cytokines cause morphological changes in nervous system which leads to cognitive impairments and neurodegeneration in brain. Natural phytochemicals have been used to treat many cognitive impairments and neurological disorders. The research work was conducted on twenty four Wistar albino rats to find out the effects of diosmetin and glabridin on hippocampus neuronal integrity. The morphometric analysis of Haematoxylin & Eosin stained sections of rat's hippocampus was done using ocular micrometry. The diameter of neurons, packed density and the total number of neurons were calculated and tabulated for statistical analysis. The statistical package SPSS version 20 was used to analyze the significance. The results show that the glabridin and diosmetin increase the number of neurons and density in Dentate Gyrus, CA1 and CA3 regions of Hippocampus. The results describe that diosmetin and glabridin have the protecting effects on hippocampus from ethanol toxicity. Increased total number of neurons in dentate gyrus region implicate that new neurons may generated in the hippocampus. which is initiated by the diosmetin and glabridin. Morphometric analysis reveals the initiation of neurogenesis and reinforcement for hippocampal neuronal integrity. Hence the Glabridin and Diosmetin may be used as a subordinate to treat the neurodegenerative disorders.

Key words: glabridin, diosmetin, neurodegeneration, hippocampus and ocular micrometry

INTRODUCTION

The dentate gyrus (DG), Cornu ammonis (CA) area 1and 3 of hippocampus have been extensively studied for spatial navigation, long term memory and behavioural activities in rodents. The pyramidal type neurons present in CA 1 and CA3 layers, where granular cells present in DG[1]. These regions are part of limbic system which suffered a lot in Alzheimer's disease and other neurodegenerative disorders[2]. Studies revealed that ethanol induced neurotoxicity in rat's brain and the effects on behavioural activities[3]. Ethanol induced neuronal damage produces many histological changes in the hippocampus regions. Of late ethanol has been used to create neuronal damage in animal's brain for experimental studies related with cognition and neuroprotcetion[4]. Glabridin is one of the active principles in *Glycyrrhiza Glabra* and diosmetin found in various natural products; both have been studied to prove the beneficial effects on hippocampal morphology. [5, 6.] Quantification of histological changes are helpful in assessing the effects of given drugs. An evaluation was done which explains the drug's effects on histological changes in the hippocampus of cold stress induced Wistar rats. Oral administration of natural drugs produces morphological changes and neuro protective effects in cold stress induced hippocampal degeneration of rats[7]. The total number of neural cells, diameter and packed density in CA1, CA3 and DG region of hippocampus are pointing tools in morphometric analysis[8]. These finding are helpful to identify the efficient drugs which protect the neurons and initiate the neurogenesis. Morphometric analyses support the behavioural refinement and reduce the disease progression ethanol induced cognitive disorders. Glabridin and Diosmetin may helpful to inhibit the action of ethanol and blocking glutamate response by ethanol[9, 10]. The purpose of the study is to analyze the quantification of morphological changes of hippocampus on ethanol induced Wistar albino rats.

MATERIALS AND METHODS

Animals and Chemicals

The experimental study was approved and conducted at Tagore Medical College and Hospital, affiliated by The TN.Dr.MGR Medical University, Guindy, and Tamilnadu, India. The animals were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Totally twenty four (n=6) 200-250gm weighed 2 months old Wistar albino rats were used in this study. Normal saline (Labthi, Thane, India), Ethanol (Nanda, Nasik, India), Glabridin and Diosmetin (Sigma Aldrich, Inc)

Experimental design

The animals were divided into four groups. Control group (I) received saline, experimental control group (II) received 35%v/v ethanol (3mg/kg/ml) and group (III) received Ethanol (35%v/v) (3mg/kg/ml) and Glabridin (50mg/kg /d/ i.p) and group (IV) received Ethanol (35%v/v) (3mg/kg/ml) and Diosmetin (4mg/ kg /d / i.p) for 28 days. After 28 days animals were sacrificed and brain tissue was harvested for Haematoxylin and Eosin staining.

Histomorphometric Analysis

Diameter of neurons: The Haematoxylin and Eosin stained slides were observed for histological changes and morphometric analysis done using ocular Micrometer. Calibration constant was set by calibration slides for low and high-power lenses. The microscopes never been changed till morphometric analysis. The stained slides were focused using 10x and 40x objectives and the diameter of the cells of CA1, CA3 and DG region of the hippocampus was calculated. The diameter of the cell was calculated using the formula:

Diameter of a cell = Axial ratio × Calibration constant,

Where,

Axial ratio = Maximum Length + Maximum Breadth / 2

The maximum length and breadth of cells was calculated using ocular micrometer then applied into the formula, the diameter of the cells in the region of CA1, CA3 and DG of hippocampus was calculated for all animals (n=6) in a group and are tabulated.

Total number of neurons: The Haematoxylin and Eosin stained sections were focused under high power objective (40x) and counting of neurons was done using calibrated micrometer. The pyramidal cells of CA3, CA1 and DG were counted. The region for calculation was selected from the serial sections made for each group randomly. We have excluded the dark stained and ruptured neurons for counting. The results were tabulated for all animals (n=6) in a group.

Packed neuronal density: The packed density of pyramidal neurons in CA1, CA3 and granular cells in DG were calculated by using the formula

Numerical Density = Average number of cells (/cubic mm) / Area of reticle (/sq mm) * (d+t) t - section Thickness in mm, d - diameter of the neurons in mm

Statistical analysis

The statistical analysis and drawing of graphs were carried out using SPSS version 20.0 [11] (). Data expressed as Mean + SEM (n = 6). One way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used for comparison in between groups. P value < 0.05 was taken as statistically significant**.**

RESULTS

Diosmetin and glabridin were given to the group III and IV to study the effects on hippocampal degeneration induced by 35%v/v ethanol (3mg/kg/ml). Table 1 shows the mean total number of neurons in CA1, CA3 and DG regions of hippocampus. The numbers of neurons were increased in group III and IV compared with group II after ethanol induction. This might be the drugs might act on and protect the neurons of hippocampus. Especially DG region has less neuronal loss after ethanol intoxication for 28 days shows statistically significant (P<.001). CA1 and CA3 regions shown a significant (P<.001) difference compared with control and experimental groups.

Ethanol intoxication for 28 days might cause neuronal loss and morphological changes also. The diameter of neurons is one of the metrics to measure after neuronal degeneration. The glabridin has beneficial effects on CA1 region where the diameter of neurons was not affected much by ethanol. Table 2 shows the results of mean diameter of neurons in hippocampus.

The mean diameters of neurons in hippocampus were analyzed by one way ANOVA to compare in between groups. The results show the F values of CA1 (F=25.11) and CA3 (F=11.52) and DG (F=3.9) which is significant (P<.05). The glabridin and diosmetin act on hippocampus and save the neurons from ethanol's effect. Fig 2 shows the Haematoxylin and eosin stained sections that less pyknotic neurons in control and drug treated groups.

The hippocampal neuronal density shown in Table-3 which explains effects of glabridin and diosmetin on ethanol induced neuro degeneration. The glabridin and diosmetin prevents the neuronal loss and diameter, ultimately density was maintained which is compared to control and positive control groups.

Table 1: **The total of number of neurons represents average number of cells present per 0.14sq mm, which is the area covered by 1 reticle**

Regions	Group I	Group II	Group III	Group IV	F value	P value
CA1	204.00±4	158.2±8.4	218.67±4	188.83±11	6.62	.000**
CA3	264.17±8	175.8±15.1	214.83±5	215.83±4.7	7.93	.000**
DG	901.33±9.35	771.7±25	832±21	872.33±21.8	11.25	.000**

Table 2: The diameter of neurons (µM) in hippocampal regions after ethanol induced neurodegeneration

Regions	Group I	Group II	Group III	Group IV	F value	P value
CA1	3.22 ±.1	2.32 ±.12	3.45 ±.05	3.39 ±.09	25.105	.000**
CA3	3.53 ±.06	2.66 ±.14	3.19 ±.13	2.88 ±.12	11.517	.000**
DG	3.25 ±.11	2.91 ±.05	3.39 ±.08	3.50 ±.08	3.903	.008 *

Table 3: The values of packed cell density in regions of hippocampus. (×10³/ cubic mm)

Regions	Group I	Group II	Group III	Group IV	F value	P value
CA1	143.33 ±2.2	107.1 ±2.02	143 ±2.2	141 ±1.8	16.034	.000**
CA3	143 ±1.8	111.83 ±3.4	143.33±1.6	143.33 ±2.4	15.845	.000**
DG	237.33 ±2.8	210.66 ±2.5	237.33 ±3.06	237.33 ±2.8	16.279	.000**

Values are expressed as Mean ± SEM, n = 6, # - non significant, * - significant (*P <0.05)* **highly significant (*P <0.001),* Statistical analysis – One Way Anova

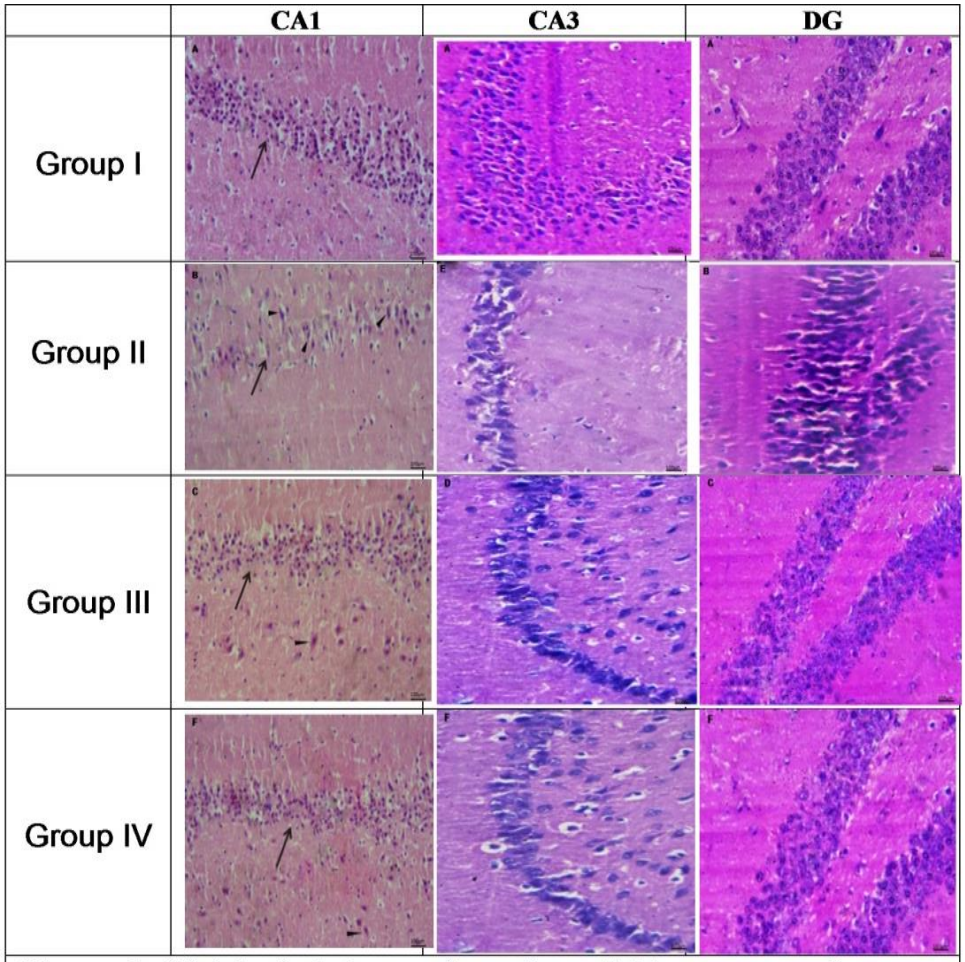

Figure -1: Histological changes in regions of hippocampus in animals groups a,b,c,d,e,f. disorganization of neuronal layer& density (moderate thick arrow) the scale bars in the figure indicate 100 µm

DISCUSSION

Morphometric analysis carried out on CA1, CA3 regions and dentate gyrus of hippocampus in all the groups. The mean number, diameter and packed density of the neurons were calculated by ocular micrometry. The results shown that increased number of neurons and density CA1 and CA3 regions. These data suggest that ethanol alters spine density and connectivity of hippocampus[12]. There was a significant reduction in the total number and diameter of pyramidal neurons in the hippocampus mainly in the sub–granular zone of dentate gyrus which was histologically proved by the present study. This may be due to the generation of new neurons[14.] Recently researchers discovered that the hippocampus is one of the region where new neurons are generated in mammals and humans in life time. The alterations of neuronal connectivity affect the spatial learning and memory. The fresh dendritic spines could form new synapses and reinforce connections between neurons[12, 13].

CONCLUSION

In this study glabridin and diosmetin is more effective in increase the number of neurons and density of hippocampus in ethanol induced neurodegenerative model animals. Glabridin and diosmetin enhance the ethanol tolerance and protect neuronal structures to preserve the physiological functions. Hippocampus is a major region suffered a lot in patients with Alzheimer's disease and epilepsy. Short term memory loss and learning disabilities are common features of neurodegenerative disorders, glabridin and diosmetin might be the drug of choice for neuro protection. These morphometric parameters explain the neuroprotcetion and neurogenesis in the hippocampus.

REFERENCES

1. Manfred C, Aertsen A. Morphological organization of rat hippocampal slice cultures. J Comp Neurol 1991;307:87-106
2. Saito M, Chakraborty G, Hui M, Masiello K, Saito M, Basavarajappa BS. Ethanol-induced neurodegeneration and glial activation in the developing brain. Brain Sci.. 2016;6(3):31.
3. Peng Wang, Qian Luo, Hui Qiao, Hui Ding, Yonggang Cao, Juan Yu et al. The neuroprotective effects of carvacrol on ethanol-induced hippocampal neurons impairment via the antioxidative and antiapoptotic pathways; Oxid Med Cell Longev.. 2016:1-17
4. Jang MH, Shin MC, Jung SB, Lee TH, Bahn GH, Kwon YK, Kim EH, Kim CJ. Alcohol and nicotine reduce cell proliferation and enhance apoptosis in dentate gyrus. Neuro report. 2002;13(12):1509–13

5. Hasanein P. Glabridin as a major active isoflavan from *Glycyrrhiza Glabra* (licorice) reverses learning and memory deficits in diabetic rats. Acta Physiol Hung. 2011;98(2):221-30.
6. Yu XQ, Xue CC, Zhou ZW, Li CG, Du YM, Liang J, Zhou SF. In vitro and in vivo neuroprotective effect and mechanisms of Glabridin, a major active isoflavan from *Glycyrrhiza Glabra* (licorice). Planta Med.2008;74(4):377-80.
7. Saravanakumar.S, Saraswathi.P, Vijayaraghavan. R. Effect of *Bacopa Monniera* on cold stress induced neurodegeneration in hippocampus of Wistar rats: A Histomorphometric Study; J Clin Diagn Res. 2015 ;9(1): AF05-AF07
8. Sivanandan R, Saraswathi P. Histomorphometric Study of *Fructus Psoralea* on ethanol induced neurodegeneration of hippocampus in rat; J Clin Diagn Res 2013;7(8): 1561-1564
9. Villa P, Dario C, Laura DF. Guaitani A. Giuseppina P. Raffaella P. Protective effect of diosmetin on *in vitro* cell membrane damage and oxidative stress in cultured rat hepatocytes. Toxicology.1992;73(2):179-189
10. Koob, GF; A role for GABA mechanisms in the motivational effects of alcohol. Biochem. Pharmacol. 2004; 68(8): 1515-1525
11. IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp.12. Mayford M, Siegelbaum SA, Kandel ER. Synapses and Memory Storage. Cold Spring Harb Perspect Biol. 2012 Jun; 4(6): a005751.
12. O'Malley AO, Murphy KJ, Regan CM. Transient spine density increases in the mid molecular layer of hippocampal dentate gyrus accompany consolidation of a spatial learning task in the rodent. Neuroscience. 2000; 99(2):229–32
13. Praag VH. Functional neurogenesis in the adult hippocampus; Nature 2002; 415:1030

Cite this article as:

Sasi Kumar A *et al*. Morphometric analysis of diosmetin and glabridin reinforces the hippocampal integrity in ethanol induced neuronal damage model rats. Int. J. Res. Ayurveda Pharm. 2017;8(Suppl 3):207-210 http://dx.doi.org/10.7897/2277-4343.083201

Source of support: Nil, Conflict of interest: None Declared