

Efficacy of Different Intensities of Aquatic Exercises in Remyelination and Neuronal Plasticity Using Cuprizone Model in Male Wistar Rats

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Abstract

Background: Multiple sclerosis is a chronic demyelinating disease of the central nervous system. Exercises as a remedy to this disorder are not widely understood. This study focuses on the effects of exercise and the addition of a preconditioning exercise program (Cn) on demyelination.

Aim and Objectives: To study and determine the neuroprotective and remyelination effects of a preconditioning exercise program and different intensities of aquatic exercises on demyelination induced by oral administration of cuprizone (Cup).

Materials and methods: Six groups of animals, each containing six rats were taken. Group I, II, and III were control, negative control (Cup), and treated with Methylprednisolone (MP) respectively. Group IV and V were treated with low (LIE) and high-intensity exercises (HIE) respectively. Group VI was treated with preconditioning exercise (Cn)+HIE. LIE included free swimming for 40 min and high-intensity exercise (HIE) included an addition of a resistance of 9% body weight and same as LIE for 5 weeks. Preconditioning (Cn) was achieved by free swimming for 40 min for 3 weeks before cuprizone administration.

Results: Cn+HIE showed a positive outcome similar to MP group with p-value <0.001 taken as statistical significance. This group showed improved areas of remyelination in histopathology and improved myelin basic protein (MBP) and reduced glial fibrillary acidic protein (GFAP) expression in corpus callosum and improved gene expression of brain-derived neurotrophic factor (BDNF) in the hippocampus region. All data were analyzed by one-way ANOVA using Sigma plot 13.

Conclusion: Therefore, high-intensity exercises with general fitness show positive effects in demyelination.

Introduction

The prevalence of multiple sclerosis is found to be increasing globally because of increased access to MRI and early diagnosis. It essentially is an inflammatory disease of the central nervous system. The precise cause is still debatable and owed to autoimmune pathology. Genetic factors have also been linked to the study (Hawkes et al. 2009; Willer et al. 2003). The treatment of Multiple sclerosis involves the use of steroids and interferon therapy. Multiple sclerosis leads to widespread demyelination because of the selective involvement of oligodendrocytes and axonal loss. Cuprizone (bis-cyclohexanone oxaldihydrazone) as a method for induction of demyelination has been successfully used (Carlton. 1967). Cuprizone, a primary copper chelator, causes selective apoptosis of oligodendrocytes and induces central nervous system demyelination in rats. A 3-week exposure causes cerebral cortical demyelination and white matter damage in mice (Skrupuletz et al. 2010). Cup was the chosen mode here, as cessation of administration of Cup leads to spontaneous remyelination, which mimics relapsing-remitting stage of MS (RRMS) (Morell P et al. 1998)

Other methods for induction of demyelination include experimental autoimmune encephalomyelitis (EAE) induced by antibodies to Myelin oligodendrocyte glycoprotein (MOG), where spontaneous remyelination cannot be studied (Shindler et al. 2008) and viral model using Theiler's virus, where the route of administration is technically complex involving intracerebral injection with an added disadvantage of difficulty in assessing the exact proportion of inflammation (Ulrich et al. 2006). Cup is one of the toxic models of demyelination, the other methods involve the use of lysolecithin, which is also given as an intracerebral injection, and leads to demyelination within 2 days of administration (Jeffery et al. 1995) as against 3 weeks for beginning and 5 weeks for completion of demyelination in Cup model (Skripuletz et al. 2010).

Methylprednisolone is the standard drug of choice for MS (Brusaferri and Candelise. 2000). MP is a chemical modification of naturally occurring glucocorticosteroid, hydroxycortisone. This drug decreases the inflammatory cycle by reducing the cytokine response, T cell inhibition, and facilitated apoptosis of immune cells that are activated.

Exercise as a disease-modifying agent and as a rehabilitation tool and one which can slow down the effects of the disease also plays a very important role. In particular, exercises have positive co-relations with neuronal plasticity and neuronal regeneration (Tong et al. 2001). According to Rossi et al. 2009, exercise was found to confer neuronal protection in experimental autoimmune encephalomyelitis. This forms the basis of the animal model of MS, with similar changes like axonal demyelination and degradation of MBP (Afzalpour et al. 2015). This study uses a Wistar rat animal model using cuprizone to induce demyelination which mimics the relapsing-remitting stage of MS and also studies the effects of exercise in oligodendrocyte degeneration in the corpus callosum and neuronal plasticity changes in the hippocampus region of the rat brain and compare the same with the neuroprotective effects conferred by methylprednisolone.

Materials And Methods

Animals:

Animals for the study were procured from Biogen Laboratory Animal Facility, Bangalore, India. The animals were maintained in an air-conditioned animal room with a 12 hour light and dark cycle and diet and water were provided ad libitum throughout the experimental period. The study was conducted between September to October 2020, after receiving proper Institutional Animal Ethical Clearance [SU/CLAR/RD/010/12/2020]. Male Wistar rats with weights ranging from 150 - 200 gm, were chosen for the study.

Chemicals:

0.2% Cup was procured from Sigma Aldrich Chemical Company, St.Louis, Missouri, USA, and hydroxypropyl cellulose(HPC) was procured from Sisco Research Laboratories, Mumbai, India. The

catalogue numbers were 69586[10099-74-8], 607995[7446-14-2], and 68015[10099-74-8] for cuprizone, MP and HPC respectively.

Experimental procedure and design:

After acclimatization for a period of 1 week, the experiment was carried out for 8 weeks for groups I to V and 11 weeks for group VI, animals were divided into six groups with six rats in each group. Group I (Control) rats were administered 1.5 mL of 1% hydroxypropyl cellulose (HPC)/kg b.w, p.o 35 days. Group II (Cup) rats were administered Cup 450 mg /kg b.w dissolved in 1.5 mL of 1% HPC, p.o for 5 weeks (Basoglu et al. 2013). Group III (Cup+MP group) rats were administered Cup 450 mg/kg b.w, p.o for 5 weeks and from 3rd week, in addition to Cup administration, MP, 20 mg/kg b.w, i.p (intraperitoneal) was included for 3 weeks. Group IV (Cup+LIE) rats were administered Cup 450 mg/kg b.w, p.o for 5 weeks, and from the 3rd week of Cup administration, free swimming with no resistance for 40 min for 5 weeks was included. Group V (Cup + HIE group) rats were administered Cup 450 mg/kg b.w, p.o for 5 weeks, and from the 3rd week of Cup administration, swimming with an added resistance of 9% body weight for 5 weeks was included. Group VI (Cn+Cup+HIE) rats were started with free swimming 40min a day, for 3 weeks. After 3 weeks, the rats were administered Cup 450 mg/kg b.w, p.o for 5 weeks and from the 3rd week of Cup administration, an exercise program same as group V was included. The Cup solution was prepared according to dosage for each day and given through oral gavage.

Exercise regimen

The rats in group VI were preconditioned with free swimming, in a circular tank with a depth of more than 50cm with a water temperature of 30±5°C, for 40 min, 5 times a week, before administration of Cup. After 3 weeks of Cup administration, rats in the Cup+LIE group were made to swim with no additional resistance for 40 min, 5 days a week, for 5 weeks (Klaren et al. 2014). For Cup+ HIE and Cn+Cup+HIE groups a resistance of 9% of body weight, in the form of a metal ring was tied around the tail of the rat and made to swim for 40 minutes, 5 days a week for 5 weeks (Almeida et al. 2009) In case of slippage of the weight from the tail, it was again reinforced and time monitored. The intensity grading based on the load was adapted from a study done by Gobatto et al. 2001

Induction of demyelination:

Cuprizone, a primary copper chelator, induces selective oligodendrocyte apoptosis by 3 weeks of administration which is followed by activation of innate neuroglial and immune cells in the brain, by astrocytes and microglial proliferation, whereby it finally leads to demyelination of distinct white and grey matter areas (Kipp et al. 2009). There is very minimal involvement of the blood-brain barrier and cells of the immune system is believed to play a role in demyelination induced by Cup (Wolf et al. 2018).

Demyelination induction was achieved by administration of 450 mg /kg b.w Cuprizone dissolved in 1.5mL of 1% HPC (Abe et al. 2015). The control group received 1.5mL of 1% of HPC.

Methylprednisolone (MP):

After 3 weeks of Cup administration, 20 mg/kg b.w of MP was administered through intraperitoneal route for 3 weeks (Cammer et al. 1999)

Tissue preparation:

After the assigned experimental period, the rats were euthanized by an overdose of 1% isoflurane. After which, the rats were perfused intracardially with 50mM phosphate-buffered saline. Brain tissue was carefully dissected and washed in saline and transferred to formalin containers for histopathology and wrapped in aluminum foil and kept in refrigeration at - 80°C for immunohistochemistry, immunofluorescence, and Quantitative real-time - Polymerase chain reaction (qRT PCR)

Luxol fast blue (LFB) staining:

.The coronal sections of brain issue was stained with Luxol fast blue (LFB). Brain tissue slides were incubated in Luxol Fast Blue Solution (0.1%) for 24 h at room temperature. The sections were rinsed thoroughly in distilled water and then dipped in Lithium Carbonate Solution (0.05%) several times. Differentiation for the sections were then continued by repeatedly dipping in alcohol reagent (70%) until the gray matter became colorless and the white matter remained blue. Then, the sections were rinsed in distilled water, followed by incubation with Cresyl Echt Violet (0.1%) for 2–5 min. The sections were rinsed quickly in distilled water, dehydrated quickly in three changes of absolute alcohol, cleared in three changes of xylene, and mounted with mounting medium. The slides were viewed under the Olympus binocular Bright field Microscope at 60x magnification.

MBP immunohistochemistry:

The tissues were immersed in ice-cold PBS followed by freshly prepared filtered 4% Paraformaldehyde (PFA) in PBS. After embedding, the tissues were cut using the Leica cryostat CM1850 and stored in cryoprotective solution (25% glycerol, 25% ethylene glycol in PBS) at -20°C. The sections were incubated with polyclonal rabbit anti-MBP (1:1,000, Abcam, Cambridge) overnight at 25°C, then treated with streptavidin-peroxidase complex (1:200). Sections were visualized by the reaction with 3,3-diaminobenzidine tetrachloride (Sigma Aldrich) in 0.1 M Tris-HCl buffer (pH 7.2). The sections were then dehydrated, mounted on a slide, and visualized under a bright field microscope (Labomed).

GFAP immunofluorescence:

Chronic demyelination usually leads to astrocyte proliferation. The corpus callosum frozen brain sections (10 µ m) were dried for 2 h at room temperature and then fixed with 4% PFA in PBS for 20 min. After blocking non-specific antibody binding with 5% non-fat dried milk for 20 min, sections were incubated overnight with rabbit anti-GFAP (1:4000, Enzo Life Sci) diluted in blocking buffer at 4 °C. Subsequently, sections were washed in TBS and incubated in a dark, humid chamber with the appropriate fluorescently labelled IgG's-Alexa Fluor 594 (1:400) combined with donkey anti-rabbit IgG Alexa Fluor 488 (1:400)

diluted in TBS-T for 2 h at room temperature. After several washes in TBS, sections were mounted with polyvinyl alcohol mounting medium. Immunofluorescence was examined using a Leica confocal laser scanning microscope (Leica Microsystems).

Gene expression level of BDNF in the hippocampus by qRT-PCR:

The total RNA was prepared from the brain hippocampus tissue using a TRIzol reagent purchased from and RNeasy® Mini kits (Qiagen, Germany). QPCR Master Mix Kit were purchased from Invitrogen, Biomedica. Complementary DNA was first synthesized from total RNA using reverse transcriptase. PCR was performed using a (Applied Biosystems, USA). The operating conditions were as follows: for glyceraldehydes-3-phosphate dehydrogenase (GAPDH), 30 cycles of denaturation at 95°C for 30sec, annealing at 58°C for 30sec, and extension at 72°C for 30 sec; for BDNF, 27 cycles of denaturation at 95°C for 30sec, annealing at 57°C for 30sec, and extension at 72°C for 30 sec. The PCR products were separated on 1.2% agarose gels and stained with ethidium bromide. The density of each band was quantified using an image-analyzing system. The expression levels were compared with each other by calculating the relative density of the target band, such as BDNF, to that of GAPDH.

The primer sequence was as in Table 1.

Table 1: showing the primer sequence for qRT-PCR BDNF in the hippocampus region

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this article

DATA ANALYSIS

All the data were represented by mean \pm SD. Comparison between groups was made using one-way ANOVA with Bonferonni's t-test with Sigma plot 13 software. P-value <0.001 was taken as statistically significant. Immunostained slides were quantified using Image J software. The expression of the BDNF gene was measured using GAPDH as an internal control.

Results

Exercise improved remyelination as seen in LFB staining of corpus callosum:

LFB staining was conducted to assess the myelin content in the corpus callosum. Widespread demyelination with increased degradation and vacuolation was evident in the Cup group (group II) against normal myelination seen in the control group. Further, it was seen that in Cn + Cup + HIE group, showed better remyelination as shown by blue staining taken by myelinated areas in the images very much similar to the control and Cup + MP group (group III) with minimal areas of remyelination seen in Cup + LIE and Cup + HIE groups.

(Fig. 1) - Effect of exercise in Cup induced changes on LFB staining in the corpus callosum region.

Exercise improves MBP expression in the corpus callosum:

MBP is the initial and reliable marker for remyelination. Immunopositivity for the same provides accurate results on the study design and experimental groups. Here, it was seen that the immunopositivity to MBP in the corpus callosum region of the rat brain was drastically reduced in the Cup group and substantially more in the Cup + MP group and Cn + Cup + HIE group. Cup + LIE and Cup + HIE showed similar minimal expression. Quantitative analysis of MBP positive cells showed a considerable decrease in the Cup group (8.267 ± 0.351) compared to the control group (30.033 ± 0.569) with a p-value < 0.001 . MBP positive cells improved in the MP group (22.967 ± 0.473) and the Cn + Cup + HIE group (21.733 ± 0.777) which showed a p-value of 0.128, with no significant changes between the experimental, Cn + HIE, and standard drug groups, showing MP and Cn + HIE produced similar effects. The other groups showed moderate improvements, Cup + LIE (13.133 ± 0.306) and Cup + HIE (17.467 ± 0.153), which showed statistical significance (p-value < 0.001) with control, Cup and standard drug group.

(Fig. 2) - Effect of exercise in Cup induced changes on MBP immunohistochemistry and quantitative analysis of expression of MBP positive cells in corpus callosum region, measured through image J software in scale bar $800\mu\text{m}$ and 100 X magnification.

Exercise shows reduced GFAP expression in immunofluorescence:

Active astrocyte proliferation is seen in chronic changes of demyelination. As seen in Fig. 4, high expression was seen in the Cup group, Cn + Cup + HIE groups showed markedly reduced expression of GFAP, where Cup + MP group shows a reduced expression of GFAP in the corpus callosum region. Cup + HIE and Cup + LIE groups showed moderate expression. Quantitative analysis of GFAP positive cells shows a definitive increase in the Cup group (112.867 ± 0.551) compared to the control group (30.550 ± 1.054), which showed statistical significance with a p-value < 0.001 . MP group showed a declined trend in expression (61.233 ± 0.643) and the Cn + Cup + HIE group showed a better response (50.367 ± 0.513) (p-value < 0.001). Cn + Cup + HIE group showed a 17.74% decrease compared to the Cup + MP group in GFAP expression, where the experimental group fared better than the standard drug group. Further, Cup + LIE (80.900 ± 1.127) and Cup + HIE (77.000 ± 0.557) showed moderate response with statistical significance (p-value < 0.001) in comparison to the control, Cup and, standard drug group.

(Fig. 3): Effect of exercise in Cup induced changes on GFAP immunofluorescence and quantitative analysis of expression of GFAP positive cells in the corpus callosum region, measured through image J software in scale bar in scale bar $100\mu\text{m}$.

Gene expression levels of BDNF in hippocampus region by qRT-PCR:

BDNF is an important marker for neuroplasticity, which was found to be least expressed in the Cup group. BDNF expression was found to be most in the Cn + Cup + HIE group compared to Cup + MP and control groups. The second least expression was found in the Cup + LIE group and comparatively moderate in the Cup + HIE group as seen in the expression of the BDNF line in Fig. 4. Quantitative analysis revealed very

much reduced expression in the Cup group (0.653 ± 0.0651) compared to the control group (2.693 ± 0.179) (p -value < 0.001). The expression was seen most in the Cn + Cup + HIE group (3.470 ± 0.0436) followed by the Cup + MP group (3.123 ± 0.0961) (p -value < 0.001). The Cn + Cup + HIE group showed a 28.85% increase compared to the control group, whereas Cup + MP showed a 15.96% increase compared to control, compared to the Cup + MP group, Cn + Cup + HIE group showed an 11.11% increase in BDNF expression, where the experimental group, Cn + Cup + HIE exceeded the standard drug group. Cup + LIE and Cup + HIE groups did not show much improvement with values of Cup + LIE group being (0.973 ± 0.121), with statistical significance in comparison to control and MP groups (p -value < 0.001), but when compared to Cup group, the p -value was 0.053, with no statistical difference and Cup + HIE group being (1.823 ± 0.0929), which showed statistical significance with control, Cup and MP groups.

(Fig. 4) Effect of exercise in Cup induced changes on the gene expression of BDNF and quantitative analysis of BDNF expression in the hippocampus region.

Discussion

The present study showed better remyelination response in the Cn + Cup + HIE group which was similar to the response produced by the standard drug, MP group. In gene expression of BDNF in the hippocampal region, Cn + Cup + HIE group showed better response than the standard drug, MP group. The other exercise groups, showed little response in remyelination and BDNF expression, which is a marker for neuronal plasticity.

For the experimental study of MS pathology, a toxic model using cuprizone can work as an appropriate model to study the remyelination process (Kalman B et al. 2007). The corpus callosum is the primary area showing white matter degeneration in this model, other brain regions like basal ganglia and hippocampus are also affected (Silvestroff L et al. 2010).

Benefits of swimming regime:

Aquatic exercises are particularly beneficial to MS patients, due to their three vital effects, which are buoyancy, whereby the load and stress on the joints are reduced, viscosity, which leads to decreased drag and multiplanar movements and the most important factor of MS is thermodynamics. As per the Uhthoff phenomenon, symptoms of MS worsen in case of the increased setting of body temperature (Frohman et al. 2013). Swimming exercise does not increase the same and maintains an optimum body temperature (Davis et al. 2010). LFB staining, one of the standard methods to visualize white matter, showed improved effects as was evidenced in increased myelination in the exercise groups, which was in accordance with a study done by Kim et al. 2020, where the demyelination in the spinal cord was studied.

Myelin Basic Protein (MBP), is important for maintaining the structural stability of myelin and is a very essential component for efficient nerve conduction (Shanshiashvili et al. 2012). Prior regular exercise has been shown to have a positive impact in reducing demyelination and axonal damage in the spinal cord of EAE animals, as well as dendritic damage in striatal neurons (Sandroff et al. 2016). A decrease in MBP is

reflective of the demyelinating status of multiple sclerosis (Mastronardi et al. 2005). The present study showed a decrease in MBP levels in the Cup group and improved MBP positivity in the Cup + MP group followed by the Cn + Cup + HIE group. Cup + HIE and Cup + LIE showed negligible improvement. Similar results in MBP expression were also seen spinal cord, where the effects of free-swimming were analyzed (Kim et al, 2020). This study was the first to throw light on the neuroprotective effects of the preconditioning exercise program along with the HIE regimen. Astrocytes are found to be promoters of a demyelinating lesion (Frohman et al. 2006), they do the same by secretion of chemokines that recruit microglial inflammatory cells, which restrict the process of remyelination. Astrogliosis is a feature seen in MS (Correale et al. 2015). GFAP, a marker for astrogliosis is found to be elevated in chronic stages of demyelination (Yamamoto et al. 2014). Better results with reduced astrocyte proliferation were seen in the Cn + Cup + HIE group. Improved remyelination was also seen by Madadi et al, where selective astrocyte ablation was tried with chronic cuprizone administration.

Immunomodulatory effects of exercise, in the form of treadmill training against neural damage and demyelination, were analyzed and found to have positive improvements (Vaynman et al. 2005) One of the most reliable and strongest effects of exercise on the brain of treated rats is the up-regulation of BDNF. BDNF is an important marker for neuronal plasticity, which increases the number and synaptic uptake of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors in experimental hippocampal slices and hippocampal neuronal cultures (Caldeira et al. 2007)

According to Pencea et al. 2001, BDNF stimulates the recruitment of supraventricular zone cells and their migration and also facilitates the differentiation of neurons. The present study showed an increase in expression of BDNF in the hippocampus of conditioned rats with a high-intensity swimming protocol, which was in accordance with a study done by Kim et al. 2017. Motor activity in the form of aquatic exercise is found to have more neuroprotective and long term effects with regards to gene expression in the hippocampus of Cuprizone treated brain of Wistar rats (Li L and Tang. 2005; Magalon et al. 2007)

Here, increased effects of neuroprotection were more evident in the Cn + Cup + HIE group. Similar results were seen in this study where among the different experimental exercise groups, preconditioning group with high-intensity exercise regimen showed promising results in minimizing demyelination and improving plasticity as shown by increased BDNF expression in the hippocampus and thereby, can be correlated to delaying or preventing secondary impairment with MS, which also surpassed the effect of methylprednisolone as seen in Fig. 4.

Conclusion

In conclusion, a prior exercise conditioning program confers neuroprotective and remyelinating effects, when subjected to demyelinating induction, as in the present study, using cuprizone, in addition to leading to better fitness. HIE showed comparative significance, the least significant effects were seen in LIE, therefore, in MS scenarios, the patient can be challenged with an HIE protocol, and in the long run, an improved general fitness confers neuroprotection also in addition to the traditional cardioprotective

effects documented since long. The relapsing-remitting stage of MS is the initial stage seen, for which the treatment involves the use of steroids and maintenance exercises in low intensity only during relapses. Instead, a challenging high-intensity exercise program can be the norm, keeping the basal body temperature constant throughout the disease course irrespective of relapse or remission. Also, this study stresses the importance of an exercise program in general, even in an otherwise healthy population to bring out better results when a neural pathology may be detected.

Declarations

ETHICAL STATEMENT:

Ethics approval and consent to participate: The study was conducted between September to October 2020, after receiving proper Institutional Animal Ethical Clearance vide letter [SU/CLAR/RD/010/12/2020]

Consent for publication:

All the authors of this manuscript hereby provide consent for publication.

Availability of data and materials:

The authors confirm that the data supporting the findings of this study are available within the article.

Competing interests:

The authors hereby declare no competing interest.

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Tables

Table 1 - showing the primer sequence for qRT-PCR BDNF in the hippocampus region

Genes	Primer right	Primer left
BDNF (153bp)	5'-CAG GGG CAT AGA CAA AAG-3'	5'-CTT CCC CTT TTA ATG GTC-3'
GAPDH (409bp)	5'ATC CCATCA CCA TCT TCC AG-3'	5'-CCT GCTTCA CCA CCT TCT TG-3'

Figures

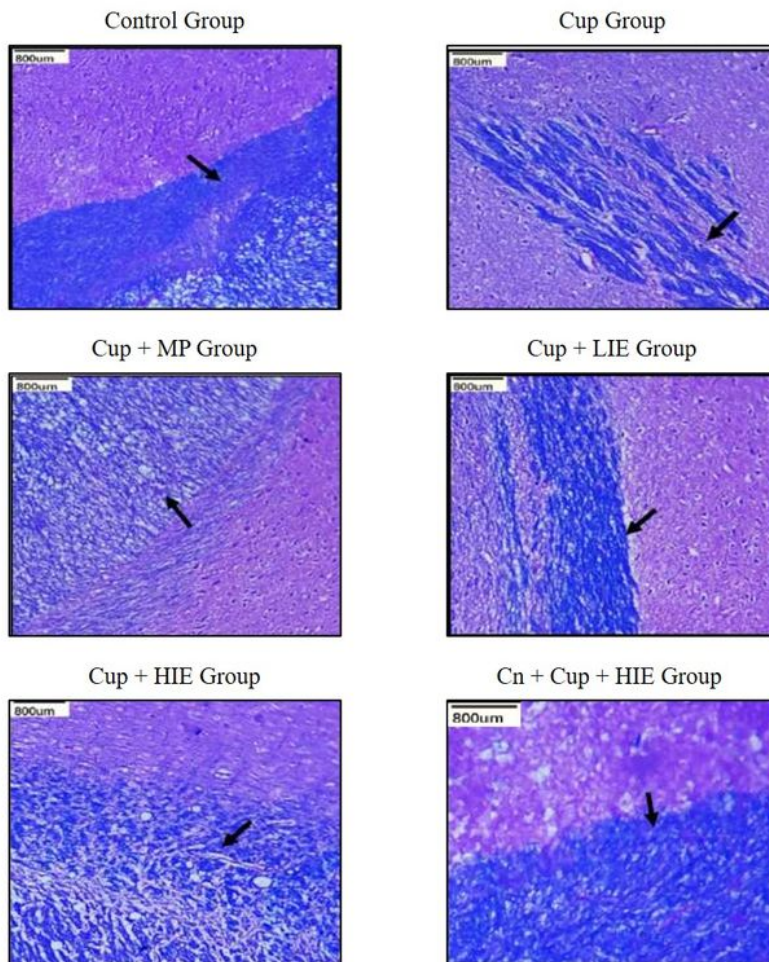


Figure 1

Effect of exercise in Cup induced changes on LFB staining in the corpus callosum region.

Control group shows normal myelination of the corpus callosum, Cup group shows more demyelination. Cup +MP group shows improved myelin formation. Cn+Cup+HIE group more remyelination similar to MP group. Cup+LIE and Cup+HIE groups show remyelination in a minimum area.

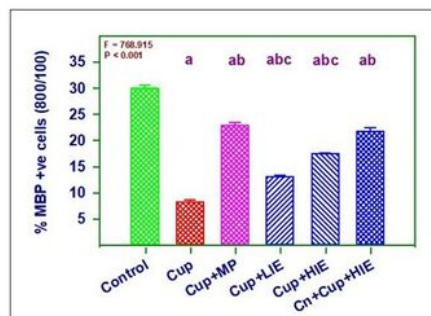
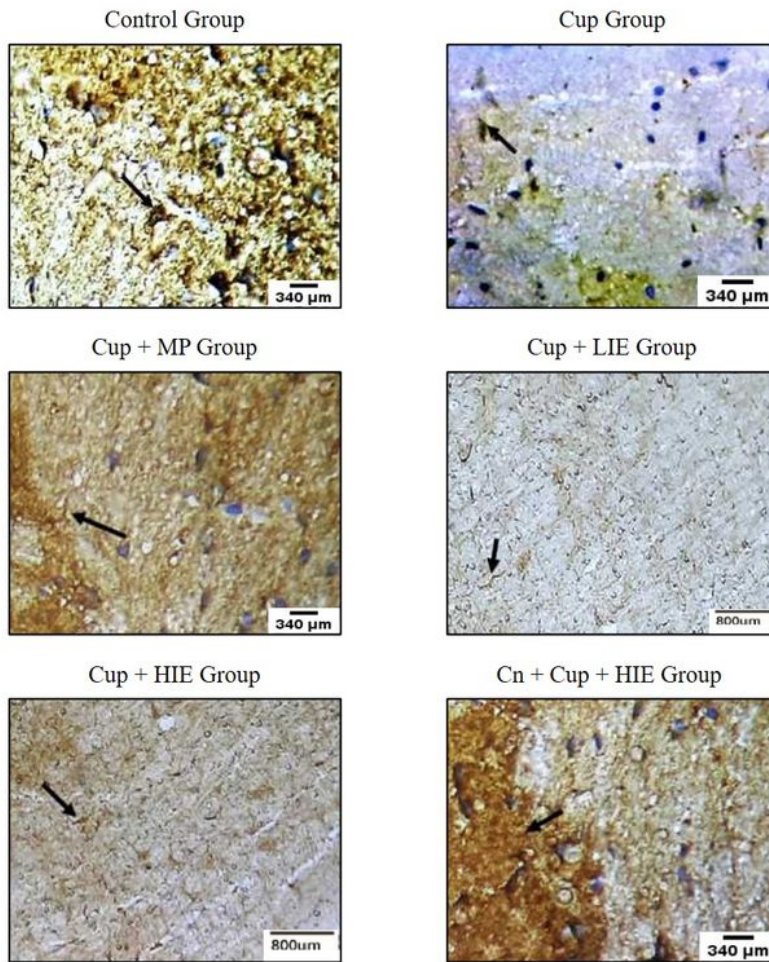


Figure 2

Effect of exercise in Cup induced changes on MBP immunohistochemistry and quantitative analysis of expression of MBP positive cells in corpus callosum region, measured through image J software in scale bar 800µm and 100 X magnification.

MBP expression in the corpus callosum of the rat brain was seen as shown by arrow mark. MBP immunopositive cells were less expressed in the Cup group, compared to the control which shows normal expression. Increased expression was seen in the Cu+MP group closely followed by the Cn+Cup+HIE group. The expression was found to be less in the Cup+HIE group followed Cup+LIE group.

The graph shows the effect of exercise in Cup induced changes on quantitative analysis of expression of MBP positive cells in corpus callosum region, measured through image J software in scale bar 800µm and 100 X magnification.

LIE = Low intensity exercise; HIE – High intensity exercise;

Cn = preconditioning; MP = methyl prednisolone.

Values are mean \pm SD (n = 6 each)

The 'F' and 'P' values are by one-way ANOVA with Bonferroni 't' test.

a Significantly different from the control group.

b Significantly different from the Cup group.

c Significantly different from the MP group.

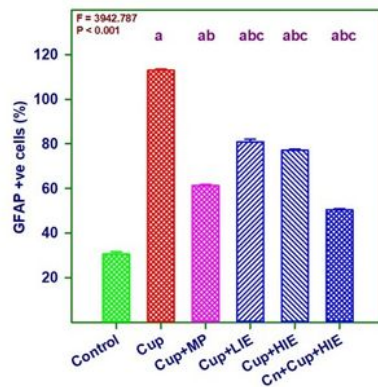
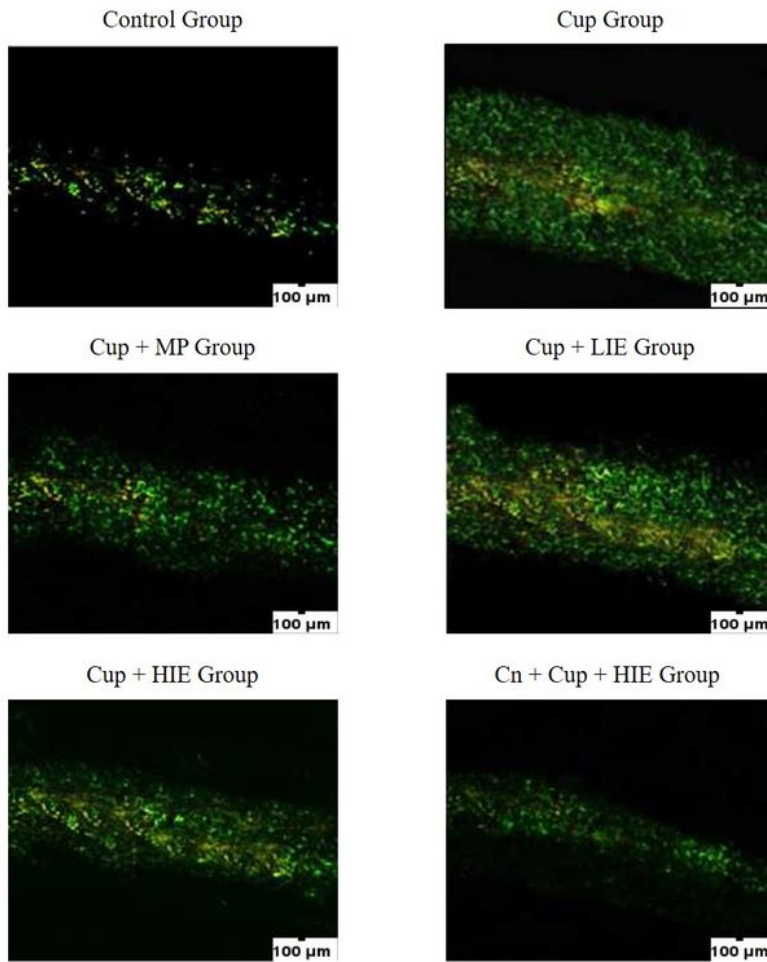


Figure 3

Effect of exercise in Cup induced changes on GFAP immunofluorescence and quantitative analysis of expression of GFAP positive cells in the corpus callosum region, measured through image J software in scale bar in scale bar 100μm.

GFAP expression in the corpus callosum region of the rat brain (scale bar 100µm) was assessed. Immunopositivity to GFAP was more expressed in the Cup group compared to the control. Less expression was seen in the Cup+MP group and the Cn+Cup+HIE group showed very much declined expression. Cup+HIE and Cup+LIE showed moderate expression

The graph shows the effect of exercise in Cup induced changes on quantitative analysis of expression of GFAP positive cells in the corpus callosum region, measured through image J software in scale bar in scale bar 100µm.

LIE = Low intensity exercise; HIE – High intensity exercise;

Cn = preconditioning; MP = methyl prednisolone.

Values are mean \pm SD (n = 6 each)

The 'F' and 'P' values are by one-way ANOVA with Bonferroni 't' test.

a Significantly different from the control group.

b Significantly different from the Cup group.

c Significantly different from the MP group.

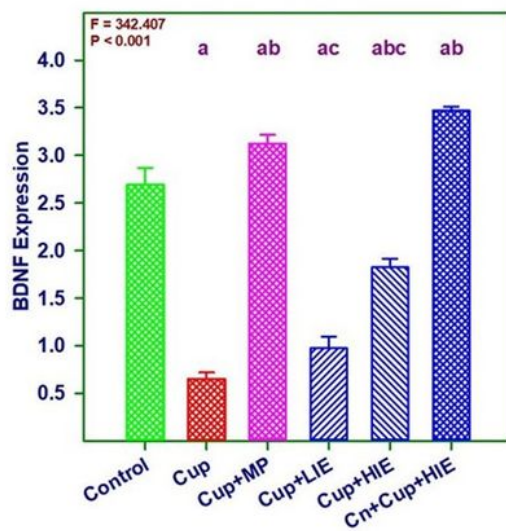
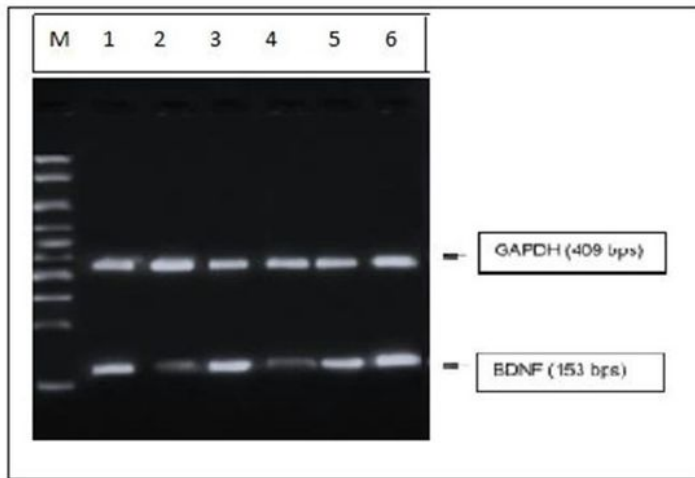


Figure 4

Effect of exercise in Cup induced changes on the gene expression of BDNF and quantitative analysis of BDNF expression in the hippocampus region.

Lane 1 - Control

Lane 2 - Cup group

Lane 3 - Cup+MP group

Lane 4 - Cup+LIE group

Lane 5 - Cup+HIE group

Lane 6 - Cn+Cup+HIE .

BDNF expression was found to be least in the Cup group, followed by the Cup+LIE group. Most expression of BDNF was found in the Cn+Cup+HIE group, followed by the Cup+MP group and Cup+HIE groups. The BDNF expression in the Cn+Cup+HIE group was found to be more than the Control group.

The graph shows the effect of exercise in Cup induced changes on quantitative analysis of gene expression of BDNF in the hippocampus region.

LIE = Low intensity exercise; HIE – High intensity exercise;

Cn = preconditioning; MP = methyl prednisolone.

Values are mean \pm SD (n = 6 each)

The 'F' and 'P' values are by one-way ANOVA with Bonferroni 't' test.

a Significantly different from the control group.

b Significantly different from the Cup group.

c Significantly different from the MP group.