The Effect of Intermittent Fasting Diet on the Hippocampus of Adult Male Mouse After Inducing Demyelination by Ethidium Bromide Injection

Efecto de la Dieta de Ayuno Intermitente en el Hipocampo de Ratón Macho Adulto Después de Inducir la Desmielinización por Inyección de Bromuro de Etidio

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SUMMARY: Intermittent fasting diet (IF) as a restrictive regimen prevents neural degeneration and stimulates overexpression of various neurotropic factors in the hippocampus of animal models. This study evaluates the potential effect of the IF in the prevention of learning and memory dysfunction and improving the alterations in the number and volume of neurons in an ethidium bromide (EB) induced mouse model of demyelination. Mice were randomly assigned into N group (Normal Diet and normal saline injection), F group (IF and normal saline injection), EBN group (Normal Diet and EB injection), EBF group (IF and EB injection). The hidden platform test was carried out based on path length, escape latency and swim speeds of mice. Stereological studies were determined by the Cavalieri and the Optical Dissector technique. Maintenance of mice on the IF results in significantly decreased the body weight and biochemical parameters, increased total number of neurons and volume of the hippocampus, and improved learning and memory parameters of adult male mice. However, IF in EBF group did not show as excellently as F group. The EBF group displayed significantly spatial memory improvement than that in EBN group. There were no statistically significant differences between EBF and EBN groups in stereological and learning parameters, though the EBF group displayed faster escape latencies, and swam faster and shorter path lengths than the EBN group in these parameters. Therefore as a conclusion, The IF fairly improved some adverse effects of EB in experimental demyelination models.

KEY WORDS: Intermittent fasting diet; Hippocampus; Demyelination; Stereology; Mice.

INTRODUCTION

Multiple sclerosis (MS) is the most common autoimmune and neuroinflammation disease of the CNS, in which the myelin sheet of the nerve fibers is destroyed. Thus it is characterized by chronic demyelination and axonal loss, inflammation, cell death and tissue damage (Versini *et al.*, 2014; Ghaffari *et al.*, 2015). Specifically, a major hallmark of MS is cognitive impairment (Khurana *et al.*, 2009). Among individuals suffering from MS, about 40 % to 70 % of patients have cognitive impairment with a significant impact on patient's daily activities and quality of life which is detectable even during the earlier phases of the disease (Ghezzi *et al.*, 2017).

Several previous studies demonstrated that the Hippocampal formation, among the various brain regions involved during the course of MS, is extensively sensitive to the detrimental effects of neuro-degeneration since hippocampal demyelination has been detected in approximately 53 % to 79 % of post-mortem brains in MS patients (Mancini *et al.*, 2017).

A majority of previous observational studies have confirmed the relationship between obesity and various inflammatory/autoimmune conditions (Ghadirian *et al.*, 1998; Versini *et al.*). Reviewing the literature indicated that nutrition and food

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patterns, especially the overconsumption of dietary fats and high energy dietary regimens, are more likely to be associated with the etiology of MS (Hedström *et al.*, 2014). Nowadays, it is accepted that weight gain in MS patients may be associated with greater disability because the overweight may aggravate the MS symptoms (Swank & Dugan, 1990; Khurana *et al.*).

In studies on rodents, the beneficial effects of some dietary restriction regimens, including intermittent fasting (IF) and caloric restriction (CR) on the various brain regions have been examined (Anson *et al.*, 2003; Martin *et al.*, 2006). It was found that the beneficial effects of IF without an overall reduction in calorie intake are as follows: Decreased body weight, increased insulin sensitivity, normalized plasma glucose, and reduced circulating leptin levels in obese and reduced size of adipocytes in both subcutaneous and visceral fat. Remarkably, neuroprotective effects were also observed in experimental models of neurodegenerative disorders, such as upregulation of neurotrophic factors such as BDNF, GDNF, IGF-1, HSP70 and GRP78 in the brain, reduced mitochondrial oxyradical production, induction of a mild adaptive cellular stress response and increased production of the growth factors (Martin *et al.*; Halagappa *et al.*, 2007). Previous studies have also concluded that IF increases the resistance of neurons against dysfunction and degeneration in animal models in terms of Huntington's, Alzheimer's and Parkinson's diseases as well as stroke (Tajes *et al.*, 2010; Manzanero *et al.*, 2014). It was also proposed that the IF is more efficacious in protecting the brain hippocampal neurons against a neurotoxin in comparison to CR (Tikoo *et al.*, 2007). As a result, it can be inferred that reducing the meal frequency without a calorie intake decrease could lead to an improved health status.

In this study we used a direct injection of Ethidium bromide (EB) into the intracerebral ventricle (ICV) as a simple model for induction of neural cells degeneration and MS disease. Since demyelination is the most common complication of MS, EB-induced demyelination model is one of the most common methods for induction of MS (Mazzanti *et al.*, 2007). The cytotoxic activities of EB result from its DNA intercalating properties. That's why, the effects of EB are not specific to myelinating cells, but can principally injure all nucleated cell types. In addition, oxidative stress plays an important role in the pathogenesis of MS (Gonsette, 2008). The EB induces reduction of the total number of neurons by production of free radicals and enhancement of oxidative stress burden (van der Star *et al.*, 2012).

Owing to the fact that little is known about the effects of IF on the preventive and therapeutic effects in MS patients and with regard to the paucity of research on the cognitive

dysfunction and morphometric hippocampal parameters in an MS mouse model under the IF, the present study was to examine the possible role of IF in the prevention of learning and memory dysfunction and to investigate whether the IF could improve the structural changes in the number of neurons and volume in the mice hippocampus and its MS-associated sub-regions.

MATERIAL AND METHOD

Animals: Experiments were performed on male Balb/c mice (about 8 weeks old) with the weight range of 25±2 g. They were kept at a controlled environmental condition with 12 h light/dark cycles and 22±2 °C room temperature and provided with standard chow and water access *ad-libitum*.

Grouping: The mice were randomly divided into four different groups (n=10 per group) after acclimatization:
N group: Normal Diet and normal saline injection.
F group: IF and normal saline injection.
EBN group: Normal Diet and ethidium bromide (EB) injection.
EBF group: IF and EB injection.

Four weeks after diet onset, the EBN and EBF groups were injected with EB and, the N and F groups were injected with an equal volume of sterile 0.9 % normal saline. Body weight was measured at the beginning of the experiment (first), the same day and time after fourth and eighth weeks; however, the food intake was daily measured (Tikoo *et al.*).

Animal model of intermittent fasting diet: At the same time, the mouse modes of the IF regimen in the F and EBF groups were deprived of food for 24 h (i.e., every other day fasting) as the calorie intake was reduced to 0 %. Chow was alternately provided or removed at 10 AM for IF animals and water was available *ad libitum* for all groups.

Ethidium Bromide-induced demyelination mouse model: The experimental model was bilaterally induced with direct single injection of 3 ml of 0.01 % ethidium bromide (Sigma, Germany) and sterile 0.9 % saline into the intracerebral ventricle (ICV) by stereotaxic devices (Ghaffari *et al.*).

Swelling and enlargement of the extracellular space, basophilic elongated structures of different size and reducing the number of cell layers were recognized twenty four hours after EB injection (Fig. 1).

Measurement of biochemical parameters: The blood was left to coagulate on ice for 15 min and was then centrifuged

for 15 min at 3000x. the plasma was stored at -80 °C until it was assayed (Tikoo *et al*.). The plasma was used to estimate the blood sugar (BS), low density lipoprotein (LDL), cholesterol (CL) and triglyceride (TG).
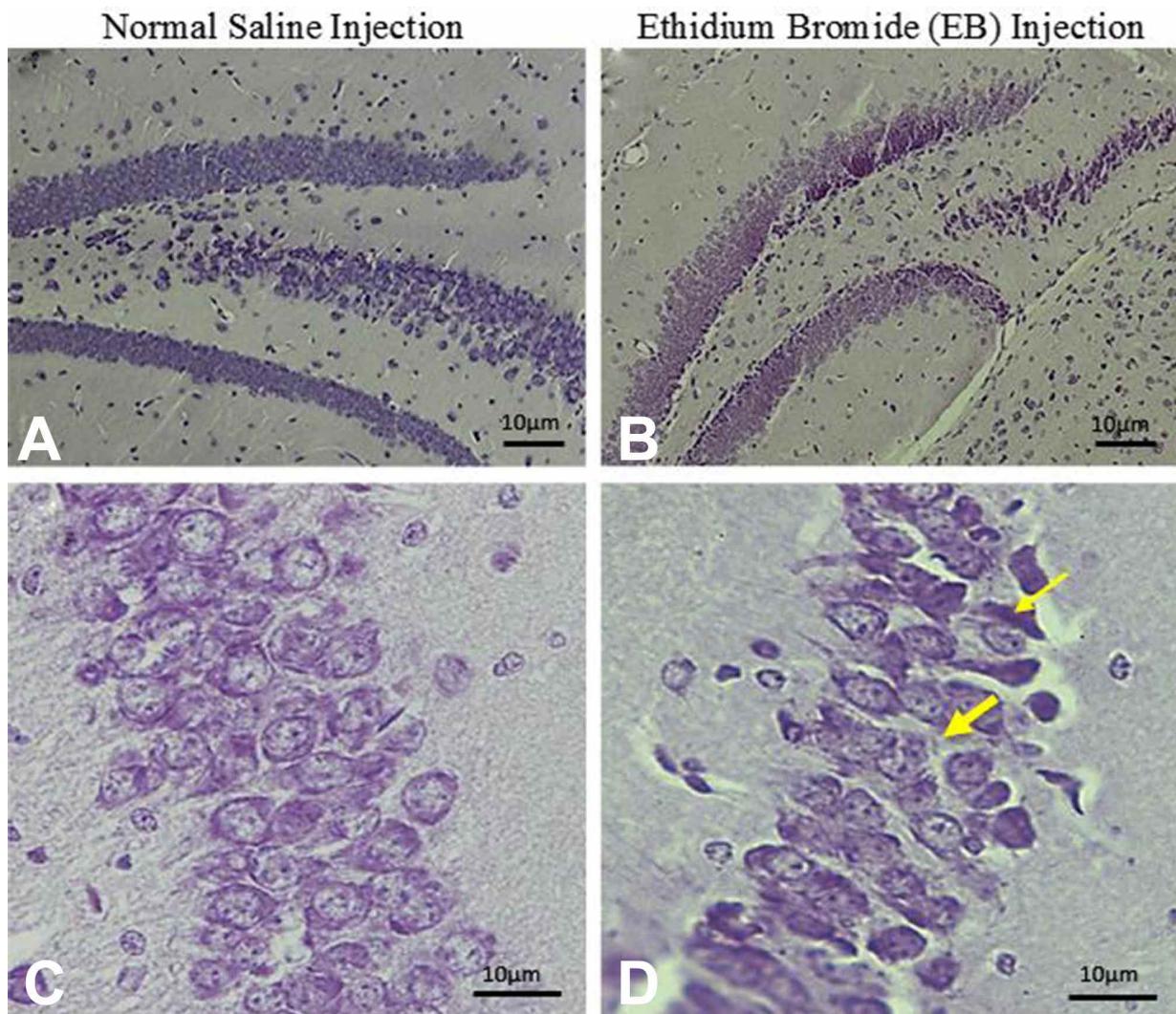

Fig. 1. Swelling and enlargement of the extracellular space, basophilic elongated structures of different size and reducing the number of cell layers were recognized twenty four hours after EB injection. The Kluver-Barrera staining methods. A, B: 40× magnification. C, D: 400X magnification.

Learning and memory evaluation: The morris water maze (MWM) consists of an iron circular pool (diameter 100 cm, height 50 cm, painted black) filled with water to a depth of 30 cm. A circular transparent hidden platform (10 cm in diameter) was submerged approximately 1cm below the water level, 10 cm off the edge of the pool at a position designated as target quadrant. The platform was kept in the same position throughout the learning trials and removed from the pool during the probe test. The swimming path length was monitored by a video camera tracking system and data were collected using Water Maze Software (Borj Sanat Azma, Iran).

Kluver-Barrera staining: The sections were rehydrated through a series of ethanol solutions and placed into distilled water for 5 min. Next, the sections were incubated in 0.1 % luxol fast blue (stains myelin) overnight at 60 °C. After that, an adequate contrast was prepared by transient immersion of sections in 0.05 % lithium carbonate solution and differentiation continued in 70 % alcohol. After rinsing with distilled water, the sections were counterstained with 0.1 % cresyl fast violet (to stain cell bodies) (Merck, Germany) working solution for 4 min. The sections were rinsed with distilled water again and dehydrated in ascending series of alcohols, then cleared in xylene for 20 min and cover-slipped

with mounting medium. myelinated fibers were stained blue, neuropils pink and nerve cells purple (Geisler *et al.*, 2002).

Anatomical definitions: The boundaries of the hippocampal structure including the dentate gyrus (DG) and the Cornu Ammonis (CA) regions were discreetly defined at all levels of sectioning according to the stereotaxic coordinates of the Paxinos and Franklin's Mice Brain Atlas. Various subdivisions of the hippocampal formation were outlined according to cell morphology (Fig. 2.A).

To clarify these regions for all mice in this study, the boundaries of the CA1 subdivision was defined by the clear morphological indication of conspicuous small and densely-organized pyramidal neurons. The CA2 was included in the CA3 region because of its difficulty in discriminating its borders from CA3. The boundaries of the CA3 subdivisions were characterized by larger and less-packed pyramidal neurons compared to the CA1 and the DG was delineated as the tightly-packed layer of small granule cells within the molecular layer.

Stereological equations: The optical design equipment consisted of a video-microscopy system made up of an Eclip-se microscope (E-200, Nikon, Japan) linked to a color video camera (CCD, Hyper HAD), a computer and a flat monitor (Platrun LG). To estimate each parameter, 9-11 sections per hippocampus were used and 10-14 microscopic fields. Microscopic fields were selected using systematic random sampling.

Volume and numbers of neurons of the total hippocampus and its subdivisions were estimated using the stereology software system (Stereolite) designed at the Histomorphometry and Stereology Research Centre (SUMS) in Iran.

The volume of the total hippocampus and CA1, CA3, and DG was estimated according to the principles of the Cavalieri-point counting method. Sections with a thickness of approximately 30 µm were selected and the interval between adjacent sections on each slide was 300 µm. The points on the image of each section were counted under 25x final magnification (Fig. 2B).

Finally, the volume (V_{ref}) of each structure was calculated based on the following equation:

V_{ref} = S P. a (P). d = Σ A. d
Where, *a* (*P*) is the area associated with each point.
Σ P is the number of points hitting in the section.
d is the distance between sections.
Σ A is the sum of the areas of the sections.

Using the Optical Disector Method, the numerical density and number of neurons (N) were manually counted in the sections containing the total hippocampus and its subdivisions. To estimate the numerical density and number of neurons, the sections with 30 mm thickness and an oil immersion objective lens (60x, numerical aperture: 1.4) were used. An unbiased counting frame with inclusion and exclusion borders was laid over the images of sections viewed on the monitor (Fig. 2.C,D).

For depth measurement (z-axis), a microcater (MT12, Heidenhain, Germany) was attached to the microscope stage. To have an unbiased counting, 5 mm was ignored in thick guard zone at the top and bottom of each section to avoid cutting artifacts occurred at the upper and lower surfaces of the tissue sections. By doing so, the height of the disector was defined as the section thickness (about 20 µm).

Hereafter, the total number of neurons (N) were calculated as the numerical density (NV: cells per mm^3) multiplied by the total reference volume (V_{ref}: mm^3).

$$N = N_V \times V_{ref}$$

The neuronal density was estimated as follows:

$$Nv = \frac{\sum Q^-}{V_{dis} \times \sum P} \times \frac{t}{BA} = \frac{\sum Q^-}{a(f) \times h \times \sum P} \times \frac{t}{BA}$$

where, $\sum Q^-$ is the number of the neuron nuclei counted within the disectors in the height of the disector (on the average, in 9 - 11 sections, 197 ± 63 granular cells and 383 ± 55 pyramidal cells were counted per hippocampus in each mouse).

$\sum p$ is the total number of counting frames in all fields.

a(f) is the sum of the frame area.
h is the height of the disector.
t is the mean thickness of the section measured using the microcator in each field (20 mm on average).
BA is the block advance (or mean section thickness = 30 µm) of the microtome.

Statistical analysis. The data was analyzed using the statistical software SPSS version 19.0.

For biochemical parameters, probe trial data and stereological estimates, we used parametric statistical analysis followed by One-way ANOVA test. The Kolmogorov-Smirnov test was used to determine the normality of the data analyzed by One-way ANOVA.
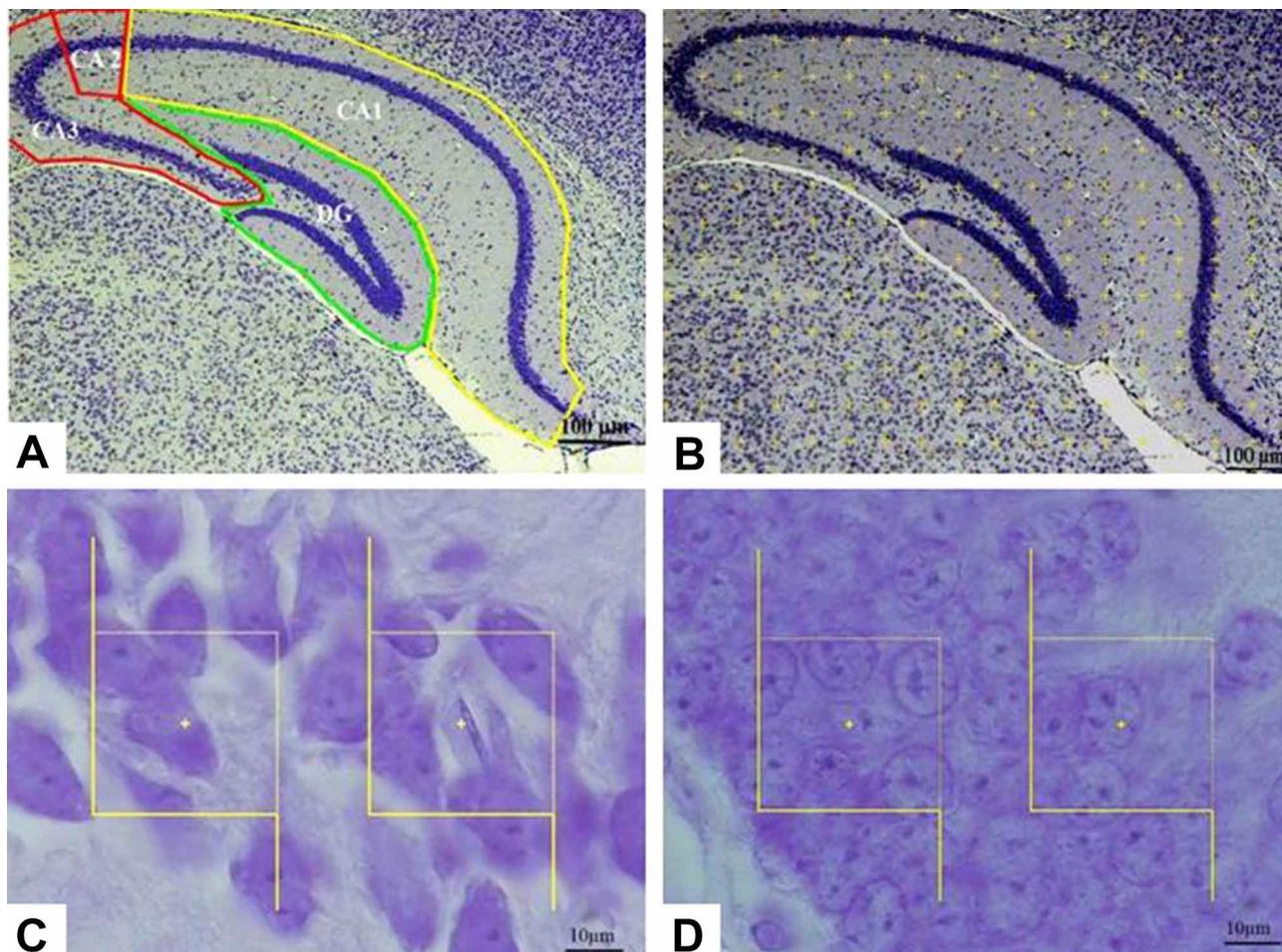
Fig. 2. A. The boundaries of the hippocampal structure including the dentate gyrus (DG) and the Cornu Ammonis (CA) regions. B. The Cavalieri principle is used to estimate the volume of the total hippocampus and its subdivisions. C, D. The number of neurons was counted in the total hippocampus and its subdivisions, using the Optical Dissector Method. Kluver-Barrera staining.

Additionally, repeated-measures ANOVA was used to compare mean body weight and behavioral parameters from the water maze experiments. Normality of variance and sphericity were evaluated using the Shapiro-Wilks and Mauchly's tests, respectively.

Differences between groups were evaluated by the post hoc LSD test. The values were presented as means ± SEM and P-value less than 0.05 was considered to be statistically significant.

RESULTS

Effect of IF on body weight: At the beginning of the experiment (first week), the body weights of the mice were almost similar in all groups (25.67±0.65 g) although, with increasing age mice in the N and EBN groups had significant increases in body weight within the fourth and eighth weeks and mice in IF (F and EBF) showed only a slight increase in body weight, which might be due to restriction of daily food intake.

The body weights of the mice were increased throughout the course of the experiment ($F_{[2, 32]} = 104.279$, $p \leq 0.001$). Besides, there was a significant day X group interaction ($F_{[6, 32]} = 20.621$, $p \leq 0.001$), indicating differences among groups within each week. There was a significant difference between groups ($F_{[1, 16]} = 24745.251$, $p \leq 0.001$) and post hoc analysis showed that the mice in the N group had significant increase in body weight as compared to the F and EBF groups ($p \leq 0.01$) and the mice in the EBN group had such an increase, as compared to the F group ($p \leq 0.04$) and EBF group ($p \leq 0.02$).

The body weights of the mice on the IF were ~13 % smaller than that of the mice on the normal diet at 8 weeks after the experiment (Table I).

Table I. The body weight (g) of the mice fed with ND and IF.

Groups	week1	week4	week8
N	25.82±0.58	28.12±1.02	30.36±1.34
F	25.64±0.98	26.18±1.14*	26.26±1.26*
EBN	25.66±0.53	27.82±0.63	29.56±0.67
EBF	25.54±0.63	25.98±0.6*	26.04±0.58*

Abbreviations: ND (Normal Diet); IF (Intermittent fasting diet); N (ND and normal saline injection), F (IF and normal saline injection), EBN (ND and ethidium bromide injection), EBF (IF and ethidium bromide injection). Data are shown as Mean ± SEM (standard error of mean); n = 10 in each group. * Significant difference with N group & EBN group (p < 0.05).

Effect of IF on biochemical parameters: After eight weeks, a significant decrease in the BS was observed in the F and EBF groups as compared to the N and EBN groups (P=0.002). Similarly, TG levels significantly decreased in F and EBF groups, as compared to the N and EBN groups (P=0.019). CL were lower in F and EBF group, as compared to the N and EBN groups (P=0.025). Moreover, LDL levels were significantly decreased in the F group in comparison to the N and EBN groups (P= 0.016) (Table II).

Effect of IF on learning and memory parameters: the mean escape latency time declined during the acquisition phase for all groups in this experiment; however, under the direct injection of EB in ICV, the mice (EBF & EBN groups) learned to locate the hidden platform with progressively longer latencies until the end of the study, while the mice administered with saline (N & F groups) were more efficient. Data were collected, after 8 weeks of being on the IF, during successive days (F (3, 48) =161.61, p≤0.001) with no day ¥ group interaction (F (9, 48) =0.621, p>0.77). A considerable main effect was evident in the variable group (F [1, 16] =9034.346, p≤0.001). Post hoc analysis showed that the F group showed significantly faster escape latencies than the EBN (p≤0.002) and the EBF (p≤0.039) groups (Fig. 3.A).

The distance traveled by mice to reach the platform (path length) decreased by each day (F [3, 48] =142.81, p≤0.001) and there were no significant differences in day x group interaction (F [9, 48] =0.63, p>0.76).

There was a significant difference between groups (F [1, 16] =19610.969, p≤0.001) and the post hoc analysis showed that the mice in the EBN group swam longer path lengths, compared to the F group (p≤0.028) (Fig. 3B).

Analysis of swim speed data in mice showed that there was a progressive increase in swim speed across days during the acquisition phase of testing for all (F[3,48]=42.771, p≤0.001). Although, there was no significant interaction between days × groups (F [9, 48] =1.204, p>0.31); There were remarkable differences between groups (F [1, 16] =19755.632, p≤0.001) and the post hoc analysis showed that mice in the N and F groups swam significantly faster than the EBN group (p≤0.024) and the EBF group swam markedly faster than those in the EBN group (p≤0.001) (Fig. 3C).

Probe trial results showed that the mice in F and EBF groups spent significantly more time in target quadrant, compared to N and EBN group (P= 0.00). The time group F spent in the target quadrant was ~13 % more than mice in N group and mice in EBF group were ~9 % more than that of the mice on EBN group at 8 weeks after the experiment (Fig. 3D).

Effect of IF on Stereological equations: Following direct injection of EB in ICV of EBF & EBN groups, a significant decrease in Mean±SEM number of neurons in the mice hippocampus and its sub-regions in comparison to N group and F group "P≤0.05". The number of neurons decreased in EBN group in comparison to the EBF group, was a significant loss of neuron numbers observed in the CA3 region (P=0.049). The CE for the number estimation was 0.05-0.08 (Fig. 4A).

The total volume of mice hippocampus and its subregions except CA3 region was significantly greater in F group compared to the N group (p≤ 0.05). Also, there was a significant decrease in the volume of mice hippocampus and its subregions in the EBF and EBN groups, compared to the F group (p≤0.05).

Table II. Blood factors (mg/dl) in the mice were fed with ND and IF after eight weeks.

Groups	Blood sugar	Triglyceride	Cholesterol	LDL
N	134±4.037	207±10.597	175±3.647	71.8±3.513
F	119±1.817*	175.8±7.235*	151.4±9.816*	60±2.51*
EBN	133.8±1.828	204.4±7.366	170.2±4.705	67.8±2.596
EBF	119.8±3.323*	177.4±6.185*	151.4±4.297*	63.8±3.569

Abbreviations: ND (Normal Diet); IF (Intermittent fasting diet); N (ND and normal saline injection), F (IF and normal saline injection), EBN (ND and ethidium bromide injection), EBF (IF and ethidium bromide injection). Data are shown as Mean ± SEM (standard error of mean); n = 10 in each group. * Significant difference with N group & EBN group (p < 0.05).
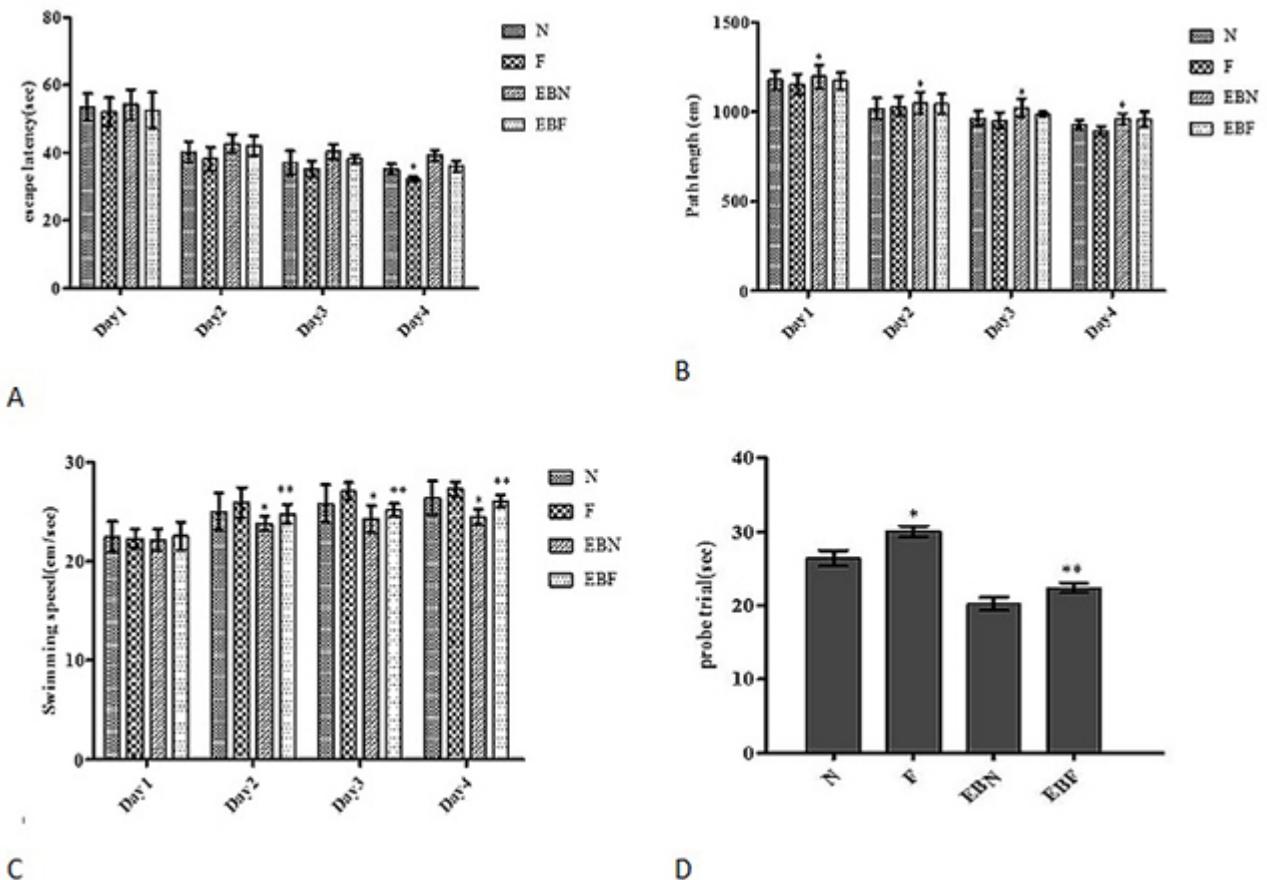
Fig. 3. A: Escape latency time (sec.) declined during the acquisition phase for all groups in this experiment; F group had significantly faster escape latencies than the EBN and the EBF groups. *Significant difference with EBF group & EBN group (p < 0.05).
B: Distance traveled (cm) by the mice to reach the platform (path length) decreased across days. The mice in the EBN group swam longer path lengths, as compared to the F group. * Significant difference with F group (p < 0.03).
C: Swim speed (cm/sec) in mice showed that there was a progressive increase in swim speed across days during the acquisition phase of testing in all groups. The mice in the N & F groups swam significantly faster than the EBN group and the mice in the EBF group swam significantly faster than the EBN group. * Significant difference with N group (p < 0.03). * * Significant difference with EBF group (p < 0.01).
D: Time (sec.) spent in the target quadrant showed that the mice in F and EBF groups spent significantly more time in target quadrant, compared to N and EBN group at 8weeks after the experiment. * Significant difference with N group (p < 0.03). ** Significant difference with EBN group (p < 0.03).
Abbreviations: N (Normal Diet and normal saline injection), F (Intermittent fasting diet and normal saline injection), EBN (Normal Diet and ethidium bromide injection), EBF (Intermittent fasting diet and ethidium bromide injection). Data are shown as Mean ± SEM (standard error of mean); n = 10 in each group.

In the CA3 region, a significant decrease of volume was observed in the EBF (p=0.046) and the EBN (p=0.013) groups compared to the N group. The CE for the volume estimation was 0.07-0.09 (Fig. 4.B).

DISCUSSION

Our results Showed maintenance of mice on the IF results in significantly decreased the body weight and biochemical parameters and improved learning and memory parameters of adult male mice. In addition, IF increased total number of neurons and volume of the hippocampus in F group, compared with N group. Though, IF in EBF group significantly improved spatial memory compared with the EBN group; however it did not show as excellently as F group.

Concerning some research on rodents, monkeys, and humans, it was detected that IF decreases the LDL levels and increases the HDL levels. Both the IF and CR regimens increase insulin sensitivity, followed by reduced plasma glucose and improved glucose tolerance. The IF reduces the
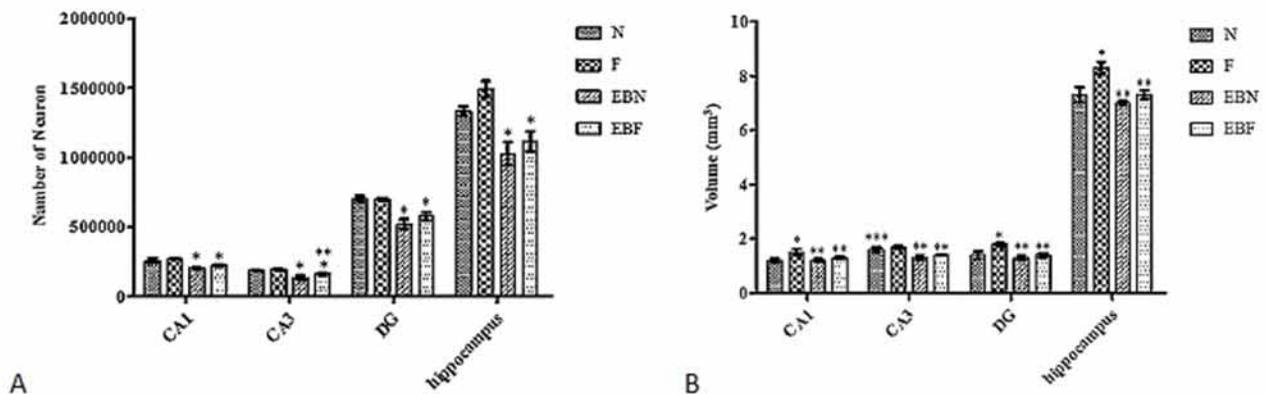
Fig. 4. A: Number of neurons in the mice hippocampus and its subregions was significantly decreased in EBF & EBN groups, in comparison to N group and F group. A significant loss of neuron numbers observed in the CA3 region of EBN group compared to the EBF group. * Significant difference with N group & F group ($p < 0.05$) ** Significant difference with EBF group ($p < 0.05$).
B: Total volume (mm^3) of mice hippocampus and its subregions except CA3 region was significantly greater in F group compared to the N group. Also, there was a significant decrease in the volume of mice hippocampus and its subregions in the EBF and EBN groups, compared to the F group. In the CA3 region, a significant decrease of volume was observed in the N group compared to the EBF and the EBN. * Significant difference with N group ($p < 0.05$) ** Significant difference with F group ($p < 0.05$). *** Significant difference with EBF group & EBN group ($p < 0.05$).
Abbreviations: N (Normal Diet and normal saline injection), F (Intermittent fasting diet and normal saline injection), EBN (Normal Diet and ethidium bromide injection), EBF (Intermittent fasting diet and ethidium bromide injection). CA (Cornu Ammonis), DG (dentate gyrus).

oxidative stress and increases cellular resistance to various type of stress. It also enhances the immune functions (Halagappa *et al*.; Tikoo *et al*.).

In agreement with previous study, we demonstrated a significant weight loss for both the F and EBF groups on the IF, in comparison with the N and EBN groups. The present survey revealed that the concentration of biochemical parameters (BS, LDL, CL, and TG) was significantly decreased in mice on the IF regimens.

In spite of a variety of mechanisms contributing to the demyelination and neurodegeneration in MS, the oxidative stress plays the most critical role (Gonsette). Also, it was demonstrated that intracerebral injection of EB (demyelinating agent) caused increased oxidative stress levels, including ROS, MDA and etc. in hippocampus, cortex and striatum (Ghaffari *et al*.). Hulst *et al*. (2015) found that there is a decrease in the hippocampal volume of MS patients. The animal studies suggest that the brain neurons of rats or mice on an IF were noticeably resistant to being obliterated by oxidative, metabolic and excitotoxic insults.

In agreement with previous studies, here the total number of neurons and volume decreased in EBN and EBF groups' hippocampus tissue following EB injection with a more decrement in EBN, compared with the F and N groups.

Moreover, our findings showed that the total volume of mice hippocampus was significantly greater in the F group,

compared to the N group and the number of neurons partially increased in the F group compared to the N group. In this regard, the variation of cell death and proliferation as well as neurogenesis after IF under basal conditions was researched by Manzanero *et al*. Their observations suggest that the extension of the meal intervals under intact conditions enhances basal cell proliferation and neurogenesis in the subventricular zone (SVZ) and hippocampus (Manzanero *et al*.).

Neuronal loss in the hippocampal region has been identified as learning and memory-associated neuropathological marker in living creatures (Mancini *et al*.). Several studies demonstrated that hippocampal impairment and decreased hippocampal volume are associated with cognitive decline such as memory dysfunction and spatial learning impairment in MS patients (Ghezzi *et al*.; Mancini *et al*.). In general, our finding revealed that the local injection of EB into ICV debilitates spatial learning and memory in the EBN and EBF groups.

Our findings provide evidence that IF would improve learning and memory deficits in mouse model of demyelination.

Studies showed that levels of the BDNF in hippocampus region increase when mice are maintained on the IF (Manzanero *et al*.). BDNF plays an important role in hippocampal synaptic plasticity and protection of neurons from damage when exposed to the neurotoxins such as EB,

which was associated with a striking preservation of learning and memory in a water maze task (Ghaffari *et al*.). Thus, IF could have a significant benefit for dysfunction and degeneration in experimental models of neurodegenerative disorders such as Parkinson's, Huntington's and Alzheimer's diseases as well as in stroke (Martin *et al*.; Halagappa *et al*.; Manzanero *et al*.) and according to the present study, in EB-induced animal model of demyelination.

CONCLUSION

Maintenance of mice on the IF results in significantly decreased the body weight and biochemical parameters, increased total number of neurons and volume of the hippocampus, and improved learning and memory parameters of adult male mice. However, IF in EBF group did not show as excellently as F group. Our finding revealed that EBF group just displayed significantly spatial memory improvement than that in EBN group. IF fairly improved some adverse effects of EB such as learning impairment at 8 weeks after the experiment. It seems that IF can alleviate the cognitive deficits and lead to learning and memory maintenance in experimental demyelination models. Therefore as a conclusion, IF has the potential to be effective at reducing MS-related symptoms.

HASSANPOUR, A.; HASSANPOUR, A.; REZVANI, M. E.; HOSSEINI, M. S. & BASIRI, M. Efecto de la dieta de ayuno intermitente en el hipocampo de ratón macho adulto después de inducir la desmielinización por inyección de bromuro de etidio. *Int. J. Morphol., 37(3)*:805-814, 2019.

RESUMEN: La dieta de ayuno intermitente (AI) como régimen restrictivo, previene la degeneración neural y la estimación de la presencia de diversos factores neurotrópicos en el hipocampo de modelos animales. Este estudio evalúa el efecto potencial de la AI en la prevención del aprendizaje y la disfunción de la memoria y mejora las alteraciones en el número y el volumen de las neuronas en un modelo de desmielinización, en ratón, inducido con bromuro de etidio (BE). Los ratones se asignaron al azar en el grupo N (dieta normal e inyección salina normal), Grupo A (AI e inyección salina normal), Grupo BEN (dieta normal e inyección BE), Grupo EBF (inyección AI y BE). La prueba de la plataforma oculta se llevó a cabo en función de la longitud del trayecto, la latencia de escape y la velocidad de nado de los ratones. Los estudios estereológicos fueron determinados por la técnica de Cavalieri y la técnica del disector óptico. En el grupo AI disminuyeron significativamente el peso corporal de los ratones, los parámetros bioquímicos, el número total de neuronas y el volumen del hipocampo, y los parámetros de aprendizaje y la memoria de los ratones machos adultos. Sin embargo, el grupo AI en BEF no se mostró tan bien como el grupo A. El grupo EBF mostró una mejora en la memoria espacial significativamente mayor que la del grupo BEN. No hubo diferencias estadísticamente significativas entre los grupos A, BE y BEN en los parámetros estereológicos y de aprendizaje, aunque el grupo EBF mostró latencias de escape más rápidas, y nado en las rutas más rápidas y más cortas que el grupo BEN en estos parámetros. Por lo tanto, como conclusión, el grupo AI mejoró bastante algunos efectos adversos de la BE en los modelos de desmielinización experimental.

PALABRAS CLAVE: Dieta de ayuno intermitente; Hipocampo; Desmielinización; Estereología; Ratones.

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