IJPSR (2022), Volume 13, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 February 2022; received in revised form, 25 March 2022; accepted, 24 April 2022; published 01 October 2022

NEUROTHERAPEUTIC EFFECT OF BERGENIN ON CUPRIZONE-INDUCED DEMYELINATION BY REGULATING NEUROLOGICAL FUNCTIONS ASSOCIATED WITH MOTOR ACTIVITY, OXIDATIVE STRESS, AND HISTOLOGICAL ALTERATIONS IN THE CORPUS CALLOSUM OF C57BL/6 MICE

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Keywords:

Bergenin, Cuprizone, Oxidative stress, Neuroinflammation

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ABSTRACT: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by Neuroinflammation, oligodendrocyte loss, and axonal pathology. Bergenin, a chief phytochemical constituent of *Bergenia* species, has been shown to exert anti-inflammatory and antioxidant effects. The cuprizone (CPZ) model is an established mouse model of MS and causes demyelination and induces motor dysfunction, cognitive impairment, and Neuroinflammation leading to damage in the myelinated neuronal region of the brain. Our study examined the potential neuroprotective effects of Bergenin in cuprizone-intoxicated C57BL/6 mice. Mice were fed with chow containing 0.2 % cuprizone for 42 days, followed by Bergenin treatment (25 mg/kg and 50 mg/kg/day, i.p) given for 42 days. At the end of the experimental period, animals were tested on behavioural activities to evaluate changes in balance and motor coordination. Mice were then sacrificed to estimate the biochemical status, and histology of brain sections was used to assess the demyelination rate and myelin inflammatory status. The aim of this study was to examine the neuroprotective effects of Bergenin on CPZ-induced alterations in the behavioural, biochemical, oxidative stress markers, and histological alterations in the corpus callosum of C57BL/6 mice. Our results suggest that Bergenincan be a useful therapeutic agent in demyelinating diseases to suppress Neuroinflammation and oxidative stress.

INTRODUCTION: Multiple sclerosis (MS) is an immunemediated inflammatory chronic neuro-degenerative disease that is mainly characterized by elevated astrogliosis, microglial activation and axonal loss [1]. MS is assessed to influence more than 2,000,000 individuals around the world, bringing about a high disability rate, particularly in young adults [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(10).3944-52

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(10).3944-52

This inflammation-mediated demyelinating disease creates a severe myelin sheath demolition in the white matter brought about by macrophages, central T-lymphocytic invasion and loss of oligodendrocytes (OLs) cells [3], which can prompt the activation of microgliosis and astrogliosis in the brain.

These lesions cause dysfunctional neurotransmission between neurons and result in neuronal dysfunctions, including visual defect, sensorimotor and autonomic inadequacies, weariness, ataxia, thinking issues and emotional concerns. Moreover, Neuroinflammation also leads to severe oxidative stress-mediated mitochondrial dysfunction, which causes damage to neurons and

glia by increasing the formation of oxygen-containing reactive species (ROS) [4, 5]. Inflammatory processes of multiple sclerosis are characterized by the development of microglia activation and mitochondrial dysfunction. Proteolytic enzymes, oxidative stress, and free radicals are released when microglia and astrocytes are activated. Primary apoptosis of neuronal cells linked with ionic channel malfunction, calcium overload, proteolytic enzyme synthesis and activation of apoptotic pathways play a role in the development of neurodegeneration in MS. It also disrupts the adenosine triphosphate transit along the axon resulting in neurodegeneration. The course of the disease is pretentious, using antioxidants and substances that influence antioxidant pathways, which reduce the severity and cause faster remission and a less pronounced course of Neuroinflammation and neurodegeneration [6, 7].

Cuprizone (CPZ) is a copper chelating agent used to incite a non-immune system model of Multiple sclerosis to examine the mechanism of demyelination. Cuprizone mainly causes demyelination in the specific region of the brain called corpus callosum (CC) [8]. The significant instrument associated with Cuprizone inebriation is the restraint of copper-subordinate mitochondrial catalysts, which prompts the weakening of oligodendrocyte cells and oligodendrocyte precursor cells (OPC) [9], which plays a significant job in the synthesis of myelin sheaths.

Copper is an essential catalytic cofactor for a group of metalloenzymes, including Cu/Zn-superoxide dismutase, cytochrome c oxidase, ceruloplasmin, or dopamine beta-monooxygenase in the brain. Thus, the copper deficiency results in dysfunction of oxidative phosphorylation and disturbs the energy metabolism of oligodendrocytes cells associated with neurons leading to apoptosis with subsequent demyelination.

Subsequently, CPZ exposure also causes behavioural changes, damages motor skills, and affects mood, as observed in clinical demyelinating disorders. The damage to myelin sheath in demyelination induced with CPZ was predictable and reached a peak in the corpus callosum after 4 weeks of consecutive CPZ exposure in rodents [10]. Hence, the CPZ model is an appropriate pharmacological model to explore myelin protection and regeneration after drug intervention [11]. The phytochemicals play an important role in the prevention and treatment of chronic diseases caused by oxidative stress due to their strong antioxidant and free radical scavenging abilities, as well as anti-inflammatory activity. One of the potential phytochemicals is Bergenin, a coumarin obtained from the species Bergenia which belongs to the family of Saxifragaceae. Plant sources have been used in traditional medicine for the curative purpose of various diseases for a long time. Bergenin is additionally found in other *Bergenia* species like *Bergenia ligulata*, *Bergenia stracheyi*, *and Mallotus japonicus*. It has been reported to possess a potential antioxidant, anti-inflammatory, antiarthritic, immunomodulatory, antinarcotic, wound healing, antidiabetic, and neuroprotective activity [12, 13, 14]. Thus, it is necessary to develop new and more effective therapeutic strategies to combat this disease. Hence the objective of this study is to explore the effects of Bergenin (BGN) in cuprizone-induced demyelination in C57BL/6 mice to assist in the studies for the development of novel natural products therapies for Multiple sclerosis.

MATERIALS AND METHODS:

Chemicals: Bergenin was purchased from TCI JAPAN. Cuprizone was obtained from Sigma chemicals. Oxidized glutathione (GSSG) reduced glutathione (GSH), NADPH, $NADP^{+,}$ NAD, H_2O_2, 5,5'-dithiobis- [2-nitrobenzoic acid] (DTNB). All other chemicals used were of analytical grade.

Animals and Drugs Administration: Studies were carried out using Male C57BL/6 mice (6-8 weeks old) weighing 20-25g each. They were obtained from the central animal house facility of Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai 113, Tamilnadu, India. They had access to food and water *ad libitium.* Animals were acclimatized to laboratory conditions before the experiment. The experimental protocols were approved by the institutional animal ethical committee (IEAC no. 02/25/2019) Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai-113, Tamilnadu, India.

Grouping of Animals: The mice were randomly assigned to the following five groups:

Group I: The control groups received the standard pellet.

Group II: The mice were fed with a powdered pellet containing 0.2% 0f cuprizone (w/w) for 42 days (6 weeks).

Group III: Cuprizone-treated mice have received Bergenin (25mg/kg of b. wt /i. p) for 42 days (6 weeks).

Group IV: Cuprizone-treated mice have received Bergenin (50mg/kg of b. wt /i. p) for 42 days (6 weeks).

Group V: The mice were treated with Bergenin (50mg/kg of b. wt/i. p) for 42 days (6 weeks).

Behavioural Assessments:

Rotarod Test: Motor coordination was assessed using the Rotarod apparatus. Animals were exposed to prior training sessions to acclimate them on the rotarod before starting the actual assessment of the drug treatments. Animals were placed on the rotating rod with a diameter of 3 cm (speed 20 rpm). The cut-off time was 120s. Three separate trials after 5 mins gap were given to each mouse. The average fall of time was recorded on days 7th, ^{14th,} 28th and 42nd days expressed as count per 2 min [15].

Balance Beam Test (BBT): The balance beam test was used to measure the motor functioning of hind limbs and sensorineural balance, which was analyzed by measuring the ability of the mice to transverse a narrow horizontal beam (1 cm x100 cm) suspended 1m above a foam-padded cushion [16]. During testing, the mice were given 2 min to transverse the beam. If they did not complete the task or if they fell off the beam, the trial was ended, and the mice were placed back into their home cages. For successful performers, the latency to cross the beam was recorded on days 7th, 14th, 28th, and 42nd days.

Biochemical Parameters:

Homogenization of Corpus Callosum: On day 42, animals were used for biochemical estimations. The animals were sacrificed, and the brain was removed by decapitation. The Corpus callosum was separated from each isolated brain. A 10% (w/v) tissue homogenate were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 x g. The aliquots of supernatant were separated and used for biochemical estimations. In this study, all biochemical estimations were performed in the cytosol fraction.

Biochemical Parameters: The total protein concentration was measured using bovine serum albumin as standard. The product of lipid peroxidation (LPO) malondialdehyde (MDA) level was measured at 535 nm by the method expressed as nmol of MDA released/min/mg protein [17]. Estimation of protein carbonyl using the method in which 2, 4-dinitrophenylhydrazine reacts with carbonyl groups of oxidized proteins to form 2, 4-dinitrophenylhydrazone and the level was expressed as nmol/mg protein [18].

The nitric oxide (NO) synthesis was measured and expressed as nmol/mg protein [19]. The enzymic antioxidants, superoxide dismutase (SOD) were measured by the inhibition of pyrogallol auto-oxidation [20,] and the catalase (CAT) activity was determined by H_2O_2 consumption [21]. The reduced glutathione (GSH) content was measured by the reaction is based on the reduction of 5,5'-dithiobis-2- nitrobenzoic acid to yellow coloured sulfhydryl compound, which was measured at 412 and expressed as μmol of GSH/min/mg protein [22].

Glutathione peroxidase (GPx) activity was determined by the coupled assay, in which oxidation of GSH with NADPH oxidation, catalyzed by GR, which was recorded at 340 nm, and enzyme activity was expressed as nmol NADPH oxidized/min/mg protein [23]. Glutathione reductase (GR) activity and protein oxidative damage which were determined by oxidized NADPH in the reaction mixture read at 340 nm and the enzyme activity expressed as μmol NADPH oxidized/min/mg protein [24, 25].

Glutathione-*S*-Transferase (GST) catalyzes the formation of the glutathione-CDNB couples which was analysed and expressed in terms of nmol CDNB conjugate formed/min/mg protein [26]. The activity of membrane bound ATPases such as Na /K^+ and Ca $^{2+}$ ATPase was estimated by the enzyme activity was expressed as μmoles of phosphorus liberated/min/mg of protein [27, 28].

Histopathological Studies:

Hematoxylin and Eosin Stain: On day 42, the animals were sacrificed by cervical decapitation after behavioral assessments, and the corpus callosum was removed and fixed in 10% formalin saline for 24 hours. Specimens were cleared in xylene, then embedded in paraffin and prepared for sectioning at 41 m thickness by microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, and examined using a light microscope [29].

Statistical Analysis: All the values are expressed as the mean standard deviation (SD) of six animals per group. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (SPSS 20 version).Values with $P < 0.05$ were statistically significant.

RESULTS:

Behavioural Assessments:

Effect of Bergenin on Cuprizone Induced Rotarod Activity in Control and Experimental Mice: CPZ induction significantly affected muscle performance, which was assessed by the rotarod test on days 7th, 14th, 21st and 42nd.

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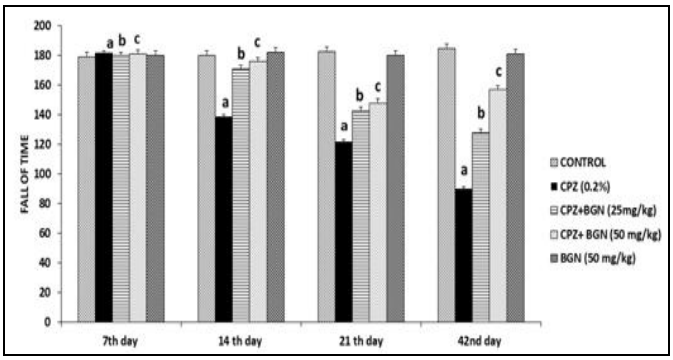

FIG. 1: EFFECT OF BERGENIN ON CUPRIZONE-INDUCED ROTAROD ACTIVITY IN CONTROL AND EXPERIMENTAL MICE. Data represents the duration spent in the target quadrant of the animal's mean values ± SD (n=6 animals in each group). [a]p<0.01, is significantly different from control. [b]p<0.05 and [c]p<0.01 is significantly different from the cuprizone exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Here, the CPZ administered mice showed (P<0.01) significant impairment in the grip strength performances on days 7th, 14th, 21st, and 42 as compared to the control group. Treatment with BGN at the doses of (50mg/kg treatment significantly (P<0.01) enhanced the muscle strength (delayed the fall of time) when compared to CPZ administered group on days 7th, 14th, 21st, and 42nd. Treatment with BGN (25mg/kg) also showed a significantly (P<0.05) improved muscular coordination on days 7th, 14th, 21st, and 42nd when compared to CPZ, administered group. However, the higher potentiation of grip strength was attained by the animals treated with BGN (50mg/kg) and BGN (50mg/kg) alone treated animals did not show any significant effect on grip strength performance **Fig. 1.**

Effect of Bergenin on Cuprizone Induced Balance Beam Walk-in Control and Experimental Mice: Intraperitoneal administration of CPZ showed a significantly (P<0.01) increased beam walk period on days as compared to control animals. Animals treated with BGN (25 mg/kg) showed a significant(P<0.05) effect in the beam walk task as compared to CPZ induced group on days 7th, 14th, 21st and 42nd. Supplementation with BGN (50 mg/kg) significantly (P<0.01) diminished the gait abnormality induced by CPZ gradually from 7th, 14th, 21st and 42nd. Thereby, the well-balanced walking movements were seen in treated groups. Furthermore, no such type of walking deficits was observed in BGN (50 mg/kg) alone treated mice; they remained as same as control animals **Fig. 2.**

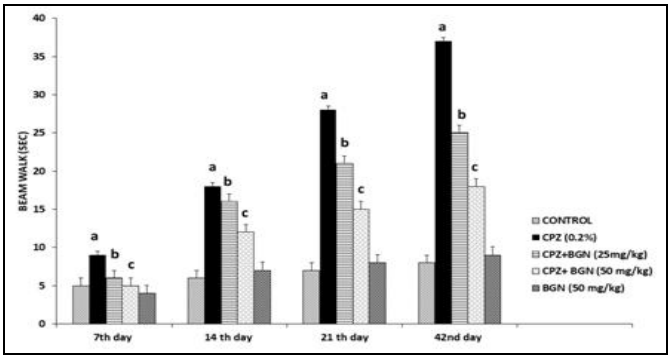

FIG. 2: EFFECT OF BERGENIN ON CUPRIZONE-INDUCED BALANCE BEAM WALK-IN CONTROL AND EXPERIMENTAL MICE. Data represents time spent in the central area of the animals, mean values ± SD (n=6 animals each group). [a]p<0.01 is significantly different from control. [b]p<0.01 and [c]p<0.01is significantly different from the CPZ exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Biochemical Observations:

Effect of Bergenin on Cuprizone Induced Changes in the Levels of LPO, Protein

Carbonyls and NO in the Corpus Callosum of Control and Experimental Mice: The CPZ induced mice showed a significantly (P<0.01) increased level of LPO, protein carbonyls, and NO as compared to control animals. However, treatment with BGN 25 mg/kg significantly (P<0.05) compared to CPZ administered animals. Also, animals treated with BGN 50 mg/kg showed a (P<0.01) significant effect by reducing the level of these oxidative stress markers compared to CPZ-induced animals. On the other hand, there were no remarkable changes observed in BGN (50 mg/kg) alone treated animals **Table 1.**

TABLE 1: EFFECT OF BERGENIN ON CUPRIZONE INDUCED ALTERNATIONS IN THE LEVELS OF LPO, PROTEIN CARBONYLS AND NITRITE IN THE CORPUS CALLOSUM OF CONTROL AND EXPERIMENTAL MICE

Groups	LPO (Units /mg of protein)	Protein carbonyl (nmol/mg of protein)	NO (Units /mg of protein)
Control	2.60±0.55	4.40±0.32	1.34±0.22
Cpz (0.2%)	10.75±0.91[a]	7.49±0.17[a]	5.62±0.28[a]
CPZ+BGN (25mg/kg)	9.41±0.16[b]	6.54±0.29[b]	4.59±0.16[b]
CPZ+ BGN (50 mg/kg)	5.63±0.22[c]	3.37±0.07[c]	2.59±0.34[c]
Bgn (50 mg/kg)	1.66±0.22	4.12±0.38	1.38±0.25

Data represent alteration LPO, carbonylated protein and NO level in corpus callosum represent mean ± SD (n=6) [a]p<0.01 is significantly different from control. [b]p<0.01 and [c]p<0.01 is significantly different from CPZ exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis.

Effect of Bergenin on Cuprizone Induced Changes in the Activity of Antioxidant Enzymes and Level of Glutathione in the Corpus Callosum Control and Experimental Mice: CPZ treatment shows a significant (P<0.01) reduction in the activities of SOD, CAT, and glutathione family enzymes (GPx, GSH. GR and GST) when compared to control-treated animals. Upon simultaneous treatment with BGN (50 mg/kg), significantly (P<0.01) improved those antioxidant enzyme activities as compared to CPZ administered animals, where BGN (25 mg/kg) treated group attained P<0.05 significant effect versus CPZ administered group. There were no remarkable changes observed in BGN (50 mg/kg) alone treated animals **Table 2.**

TABLE 2: EFFECT OF BERGENIN ON CUPRIZONE INDUCED ALTERATIONS IN THE ACTIVITY OF ENZYMIC ANTIOXIDANTS AND THE LEVEL OF GLUTATHIONE IN THE CORPUS CALLOSUM OF CONTROL AND EXPERIMENTAL MICE

Groups	SOD (Units /mg of protein)	CAT (µmol of H_2O_2 reduced/min/ mg protein)	GPX (nmol of GSH consumed /min/mg protein)	GST (nmol of CDNB conjugated/min/mg protein)	GR (µmol of NADPH oxidized/min/m g protein)	GSH (µmol of glutathione/min/ mg protein)
Control	24.69± 0.23	5.39±0.46	30.44±0.44	18.57±0.38	2.47±0.38	35.64±0.44
CPZ (0.2%)	10.24±0. 10[a]	1.37±0.19[a]	14.59±0.24[a]	5.25±0.13[a]	0.36±0.22[a]	17.71±0.91a
CPZ+BGN (25mg/kg)	15.64±0. 26[b]	2.53±0.28[b]	21.63±0.35[b]	11.55±0.41[b]	1.35±0.34[b]	19.91±0.54[b]
CPZ+ BGN (50 mg/kg)	22.54±0. 37[c]	2.65±0.38[c]	36.43±0.39[c]	20.64±0.46[c]	1.99±0.37[c]	28.77±0.15[c]
BGN (50 mg/kg)	20.65±0. 39	5.42±0.40	29.38±0.42	18.52±0.49	2.53±0.41	32.82±0.43

Data represent mean values ± SD (n=6 animals in each group). [a]p<0.01 is significantly different from control. [b]p<0.01 and [c]p<0.01 is significantly different from CPZ exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

Effect of Bergenin on Cuprizone Induced Changes in the Membrane-bound Enzymes Na /K^+ and Ca $^{2+}$ ATPase Activity in the Corpus Callosum of Control and Experimental Mice: On CPZ administration, there was a significant (P<0.01) elevation in *Na /K^+ And Ca $^{2+}$ ATPase* activity as compared to control animals. Upon treatment with BGN (25 mg/kg), significantly

(P<0.05) attenuated the *Na /K^{+} And Ca $^{2+}$ ATPase* enzyme activity when compared to CPZ induced animals, where BGN (50 mg/kg) showed a significant (P<0.01) shows an improvement in the *Na /K^{+} And Ca $^{2+}$ ATPase* enzyme activity as compared to CPZ induced animals.

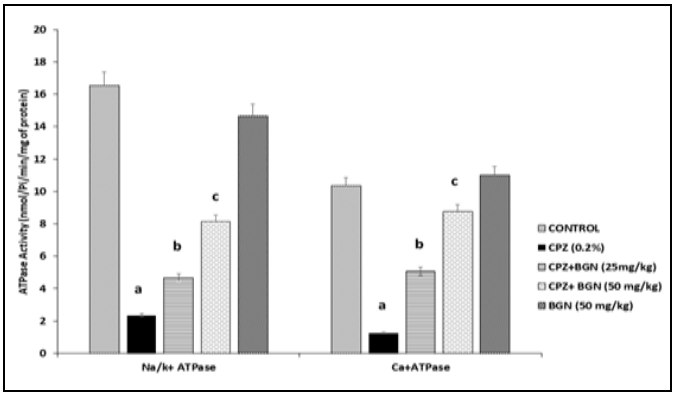

FIG. 3: EFFECT OF BERGENIN ON CUPRIZONE INDUCED ALTERATIONS IN THE LEVELS OF ENZYMES NA+/K+ATPASES AND CA2+ATPASES IN THE CORPUS CALLOSUM OF CONTROL AND EXPERIMENTAL MICE. Data represent mean values ± SD (n=6 animals in each group). ^{a}p<0.01, is significantly different from the control. ^{b}p<0.05 and ^{c}p<0.01 is significantly different from CPZ exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

The membrane-bound enzymes were enhanced by the animals treated with BGN (50 mg/kg). Further, the activity of *Na /K^{+} And Ca $^{2+}$ ATPase was* calculated to be normal in BGN (50 mg/kg) alone treated animals **Fig. 3.**

Histological Examination of the Corpus Callosum:

Effect of Bergenin on CPZ Induced Pathological Changes in the H & E-Stained Corpus Callosum of Control and Experimental Mice: Haematoxylin and eosin-stained Corpus callosumsections of control and experimental groups were depicted in **Fig. 4**. The control group exhibited normal histology of Corpus callosum (A). Section of CPZ induced group exhibited severe corpus callosum bundle degeneration with an inflammatory apoptotic lesion (B).

Animals who received BGN (25mg/kg) lost oligodendrocyte cells with few surviving neurons and disassembled corpus callosum bundles (C). Animals treated with BGN (50 mg/kg) against CPZ exhibited normal neurons with reduced inflammatory lesions (D). Animals treated with BGN (50 mg/kg) alone resemble that of control histo-architecture (E) **Fig. 4.**

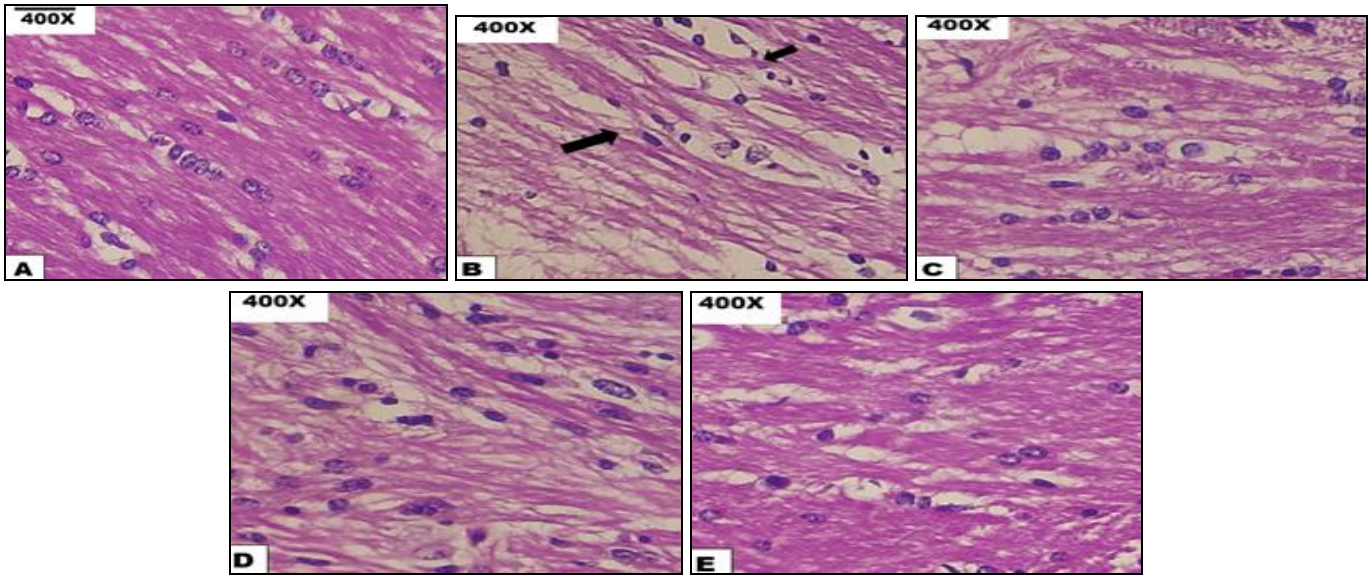

FIG. 4: EFFECT OF BERGENIN ON CUPRIZONE-INDUCED HISTOLOGICAL CHANGES IN CORPUS CALLOSUM SECTIONS OF CONTROL AND EXPERIMENTAL MICE. Haematoxylin and eosin-stained sections were visualized under the microscope at a magnification of 400x. Fig. A: Control mice showing normal bundle structure corpus callosum. Fig. B: CPZ induced mice showing a greater extent of inflamed apoptotic bundle oligodendrocyte of the corpus callosum. Fig. C: CPZ+ BGN (25 mg/kg b. wt) treated showing mild recovery of degenerative cells. Fig. D: CPZ+ BGN (50 mg/kg b. wt) treated showing reduced number of damaged cells and recovering positive healthy. Fig. E: BGN (50 mg/kg b. wt) alone administered to mice showing no inflammatory alterations they resemble the control histological.

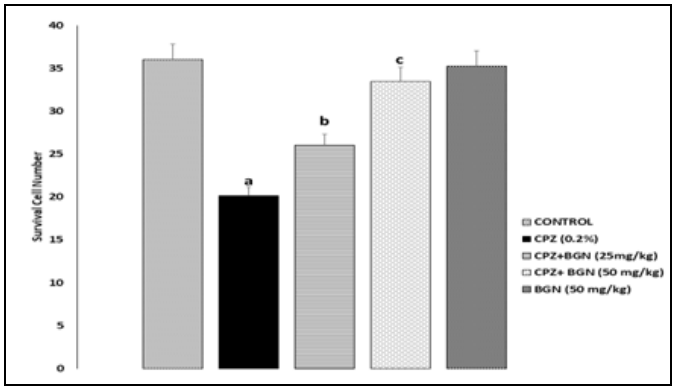

FIG. 5: SURVIVAL CELL COUNT ON H&E STAINING OF THE CORPUS CALLOSUM IN CONTROL AND CUPRIZONE-INDUCED MICE. Data represent mean values ± SD (n=6 animals in each group). [a]p<0.01 is significantly different from control. [b]p<0.01 and [c]p<0.01 is significantly different from CPZ exposed group; one-way ANOVA with Tukey's post hoc test was used for statistical analysis

DISCUSSION: The present study aimed to evaluate the neuroprotective properties of Bergenin in mice with cuprizone-induced demyelination in the corpus callosum. The cuprizone model is a commonly used animal model for studying myelin loss in the CNS that reflects the whole spectrum of MS pathology [30]. Cuprizone toxic effect causes motor dysfunction, demyelination, and glial activation and inhibits the brain copper-dependent mitochondrial enzyme, cytochrome oxidase [31]. Furthermore, Cuprizone induces demyelination lesions through oxidative stress and mainly targets the mature oligodendrocytes [32].

Apoptosis of oligodendrocytes is highly related to mitochondrial dysfunction due to cuprizone toxicity. The study mainly focuses on the neuroprotective effect of Bergenin a plant-based therapeutic compound, against cuprizone-induced toxicity onantioxidative enzyme dysfunction relates to the spreading of demyelination and the malfunctioning respiratory chain Complex leading to neuron degeneration and motor dysfunction. The mice exposed to CPZ intoxication have undergone neurobehavioral changes, which were elucidated by, the rotarod and balance beam walk test to examine the excellence of Bergenin because of its neuroprotective activity on 42nd-day reading was recorded by having the periodical trials on 7th, 14th, 28th and 42 and day. In the present study, administration of CPZ for 42 days significantly raised the mean fall-off time in cuprizone-treated mice significantly and increased the mean fall-off time in the Rotarod task depicting the motor coordination dysfunction similarly. The balance beam test of cuprizone intoxication treated mice showed a reduced body balance performance, a greater period of immobility, thus indicating motor impairment. Our results showed that cuprizone-fed mice show significantly decreased behavioural activity compared to the control group, whereas the Bergenintreated groups (25mg/kg and 50 mg/kg) show significantly increased activities compared to cuprizone treated group in the 7th, 14th, 28^{th,} and 42nd day. The first line of defense against ROS is antioxidant enzymes like (SOD, CAT, GPX, GR, GSH and GST). After cuprizone treatment, the levels of antioxidant enzymes (SOD, CAT, GPX, GR, GSH and GST) in this research reduced considerably, with a commensurate rise in oxidative stress indicators (MDA and NO). Previous research has revealed that oxidative stress plays an important role in the pathophysiology of a variety of neurodegenerative diseases [33]. Oxidative stress is caused by an imbalance in intracellular biochemical redox activity, which eventually compromises the cell's integrity and function. Because of its relatively high oxygen use and low antioxidant level, the CNS is particularly vulnerable to oxidative stress [34]. Assessments of oxidative stress indicators in specific brain areas are commonly used to determine the amount of oxidative stress.

However, co-administration of BGN with cuprizone significantly improved antioxidant (SOD, CAT, GPX, GR, GSH and GST) and oxidative stress indicators (MDA and NO), consequently modulating the neurotoxicant's detrimental efficacy. The Cuprizone-induced copper deficit is detrimental to neuronal functions in the brain, and the subsequent disturbance of energy metabolism through ATP production in oligodendroglia leads to cellular apoptosis [35]. These results are in satisfactory simultaneousness with recognitions from various authors where Bergenin regulates GSH metabolism by elevating glutathione-S-transferase and glutathione reductase activities. In addition, the GSH-based antioxidant system had a protective effect against NOX1-induced oxidative stress, and Bergenin attenuated the morphine-induced withdrawal symptoms *via* the modulation of NO synthesis [36].

Bergenin being a widely known free radical scavenger, succeeded in significantly decreasing LPOs, increasing GSH content, and restoring the redox activities of cuprizone-intoxicated animals. The model of demyelination shows a lapse in Na^+/K^+ ATPase and Ca^{2+} ATPase levels would be required to incline demyelinated axons to import harmful degrees of Ca^{2+}. The ATPase is predicted to have a protective effect, either by preventing axonal degeneration through direct action on axons or by blunting the activity of microglia and macrophages in MS [37]. In this study, the level was decreased in CPZ treated groups, whereas the level was abrogated in Bergenin treated group, but a significant increase was noticed in 50mg/kg/ b.wt treated groups. The histomorphology outcome in this present study showed reduced corpus callosum bundle and neuronal loss compared with the control group. Cuprizone worsened lipid peroxidation in the brain tissue, which happened due to an increased number of ROS and a disrupted antioxidant defense mechanism.

However, when cuprizone and BGN (25 mg/kg and 50 mg/kg) were given together, the corpus callosum morphological characteristic was restored with minor degenerative alterations that were essentially identical to the control. BGN (50 mg/kg) reduces cuprizone toxicity by scavenging free radicals and as a result, boosts the body's antioxidant defense system. Furthermore, an increase in free radicals in brain tissue has been linked to a significant reduction in the integrity and functioning of cell membranes and organelles. Bergenin administration significantly improved balance and motor coordination in cuprizone-intoxicated animals. These results are online with the ability of Bergenin to prevent motor impairment resulting from cuprizone toxicity. The efficiency of Bergenin in improving motor dysfunction of cuprizone-intoxicated mice could be correlated to the remyelination, antioxidant, anti-inflammatory, and mitochondrial-enhancing properties of Bergenin observed.

CONCLUSION: In conclusion, the current study demonstrated the effect of Bergenin on the cuprizone model of MS *via* alleviating demyelination, oxidative stress, and neuro-inflammation. Our data also suggest that Bergenin is a potential therapeutic drug and consequently supports restoration effect as evidenced by improved neurobehavioral, oxidative stress, and histological alterations in cuprizone-intoxicated animals. Therefore, these findings highlight the potential neuroprotective effect of Bergenin as a therapeutic option for MS**.**

Funding: This research work receives no funding or financial assistance from any individual or organization.

ACKNOWLEDGEMENT: Nil

CONFLICTS OF INTEREST: The authors declare that there are no conflicts of interest regarding the publication of this paper.

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How to cite this article:
Murugan A and Thangarajan S: Neurotherapeutic effect of bergenin on cuprizone-induced demyelination by regulating neurological functions associated with motor activity, oxidative stress and histological alterations in the corpus callosum of c57bl/6 mice. Int J Pharm Sci & Res 2022; 13(10): 3944-52. doi: 10.13040/IJPSR.0975-8232.13(10).3944-52.