DARK COCOA NEURO-ENHANCING ROLE IN ETHIDIUM BROMIDE-MULTIPLE SCLEROSIS INDUCED MICE MODEL: BEHAVIORAL, BIOLOGICAL, AND HISTOPATHOLOGICAL ASPECT

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Abstract

Ages ago, treatment of several ailments as neurodegenerative diseases was established using natural resources, from both animal and plant origins. Till now susceptibility of cognition to wain either by natural/ pathological aging or oxidative stress is a major concern and is continually increasing. One of these major health concerning neurodegenerative diseases in addition to its treatment, is MS, and has been a challenge for many years. Some dietary components that contain polyphenols as dark chocolate (DC) have shown that if obtained in proper amounts could enhance the cognition process. Thus, we aimed to assess a more natural treatment/prophylaxis with reduced side effects, using DC as a main nutrition component in ethidium bromide-treated mice. Treatment was assessed through the measurement of neuroinflammatory and remyelination parameters to establish the efficacy of the formulation in both treated and untreated mice. Mice that received DC in their diet (both control and MS-induced) showed lower levels of TNF, IL17 &6, glycophore, and higher levels of protein thiols and Nrf2, and brain proteolipid protein when compared to their respective controls, they also showed better cognition in Morrice Maze behavioral test, and improved Histopathological findings. Hence, from the aforementioned revelations, DC represents a safe promising natural neuro-enhancing agent that can be used to improve the prognosis of MS.

Keywords: *MS; Dark chocolate; anti-inflammatory; antioxidant*

1. Introduction

Not so long ago, medications' active ingredients were obtained from natural origins, including animals and plants, among the first nations using such medications was the Egyptians, where Egyptian herbal remedies have been dated back to 2900 BC [1]. This natural remedial system led to the evolution of medicinal plants' science, which aided in the

improvements of spectroscopic methodologies, and means of active material identification, which in return account for the intended effect of health improvement [2].
The tendency of awareness to decrease via natural/ pathological aging, with/without oxidative stress, is unceasingly increasing. And if the awareness tapering off lingers, the risk of developing neurodegenerative diseases increases, especially in old age.
Neurodegenerative diseases are multiplex ailments that affect the patient's life quality, and if left untreated can cause organ and muscle dysfunction, and ultimately lead to mortality [3]. Neurodegenerative diseases are generally divided into two subtypes: acute and chronic ones, the former primarily includes stroke and brain injury, and the latter mainly includes Parkinson's disease (PD), Alzheimer's disease (AD), and Multiple Sclerosis (MS) [4]. MS is an accepted immune-mediated demyelinating disease for the central nervous system (CNS) with multiple external and internal causes [5]. The therapy of these neuron degeneration ailments, especially MS, has been in opposition for many years [6]. Some nutritional components that contain polyphenols, and caffeine as dark chocolate (DC), have shown that if obtained in proper amounts could hinder the oxidative processes in several conditions, including the enhancement of the cognition process [7].

2. Aim of the work

The assessment of a more effective neuroprotective/prophylactic natural source with reduced side effects. This was accomplished through the use of DC as a main nutritive component in ethidium bromide (EB) MS-induced mice. DC efficacy will be assessed through the measurement of brain neuro-inflammatory and remyelination parameters to establish the efficacy of the formulation in both treated and untreated mice.

3. Materials and methods

Experimental design and procedures:
The Research and Ethics Committee for Experimental and Clinical Studies at the Faculty of Pharmacy Future University in Egypt, Cairo, Egypt approved our study. Its approval number is; REC-FPFUE 6/2023. This study followed the guidelines of the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines, EU Directive 2010/63/EU for animal experiments. Twenty-four adult male Wistar mice with body weight between 25-30 gms, were obtained from the National Institute of Research in Egypt animal facility. Animals were placed in a controlled temperature/ alternating day and night (using artificial fluorescent light) controlled housing. Mice were fed a standard food, ad libitum. These circumstances were assessed on day-to-day bases to make sure of their safety.
A veterinarian checked the mice to make certain of the absence of any changes or ailments. After the purchase, the mice were left for 7 days as an adjustment period before we started.

MS induction
Mice were prepared for stereotaxic surgery, through injecting ketamine (500mg/kg) for anesthesia. On losing the righting reflex, the mice were put on the stereotaxic frame. We then cleaned the scalp using 70% alcohol, and a 1.5 cm long cut, using a blade, was done. For induction of MS, EB was used, by dissolving it in 0.9% saline (10 μg/μL, 3 μL/animal). To

make sure of EB release, the needle used for injection remained in its place for five minutes. On completing the injection, the scalp was cleaned using iodine then sutured, then the mice were placed in their cages for recovery [8].

For monitoring MS induction:

Following the twenty days of MS, the following symptoms were assessed according to the scale:

Table 1: Scale of MS symptoms

Scale	Symptom
0	No clinical signs
1	Weak or flaccid tail
1.5	Limp tail and unsteady gait
2	Unsteady gait (ataxia), hind limb paresis
2.5	Clumsy gait, hind leg paresis (partial dragging)
3	Paraparesis of one or two hind limbs
3.5	Paraparesis of one or two hind limbs, forelimb weakness
4	Paraparesis with fore-limb involvement
4.5	Paraparesis of hind limbs, paresis of forelimbs (cannot move or groom)
5	Moribund or dead

The study was comprised of 24 mice, which were divided into 4 groups as follows:

- Group 1: Healthy mice with a normal diet. (n=6)
- Group 2: Healthy mice with DC in their diet, at a dose of (500mg/kg) orally (n=6) [8].
- Group 3: MS-induced untreated mice (n=6)
- Group 4: MS-induced mice treated with DC at a dose of (500mg/kg) orally (n=6) [9].

-For the assessment of spatial memory Morris maze test was carried out, three times, throughout the three weeks after the accommodation period.

1. Neurodegeneration estimation:

Sera Elisa assessment of tissue necrosis alpha (TNF), using TNF alpha mouse ELISA kit, catalog number (ab100747), glycophore using Mouse Glycophorin-A ELISA Kit`, catalog number (EM0812), interleukin 17 (IL 17) using Invitrogen IL-17 Mouse ELISA Kit, Catalog number (BMS6001), IL 6 using Mouse IL6 ELISA Max standard set, catalog number (431301), protein thiol using Thiol Microplate assay kit, catalog number (# U " o), The nuclear factor erythroid 2 (NrF2) using Mouse Nrf2 ELISA colorimetry kit, catalog number (MBS2516218), and proteolipid protein brain tissue-PLP, using mouse PLP ELISA kit, catalog number (RK07909).

2. Histological examination:

Histopathological changes of the brain were also carried out to estimate the effect of DC on decreasing the rate of neuron degeneration. Then, the samples for autopsy were obtained from the mice's brains and then fixed in 10% formol saline for 24 hours. Tap water was used for washing and then for dehydration; serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used. Clearance of the specimens was done in xylene and then embedded in

paraffin at 56o in a hot air oven for 24 hours. Paraffin beeswax tissue blocks were prepared for sectioning, at 4 µ thickness by rotary LEITZ microtome. The obtained tissue sections were collected on glass slides, de-paraffinized, and stained by hematoxylin &eosin stain [10] for examination through the light electric microscope.

3. Statistical analysis:

Our study data were assessed as mean ± standard deviation. The normality of sample distribution was carried out using the Kolmogorov-Smirnov test. Furthermore, the statistical difference between the study groups was carried out using one-way analysis of variance (ANOVA), followed by Bonferroni's post. hoc Quantitative -tests were done using SPSS software. The Significant association between the study parameters was done using Pearson's correlation coefficient.

4. Results:

Biochemical analysis:

Any alterations in the gait & posture of the mice were observed and noted as shown in Table 2, where the healthy (gps 1&2) showed no decline nor change in cognition or behavior, unlike those which suffered from MS (gps 3&4) and showed flaccid tail, unsteady gait, and hind leg paresis. Moreover, the MS-induced mice that were not receiving DC (group 3), showed further deterioration as shown in the form of paraparesis of both hind and forelimbs.

Groups		Brain weight	Maze test week 1	Maze test week 2	Maze test week 3
Control mice not receiving DC	Mean	395.17	19.00	18.17	18.83
	N	6	6	6	6
	Std. Deviation	11.83	2.37	1.47	2.40
Control mice receiving DC	Mean	408.50	18.17	16.67	14.00
	N	6	6	6	6
	Std. Deviation	8.07	1.47	1.37	1.41
MS mice not receiving DC	Mean	462.33^{ab}	110.50^{ab}	123.67^{abc}	173.33^{ab}
	N	6	6	6	6
	Std. Deviation	10.67	10.05	8.73	10.91
MS mice receiving DC	Mean	441.50^{abc}	104.50^{ab}	87.33^{abc}	61.33^{abc}
	N	6	6	6	6
	Std. Deviation	6.06	8.19	9.67	6.62

Brain weight was measured in grams; Weeks' results were estimated as seconds. DC; dark chocolate.

a: significant from Gp1 at $p<0.05$

b: significant from Gp2 at $p<0.05$

c: significant from Gp3 at $p<0.05$

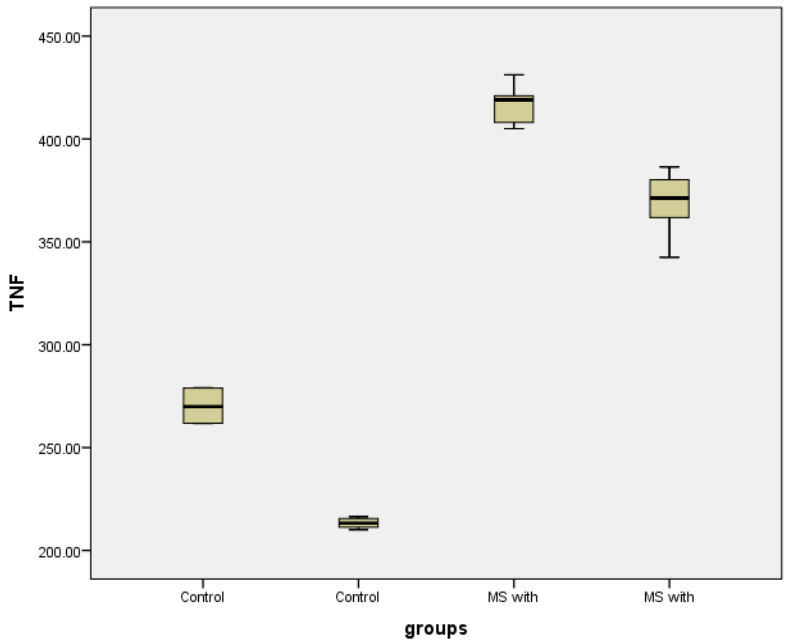
Brain weight and Morrison's maze test's results were both assessed and placed in Table 3; regarding the brain weight amongst the study group measured after the proper mice' sacrifice, there were only significant elevations in the brain weight of gps 3&4 (MS-induced groups), when compared to their both healthy mice groups (gps 1&2).

groups		TNF	Glycophore	IL17	IL6	pThiol	Nrf2	Proteolipoprotein
Control mice with no DC	**Mean**	270.22	2.67	64.13	23.88	0.97	298	3.43
	N	6	6	6	6	6	6	6
	Std. Deviation	8.07	0.14	10.19	2.48	0.12	10.29	.12
Control mice with DC	**Mean**	213.30[a]	1.96[a]	32.65[a]	14.58[a]	1.45[a]	376.5[a]	3.07
	N	6	6	6	6	6	6	6
	Std. Deviation	2.41	0.19	8.71	1.849	0.09	8.19	0.21
MS mice with no DC	**Mean**	417.20[ab]	11.75[ab]	138.58[ab]	86.87[ab]	0.38[ab]	92.67[ab]	9.89[ab]
	N	6	6	6	6	6	6	6
	Std. Deviation	9.52	1.06	10.43	7.49	0.06	6.25	0.96
MS mice with DC	**Mean**	368.92[abc]	9.89[abc]	119.32[abc]	58.23[abc]	0.57[abc]	212.17[abc]	4.75[abc]
	N	6	6	6	6	6	6	6
	Std. Deviation	16.38	1.43	6.16	8.68	0.12	19.13	0.09

a: significant from Gp1 at p<0.05
b: significant from Gp2 at p<0.05
c: significant from Gp3 at p<0.05
Gp 1: Control mice (receiving normal diet)
GP 2: Control mice (receiving normal diet+ DC as 500mg/kg body weight)
Gp 3: MS mice (receiving normal diet)
Gp4: MS mice (receiving normal diet+ BG as DC as 500mg/kg body weight)

As for the maze test results, the timing for the mice to reach the maze's end was assessed in seconds throughout three weeks after the MS stimulation and accommodation periods. There was an overall elevation in the time within the overall MS-induced mice (gps 3&4) when compared to both controls (gps 1&2), with a significant decline amongst MS-induced mice receiving DC (group 4) when compared to those of group 3 (MS-induced mice that were not receiving DC).

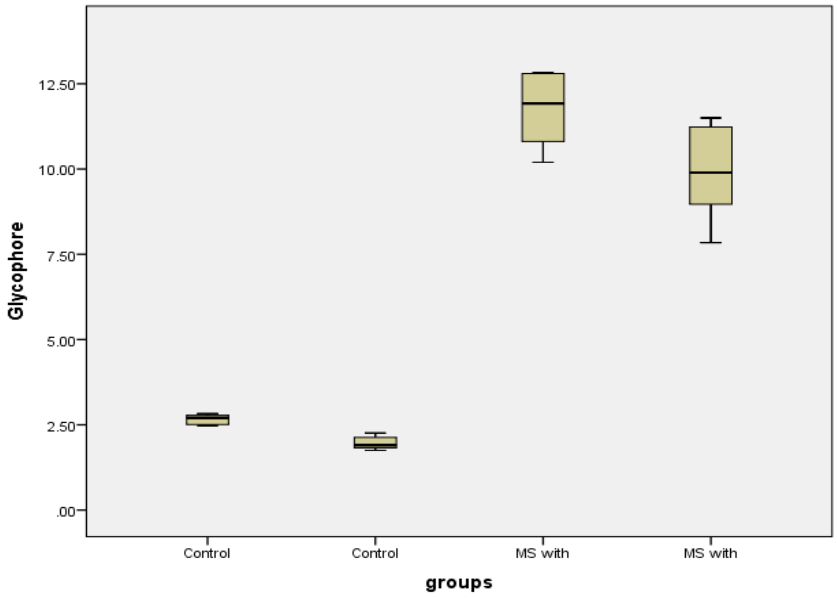
Anova's table of the assessed sera and brain parameters is shown in Table 4. Regarding sera protein levels; TNF levels were significantly elevated in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2) while showing a significant decline in both the control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 1.



1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC TNF; Tissue necrosis factor.

Figure (1); TNF levels within the study groups

In the case of Glucophore concentrations, they were significantly increased in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2), while showing a significant decline in both the control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 2.

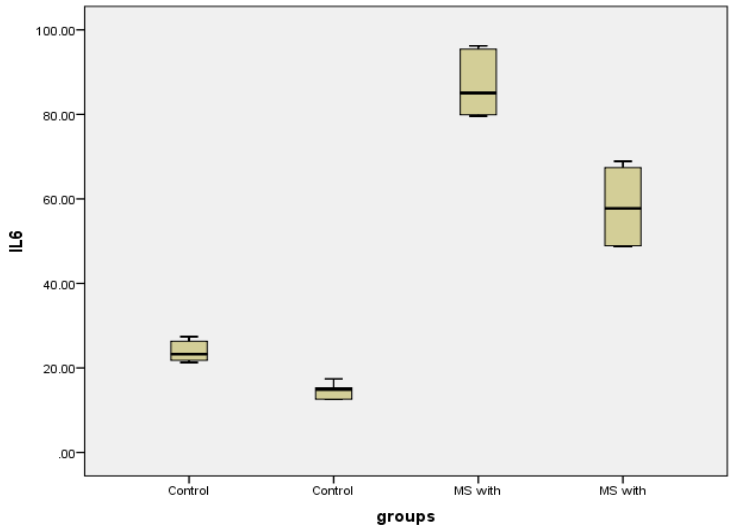


1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC.

Figure (2); Glycophore levels amongst the study groups

egarding IL 17 levels, they were significantly elevated in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2), while showing a significant decline in both the

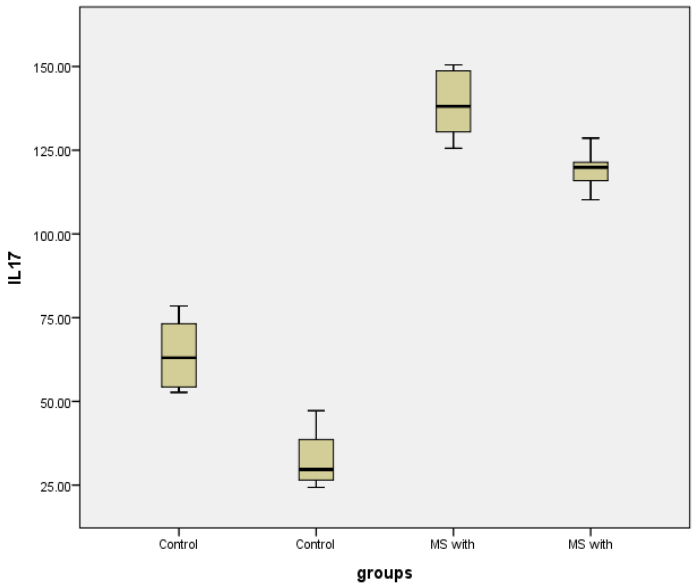
control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 3.



1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC
IL 6; Interleukin 6.

Figure (3); Interleukin 6 levels amongst the study groups

As for IL 6, its levels were significantly elevated in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2), while showing a significant decline in both the control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 4.

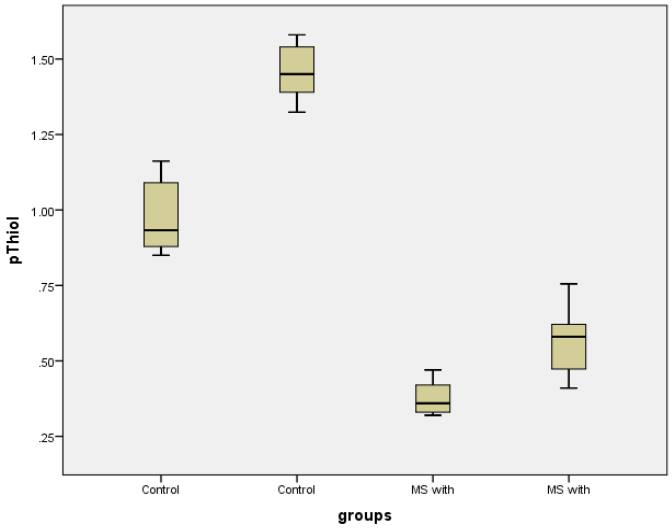


1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC
IL 17; Interleukin 17.

Figure (4); Interleukin 17 levels amongst the study groups

In the case of protein-thiol, its levels showed significant elevation in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2), while showing a significant decline in

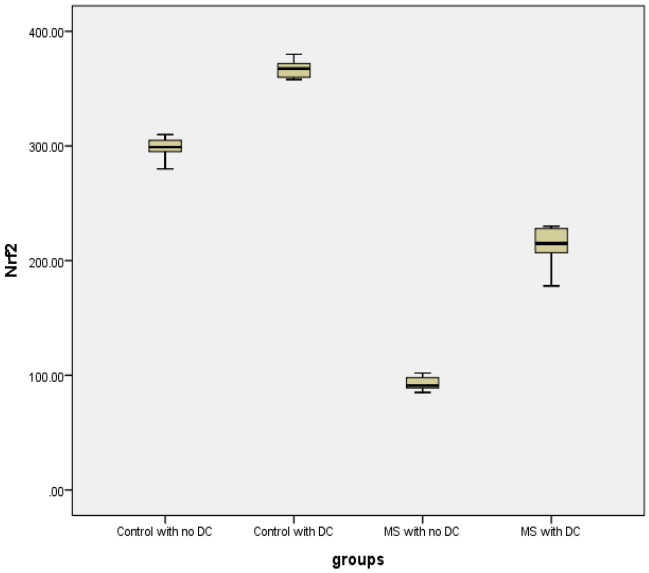
both the control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 5.



1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC
PThiol; protein thiol.

Figure (5); Protein thiol levels amongst the study groups

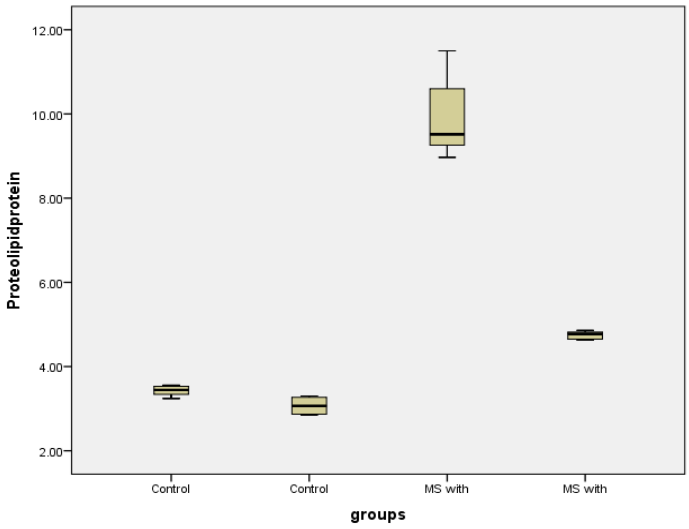
As for Nrf2, it showed a significant increase in the control group taking DC (group 2) on comparing its levels to group 1, and showing a significant decline in the MS-induced mice (groups 3& 4) when compared to the control groups (groups 1& 2). Furthermore, its levels showed a significant increase in MS group 4 taking DC, when being compared to the MS control group 3 (figure 6).



1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC.

Figure (6); Nuclear factor erythroid 2-related factor 2 levels in the study groups

Regarding brain PLP, its levels were significantly decreased in MS-induced mice (gps 3&4) when compared to the control ones (gps 1&2), while showed a significant increase in both the control and MS-induced mice which were receiving DC (gps 2& 4) when compared to their respective controls (gps 1& 3) as shown in figure 7.

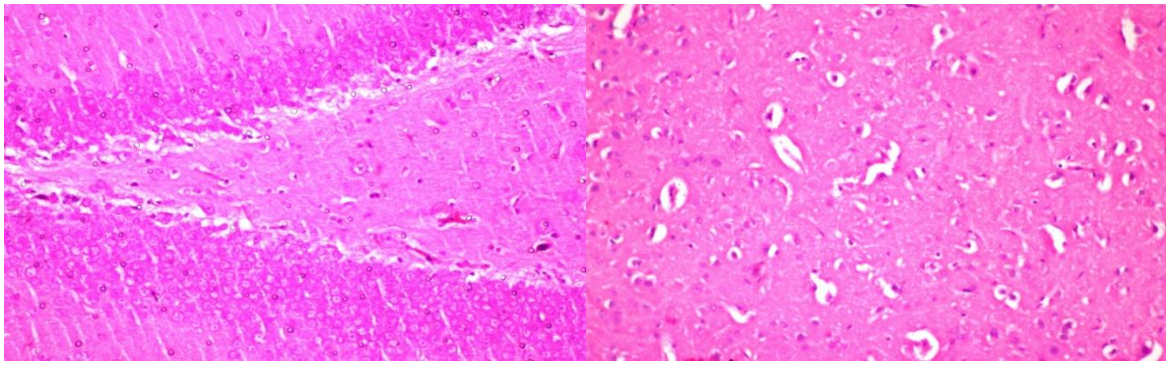


1; Control mice, 2; Control mice receiving DC, 3; MS mice, 4; MS mice receiving DC.

Figure (7); Proteolipoprotein levels in the study groups

Histopathological observations:

Controlled groups (gps 1&2) showed normal histopathological findings in the cerebral cortex, hippocampus, and cerebellum as shown in Figures 8 and 9 respectively.



Figures (8&9); Normal histopathological findings in control mice and control mice receiving DC, respectively (H&E ×40).

Regarding MS-induced mice who were not receiving DC (gp3) histopathology assessment of the hippocampus and the cerebral cortex revealed nuclear pyknosis and vascular degeneration in most neurons (Figure 10).

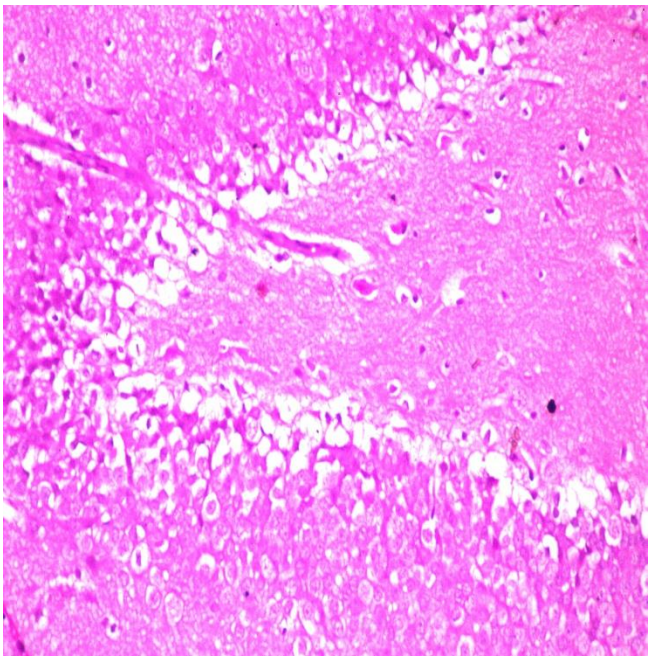


Figure 10; Hippocampus of MS induced mice.

Finally, for MS-induced mice receiving DC (gp 4), some of the neurons showed nuclear pyknosis and cytoplasmic vascular degeneration which was located in the Fascia dentate and hippocampus' Hilus, as shown in Figure 11.

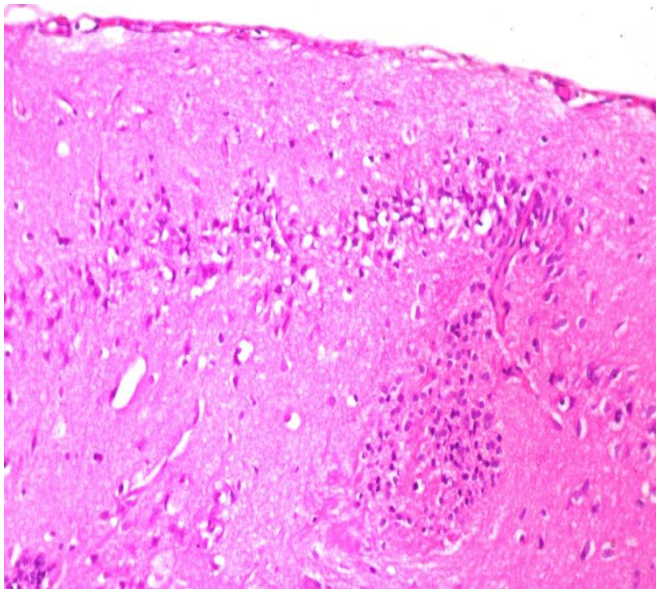


Figure 11; Hippocampus of MS induced mice receiving DC.

Correlation between the measured parameters was placed in Table 4, where on performing Pearson's correlation among the study parameters, a significant positive association was found between TNF, Glycophore, IL 17, IL 6, PLP, and brain weight. In addition, the aforementioned parameters were significantly negatively associated with protein-thiol and Nrf2s.

Table (4) Pearson correlation of the assessed parameters

		TNF	Glycophore	IL17	IL6	pThiol	Nrf2	Proteolipid protein
TNF	**Pearson Correlation**	1	.951**	.972**	.962**	-.955**	-.968**	.836**
	Sig. (2-tailed)		.000	.000	.000	.000	.000	.000
Glycophore	**Pearson Correlation**	.951**	1	.949**	.949**	-.888**	-.915**	.808**
	Sig. (2-tailed)	.000		.000	.000	.000	.000	.000
IL17	**Pearson Correlation**	.972**	.949**	1	.937**	-.941**	-.939**	.792**
	Sig. (2-tailed)	.000	.000		.000	.000	.000	.000
IL6	**Pearson Correlation**	.962**	.949**	.937**	1	-.889**	-.973**	.893**
	Sig. (2-tailed)	.000	.000	.000		.000	.000	.000
pThiol	**Pearson Correlation**	-.955**	-.888**	-.941**	-.889**	1	.922**	-.761**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000
Nrf2	**Pearson Correlation**	-.968**	-.915**	-.939**	-.973**	.922**	1	-.920**
	Sig. (2-tailed)	.000	.000	.000	.000	.000		.000
Proteolipid protein	**Pearson Correlation**	.836**	.808**	.792**	.893**	-.761**	-.920**	1
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	
Brain weight	**Pearson Correlation**	.862**	.899**	.850**	.880**	-.766**	-.848**	.830**
	Sig. (2-tailed)	.000	.000	.000	.000	.000	.000	.000

**. Correlation is significant at the 0.01 level (2-tailed).

IL, Interleukin;TNF, tissue necrosis; Nrf2, Nuclear factor erythroid 2-related factor 2**;** *pThiol, protein thiol.*

4. Discussion

MS is currently accepted as one of the most common diseases affecting the CNS of adolescents with a complex and still uncertain pathogenic outcome. And adding to the fact that the pathogenic process of MS is complicated, it also includes immune system stimulation, especially that of adaptive immunity [11]. The aforementioned stimulation involves reactive oxygen species (ROS) release and in return tissue damage. Moreover, infiltrated microglia and macrophages will release numerous pro-inflammatory effectors and free radicals, such as superoxides, hydroxyl radicals, hydrogen peroxides, and nitric oxide [12].

Thus, the use of a safe anti-oxidizing agent is crucial to delay MS symptoms and progression, hence we assessed such an effect of DC on MS-induced mice and compared them to their respective controls.

Pro-inflammatory mediators as TNF-α, IL 17, and IL 6 showed a significant increase in MS-induced mice when compared to their controls. However, MS-induced mice that were taking DC showed a significant decrease when compared to MS mice that were not taking DC. In addition, they were positively associated with each other by conducting Pearson's correlation.

This could be explained as ROS and T cells are closely intertwined, where not only oxidative stress regulates disease outcome in MS patients by acting directly on the CNS, but can also have its role in the burden of the disease by shaping the immune response in organs other than those in the CNS [13].

Moreover, cells that belong to the adaptive immune response, especially $CD4^+$ T helper (T_h) cells, have a crucial role in MS pathogenesis [13]. T cell activation depends on the attachment of T cells, T cell receptors (TCRs) with antigens, which are found on the surface of antigen-presenting cells (APCs), which include macrophages, B cells, and dendritic cells (DCs). Activation of T cells through the TCR and co-receptor CD28 (CD80/86 ligand) promotes transcriptional cascades – which in return promotes the release of cytokines such as IL-2, IL 17, and IL -6, all of which are crucial for T cell proliferation and activation [14]. Cytokines are also key regulators of T cell differentiation towards one of several T_h cell subtypes, including T_{h1}, T_{h2}, T_{h17}, and inducible T_{reg} cells. IL 6, IL 12, and TNF are important for T_{h1} differentiation, while IL-4, IL-2, IL-7, and IL 17 are important in thymic stromal lymphopoietin-derived T_{h2} differentiation [15]. It had been thought that T_{h1} cells were the main effector T cell subtype in MS, but other studies had shown further evidence regarding the pathogenic effect of T_{h17} cells in MS pathogenic processes [15]. Hence the overall activation of T cell subtypes, results in the production of inflammatory cytokines, enhancing neurodegeneration, which is in coherence with our findings of elevated levels of such cytokines in MS-induced rats. However, the dietary intake of DC, in both groups receiving it, was capable of showing a significant decrease in the aforementioned cytokines.

Regarding oxidative stress and protein thiols, its levels were significantly decreased in MS-induced mice, however, with a significant increase in mice receiving DC in their diet. This could be explained as ROS and redox states are essential for immunological response and T-cell function, where T-cells can sense redox stress (16). T-cell receptor binding, as mentioned before, promotes the release of ROS, this production affects T-cells differentiation and function through the release of pro-inflammatory cytokines [17], and the depletion of antioxidants, mainly GSH, which results in further T-cell function impairment [18]. The decrease in GSH will in return result in a further decrease in other cysteine based antioxidants residues as protein thiols [19], hence exacerbating the oxidative stress state found in MS, which was in return in accordance with our findings, however, the protein thiol levels improved through the inclusion of DC in the mice' diet, and hence decreasing the oxidative stress-inflammatory state.

Adding to the aforementioned oxidative stress mechanisms, body cells are capable of neutralizing any excess ROS thus allowing protection against injury, this is known as oxidative stress response which is mostly done by Nrf2, where it aids in maintaining cellular homeostasis [20]. As an anti-inflammatory response, Nrf2 increases phase II detoxifying enzymes as GSH and antioxidant proteins as protein thiols [21]. And since inflammation is associated with oxidative stress, it was found that mice with low Nrf2 levels had worse disease pathogenic outcomes, with the aid of T cells, in numerous inflammation-induced animal models, one of which was of experimental asthma [22]. In addition to being expressed in different body organs, Nrf2 plays a crucial role as an anti-inflammatory agent by delaying brain cell aging and death, and low levels of Nrf2 had been found in neurodegenerative

diseases such as AD and Parkinson's [23]. Thus, all of the previous, aid our findings of low Nrf2 levels in MS-induced mice, and its significant elevation on the use of DC (as its active ingredients induce the production of anti-oxidizing agents including GSH, protein thiols, and Nrf2, together with decreasing pro-inflammatory cytokines). Moreover, the previous explains the positive association of these anti-oxidizing agents with each other together with their negative association with pro-inflammatory parameters such as TNF, IL 17, and IL 6, through the correlation analyses conducted in our study.

Moreover, ROS, reactive nitrogen species (RNS), and reactive chlorine species (RCS) interact with most bioactive molecules, including proteins due to their high intracellular concentrations [24]. As a result, advanced oxidation protein products (AOPP) increased and the total thiol (SH) group level declined in a study conducted on the cerebrospinal fluid (CSP) and sera of clinically isolated syndrome relapsing remittent multiple sclerosis (RRMS) patients with respect to the control group [25], which was in coherent with our findings of decreased protein thiols and Nrf2 in MS induced mice. In addition to the previous, it had been found in a study also conducted on the CSF and sera of MS patients that the concentrations of protein carbonyls as glycophore and protein-HNE adducts were increased, indicating axonal damage resulting from the oxidative stress environment [26], which was also in accordance with our findings of elevated Glycophore levels in MS induced groups. However, the use of DC in the mice's diet improved the neuronal damage, resulting in decreased levels of glycophore in the groups receiving it in their diet.

To estimate the effect of DC on axonal regeneration, we assessed one of the major remyelination proteins, which is proteolipid protein, which was significantly decreased in MS-induced groups, evident of degeneration, while in mice receiving DC in their diet, proteolipid protein levels were significantly improved, indicating myelin regeneration. This could be explained as axonal degeneration is one of the causes of disability found in neuropathy associated with inherited demyelination, caused by a mutation in one of the genes that encode several myelin proteins, one of which is proteolipid protein [27]. In such ailments, the physiological defect occurs in the myelin sheath of Schwann cells, this causes alterations in the Schwann cells' interactions with their axons, which will result in abnormal physiology of such axons, leading to abnormal axonal transport, phosphorylation of neurofilaments, and as an end result axonal damage [28]. Such mutations in myelin proteolipid protein, which is produced mainly in myelinating oligodendrocytes, lead to several neurological diseases that are associated with nystagmus and hypotonia and can evolve into spastic quadriparesis, cognitive impairment, and ataxia, with spastic paraparesis, that is characterized exclusively by leg spasticity and weakness [29]. Axonal injury had also been seen in mice CNS, whose proteolipid protein gene had been inactivated [29]. Hence, aiding our findings regarding proteolipid protein, together with the suggested neuroprotective role of DC.

All of the aforementioned findings aid our work and findings, especially the histopathological ones, where MS-induced mice showed significant histopathological and behavioral changes when compared to their respective controls, together with an increase in their brain weight. However, the histopathological and behavioral` alterations were improved in MS mice receiving DC.

Hence, DC showed prospect as a safe, non-toxic, natural, dietary-neuroprotective agent, through decreasing the inflammatory-oxidative stress milieu (TNF, 1l 17 and IL 6), while increasing anti-oxidants (protein thiol and Nrf2), together with decreasing elements of demyelination (Glycophore) and favoring those of remyelination (proteolipid protein), through their assessment, together with improved behavior and histopathological findings.

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