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ORIGINAL CONTRIBUTION

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The modulation effect of green tea and pumpkin oils on hyperlipidemia, oxidative stress, and hematological abnormalities in an experimental multiple sclerosis rat model

Nahed S. Lamloum[1], Hanan A. Soliman[1], Rasha Rashad Ahmed[2], Osama M. Ahmed[3] and Mohamed Y. Zaky[3*]

Abstract

Background Multiple sclerosis (MS) is a chronic inflammatory condition that can impair the body's physiological functions. Although many diseases have been successfully treated with herbal treatments for a long time, the majority of the herbs utilized have unclear mechanisms. Therefore, this study aimed to examine the modulation effects of green tea oil (GTO) and pumpkin oil (PO) on hyperlipidemia, oxidative stress, and hematological abnormalities in an experimental multiple sclerosis rat model.

Methods Forty albino male Wistar rats (weighing 120–140 g) were divided into four groups of six each: group 1, the control group; group 2, the myelin oligodendrocyte glycoprotein (MOG)-injected group; and groups 3 and 4, the MOG-injected groups treated with GTO and PO at 5 mg/kg b.w., respectively. At the end of the experiments, animals were anesthetized with diethyl ether inhalation, and blood samples were collected from the jugular vein. A Beckman Coulter was then used to determine the differential complete blood counts. The obtained serum was rapidly collected and stored at 20 °C to assess the lipid profile and oxidative stress and antioxidant biomarkers.

Results Our findings showed that GTO and PO treatment produced a significant reduction in total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and very low-density lipoprotein-cholesterol (VLDL-C) levels. Furthermore, GTO and PO treatment alleviated the elevated cardiovascular risk indices 1 and 2. Thiobarbituric acid reactive substance (TBARS) concentration significantly decreased and glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels significantly increased in rats injected with MOG and treated with GTO and PO. Furthermore, after GTO and PO treatment, the reduced red blood cells (RBCs) count, hemoglobin content (Hb%), lymphocyte percentage, and hematocrit (HCT) of MOG-injected rats increased, while the elevated white blood cells (WBCs), platelet, and neutrophil percentage substantially declined.

Conclusion Collectively, our research revealed that GTO and PO may be capable of modulating hyperlipidemia, oxidative stress, and hematological abnormalities in the MS rat model.

Keywords Multiple sclerosis, Green tea oil, Pumpkin oil, Hyperlipidemia, Oxidative stress, Hematological abnormalities

*Correspondence:
Mohamed Y. Zaky
mohamedzaki448@science.bsu.edu.eg
Full list of author information is available at the end of the article

Introduction

Multiple sclerosis (MS) is a neurological inflammatory disease marked by demyelinating lesions in the central nervous system (CNS). It frequently presents as recurrent episodes of the nervous system, either with or without the gradual worsening of neurological symptoms in between relapses [64]. MS is a disease with numerous causes, including genetic and environmental factors, as well as unknown causes [13]. Myelin and axons are partially or completely destroyed by MS's attacks on myelinated axons in the CNS [28]. Active leukocytes can cross the blood–brain barrier (BBB) in MS. A weakened BBB pathway allows mononuclear cells to enter the CNS. The release of various inflammatory cytokines and chemokines by glial cells is responsible for myelin loss, oligodendrocyte integrity deterioration, and axonal loss. These incidents have a significant impact on progressive neuronal atrophy [11]. Chronic inflammatory processes that define the pathophysiology of MS disrupt the immune systems that control and restrain the inflammatory cascade to prevent irreversible tissue damage [9].

Lipids, which play a crucial role in mammalian cells, are present in a variety of body tissues, with the highest concentration in nerve tissues [29]. Various lipid species serve a variety of purposes in the CNS. Glycerophospholipids and glycosphingolipids, for instance, contribute to membrane biogenesis. Cholesterol promotes the formation of synapses in neurons of the CNS [62]. Furthermore, several neurological disorders have also been associated with abnormalities in lipid metabolism. In order to comprehend the molecular processes occurring at the onset of the disease and possibly aid in the diagnosis and prognosis of MS, the study of lipids at the onset of the disease may be an essential tool [46].

Oxidative stress involves a crucial pathway that contributes to numerous pathological processes, such as MS [51]. It is essential to maintain a balance between antioxidant defense and reactive oxygen species (ROS) generation to prevent structural damage to the CNS. During the last decade, limited studies have examined the role of ROS in the CNS and its relationship to both healthy and unhealthy signaling pathways that affect neurons [63].

The MS research community has shown considerable interest in identifying MS and its subtypes' physiological variations [10]. Numerous case–control studies have linked hematological and cerebrospinal fluid (CSF) biomarkers to the presence or absence of MS [49]. The utility of these biomarkers as clinical MS prognostic indicators, such as disease progression, is also possible. Prior research [10] has identified CSF and hematological biomarkers associated with MS subtypes. The complete blood count (CBC) measures blood cells values such as hemoglobin (Hb%), red blood cells (RBCs) count, hematocrit (HCT), platelet count, and white blood cells (WBCs) count.

Although many diseases have been successfully treated with herbal remedies for a considerable amount of time, most herbs used have undefined mechanisms. Most of the therapeutic efficacy evidence for many conventional plant treatments for MS is subjective [5]. GTO is the second most popular beverage in the world. Its oil contains polyphenol and catechin compounds, which both possess powerful antioxidant properties [44]. PO has antioxidant activity due to its ability to scavenge free radicals and prevent lipid peroxidation in cell membranes [17]. In addition, PO may have antiatherogenic properties because it contains polyunsaturated fatty acids, phytosterols, tocopherols, and beta-carotene [61]. Therefore, this study aimed to investigate the potential modulation effects of GTO and PO on hyperlipidemia, oxidative stress, and hematological abnormalities in a staged MS model in rats.

Materials and methods

Reagents and antibodies

Sigma Chemicals Company was commissioned to procure MOG35–55, pertussis toxin, and complete Freund's adjuvant (CFA) manufactured by sigma Chemicals Company and delivered by Egyptian International Center for Import (Nasr City, Cairo, Egypt). All of the remaining chemicals and reagents were of analytical quality.

Experimental animals

We randomly selected 40 albino male Wistar rats weighing between 120 and 140 g. They were observed for approximately 14 days prior to the start of the experiment. The animals were housed in stainless steel cages at a normal temperature and were provided with food and water *ad libitum*. The experiments were conducted per the animal care guidelines and the recommendations of the Experimental Animal Ethics Committee of the Faculty of Science at Beni-Suef University, Egypt (Ethical Approval number: BSU/FS/2017/12).

MS induction

The MS rat model was developed in accordance with previous reports by Fonseca-Kelly et al. [24]. First, the rats were rendered unconscious with ketamine and xylazine (Sigma Chemicals Company, Nasr City, Cairo, Egypt). The subjects were then subcutaneously administered two doses of 300 mg of MOG35–55 peptide emulsified in CFA at two different locations on the back. An equal volume of phosphate-buffered saline (PBS) was administered to the control animals. On day 0 and day 2 after vaccination, MS rats and control rats were injected

intraperitoneally with 0.1 mL of PBS containing 200 ng of pertussis toxin after 48 h.

Green tea and pumpkin oils dose preparation

The GTO and PO manufactured by the Nefertari Natural Body Care Line (Giza, Egypt) were prepared by dissolving 5 mg in 1% carboxy methyl cellulose solution (CMC).

Experimental protocol

There were four groups of rats ($n=6$/group) (Fig. 1). (1) Normal control rats, (2) MS-induced rats, and (3) and (4) MS-induced rats treated with GTO and PO at 5 mg/kg b.w., respectively [27]. As a vehicle, 5 mL of 1% CMC was orally administered to both normal control and MS-induced rats. The GTO- and PO-treated MS-induced groups received daily oral by gavage supplements of 5 mg/kg b.w. GTO and PO. The treatment was initiated upon the appearance of the initial clinical symptoms and continued for 21 days. Clinical symptoms of the disease initiated between days 12 and 19 post-immunization.

Blood sample collection

In order to obtain the samples, all animals were anesthetized with diethyl ether and euthanized. Blood was drawn from the jugular vein and placed in plain tubes and EDTA tubes containing an anticoagulant. A Beckman Coulter was then used to determine the differential complete blood counts. Serum was isolated from blood samples in plain tubes to assess lipid profile and oxidative stress parameters.

Biochemical assays

The lipid peroxidation (LPO) was measured as thiobarbituric acid (TBARS) in accordance with Ohkawa and Ohishi's method [47]. Dutta et al. [21] utilized a chemical technique to detect the glutathione (GSH) content. Superoxide dismutase activity (SOD) was evaluated using a method developed by Fridovich [25]. The activity of glutathione peroxidase (GPx) was determined chemically using a method developed by Paglia [48]. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were calculated using Spinreact Company reagent kits (Spain). The serum low-density lipoprotein cholesterol (LDL-C) concentration was measured using Friedewald et al. [26] previously described method. Very low-density lipoprotein-cholesterol (VLDL-C) concentration was calculated by the method of Tietz [59], VLDL − C concentration (ðmg/Dlþ) = TG/5. From the following calculations, cardiovascular risk indicators were generated [50] as follows:

$$\text{Cardiovascular risk index 1} = \frac{\text{TCh conc.}}{\text{HDL} - \text{Ch conc.}}$$

$$\text{Cardiovascular risk index 2} = \frac{\text{LDL} - \text{Ch conc.}}{\text{HDL} - \text{Ch conc.}}$$

As part of the CBC, RBCs, HCT, WBCs, platelets, Hb%, neutrophils, and lymphocytes were measured. These measurements can be generated by any of the widely used automated counters, including those manufactured by Abbott, Bayer, Beckman-Coulter, and Technicon.

Statistical analysis

The results were displayed as the mean ± standard deviation of the mean (SEM). IBM SPSS Statistics 20 was used to conduct statistical analysis (IBM Corporation, NY, USA). An analysis of variance (ANOVA) and Duncan's

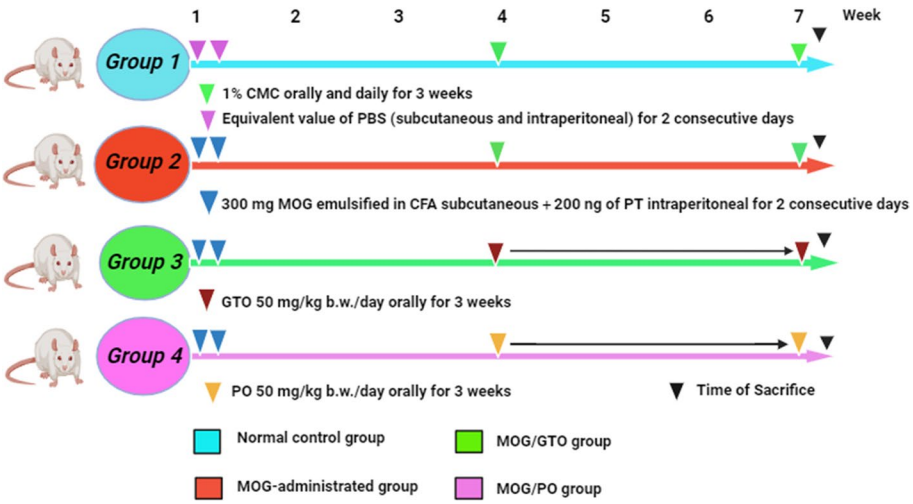

Fig. 1 Schematic figure of the animal grouping and experimental design

multiple-range test were used to assess the data. *P* values under 0.05 were considered statistically significant.

Results

GTO and PO improved lipid profile abnormalities in MS-induced rats

Compared to the normal control group, rats injected with MOG exhibited a significant ($P < 0.05$) increase in T.C, TG, HDL-C, LDL-C, and VLDL-C levels, as well as cardiovascular risk indices 1 and 2. Nevertheless, the oral administration of GTO and PO to MS-induced rats significantly ($P < 0.05$) reduced the elevation of T.C, TG, HDL-C, and LDL-C levels relative to MOG-injected rats. Furthermore, GTO and PO treatment alleviated the elevated cardiovascular risk indices 1 and 2 (Fig. 2).

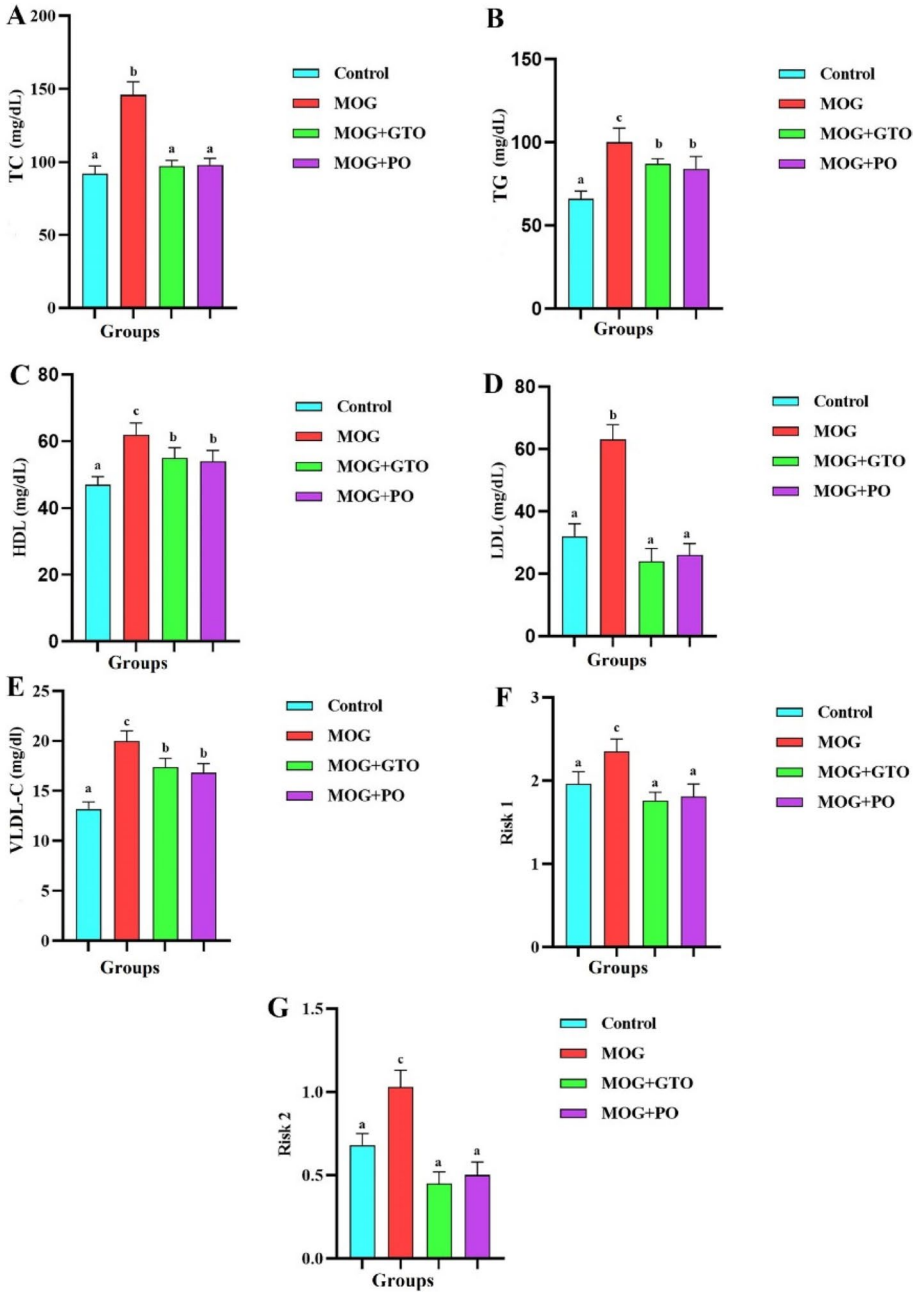

Fig. 2 Effect of GTO and PO on serum (**A**) TC, (**B**) TG, (**C**) HDL-C, (**D**) LDL-C, (**E**) VLDL-C, (**F**) risk factor 1, (**G**) risk factor 2 in MOG-injected rats. Symbolically equivalent means are not significantly distinct

GTO and PO reduced oxidative stress and enhanced antioxidant markers

Comparison to normal rats, rats injected with MOG had significantly ($P<0.05$) higher serum TBARS levels and significantly ($P<0.05$) lower GSH level and GPx and SOD activities. However, GTO and PO treatment of MOG-injected rats significantly ($P<0.05$) reduced the elevation of TBARS levels while also increasing the suppressed GSH level, and GPx and SOD activities (Table 1).

GTO and PO improved complete blood count abnormalities in MS-induced rats

Rats injected with MOG had a significant ($P<0.05$) decrease in Hb% and HCT values and a non-significant ($P > 0.05$) decrease in RBCs values when compared to normal rats. Also, they exhibited a significantly ($P<0.05$) lower number of lymphocytes as well as a significant increase in WBCs and platelet counts as well as the neutrophil values when compared to normal rats. In contrast, orally-gavaged GTO and PO significantly ($P<0.05$) decreased the elevated WBCs, platelets, and neutrophils levels ($P<0.05$) and improved the lowered Hb%, RBCs, HCT, and lymphocyte levels compared to the MOG-injected rats (Figs. 3 and 4).

Discussion

This study focused on the moderating effects of GTO and PO on hyperlipidemia, oxidative stress, and hematological abnormalities in rats induced by MOG. Compared to the normal control group, TC, TG, HDL-C, LDL-C, and VLDL-C levels in the serum of rats injected with MOG were abnormally increased. Despite the chronic inflammatory nature of MS, it is still unknown whether and how lipoprotein levels are altered in MS patients, as well as whether variations in diet and body composition influence the progression of the disease. Some studies argue that MS patients' lipid profiles should demonstrate alterations [45, 65]. In addition, Weinstock et al. [65] found correlations between disability and magnetic resonance imaging measures in MS and the components of the serum lipid profile, including TG, HDL-C, LDL-C, and TC. They discovered that elevated levels of LDL-C, TC, and HDL-C were associated with increased inflammatory activity in MS patients. Çomoğlu et al. [19] found that compared to healthy individuals of the same age and gender, MS patients had higher levels of TG and TC. In the present study, the administration of GTO orally to rats injected with MOG significantly reduced the increase in TC, TG, LDL, HDL, and VLDL levels compared to normal control rats. Our obtained data correspond to previous publications [35, 67]. It has been discovered that GT catechins can significantly reduce plasma levels of TG, TC, and LDL-C [67]. In addition, Bursill et al. [15] demonstrated that GT catechins inhibit key enzymes involved in lipid biosynthesis and limit TC absorption in the intestine, leading to improved blood lipid profiles. Also, the PO treatment improved the lipid profile, nearly restoring it to normal levels. These findings are consistent with reports by Tzortzakis et al. [61] and El-Adawy et al. [22]. Due to the presence of polyunsaturated fatty acids, phytosterols, tocopherols, and B-carotene, they concluded that pumpkin seeds may have anti-atherogenic properties. In addition, Abuelgassim and AlShowayman [2] found that rats treated with PO exhibited a significant reduction in their serum concentrations of TC and LDL-C, providing support for this claim. This decline was due to the high phytosterol concentration in pumpkin seeds, which prevents cholesterol absorption by the small intestine. Agatemor [3] and Al-Masri [6] attributed the hypolipidemic action of PO to the existence of unsaturated fatty acids, which are essential for lowering blood cholesterol in humans and rats and may be associated with decreased cholesterol synthesis and/or increased cholesterol catabolism in the liver. Moreover, Sedigheh et al. [52] credited the fiber content of pumpkin as the cause of this lipid-lowering effect. Dietary fibers lower plasma LDL-C levels by preventing the absorption of bile acids and cholesterol and by increasing LDL receptor activation [8].

In MS, oxidative stress is associated with the destruction of myelin and axons, which leads to clinical symptoms [66]. Also, increased ROS production induces oxidative stress, a prevalent pathogenic trait of various neurological conditions, including MS. In the current

Table 1 GTO and PO reduced oxidative stress and enhanced antioxidant markers

Parameters Groups	LPO (nmol/ mg protein/ hr)	GSH (mmol/mg protein)	GPx (U/mg protein)	SOD (U/mg Protein)
Control	76.0 ± 2.54^{a}	132.1 ± 3.4^{b}	231.3 ± 8.83^{c}	18.3 ± 0.53^{b}
MOG	116 ± 5.3^{b}	66.0 ± 4.8^{c}	153.1 ± 9.4^{a}	7.30 ± 0.62^{c}
MOG + GTO	85.1 ± 3.11^{c}	74.8 ± 2.12^{a}	175.9 ± 23.5^{b}	9.85 ± 0.35^{a}
MOG + PO	95.2 ± 4.0^{c}	103.9 ± 3.9^{a}	208.1 ± 9.8^{b}	15.55 ± 0.60^{a}

Data is presented as the mean ± SE. Means, which share the same symbol(s), are not significantly different

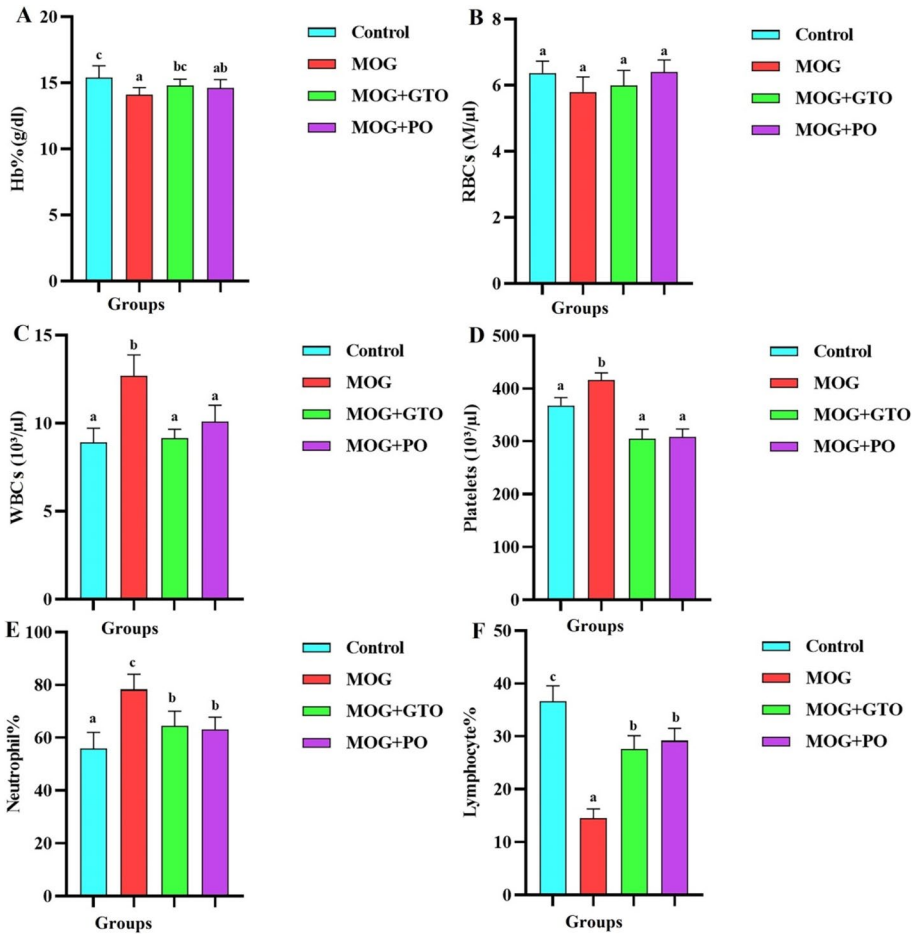

Fig. 3 Effect of GTO and PO on (**A**) Hb%, (**B**) RBCs, (**C**) WBCs, (**D**) platelets, (**E**) neutrophils, and (**F**) lymphocytes counts of MOG-injected rats. The means that share the same symbol(s) are not significantly different

study, administration of MOG to normal rats led to a significant increase in serum LPO and a decrease in SOD and GPx activities and GSH levels. Our findings were validated by prior research [12, 16]. TBARS, a lipid peroxidation marker, increased by 81% in the serum of MS patients. Lack of lipophilic antioxidants in the blood of MS patients may impair the bioenergetics of reparative remyelination and contribute to neurodegeneration [38]. In addition, several studies have demonstrated that oligodendrocytes have particularly low GSH level, making them more vulnerable to the effects of oxidative stress [20]. Significantly decreased SOD activity was observed in the erythrocytes of MS patients, suggesting that their enzymatic defense mechanisms against oxidative stress were compromised [69]. In addition, Tasset et al. [58] reported that several oxidative stress markers were elevated in the peripheral blood of relapsing–remitting MS (RRMS) patients, whereas GPx, GST, and total antioxidant capacity were reduced. In the present research, the administration of GTO to rats injected with MOG reduced the increase in LPO and improved the depletion of SOD, GPx, and GSH activities. These results are consistent with other studies [32, 40]. In animal models of chemically induced oxidative stress, GTO has been demonstrated to improve the activity of natural antioxidant enzymes such catalase, superoxide dismutase, and/or glutathione antioxidant enzyme systems [55, 56]. Green tea polyphenols can activate antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (SOD), according to Tsao's research [60]. In contrast, the administration of PO to rats injected with MOG improves the elevation of LPO and depletion of SOD, GPx, and GSH levels. These findings are consistent with previous research [23, 43]. It has been discovered that PO contains potent antioxidant substances, such as vitamin E and gamma-tocopherol. This may explain why GSH, SOD, and GPx activities have risen [33]. Also, Al-Zuhair et al. [7] reported that PO supplementation improved non-enzymatic antioxidants GSH

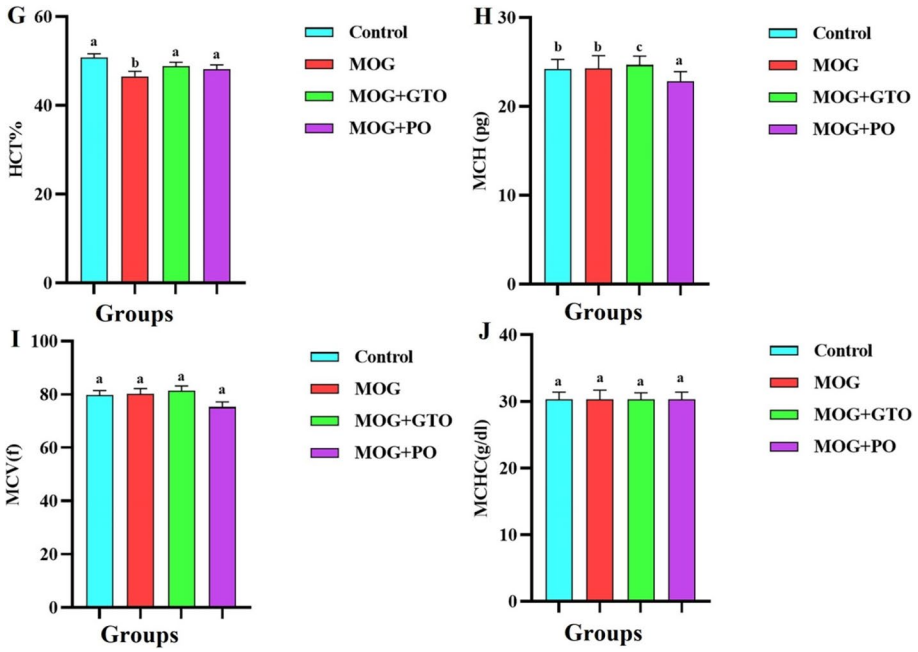

Fig. 4 Effect of GTO and PO on (**G**) HCT, (**H**) MCV, (**I**) MCH, and (**J**) MCHC values of MOG-injected rats. The means that share the same symbol(s) are not significantly different

and Vitamin C levels as well as enzymatic antioxidant SOD, GST, Chloramphenicol acetyltransferase (CAT), and GPx.

This study revealed that the hematological profile of rats injected with MOG differed from that of the normal control group. Consistent with previous findings, we observed a significant decrease in Hb% and HCT values and a non-significant decrease in RBCs values when compared to normal rats. Our results also showed a significant decrease in lymphocytes, as well as a significant increase in platelets, WBCs, and neutrophils [36, 42, 54]. The RBC count in MS patients was not significantly lower than in controls, but it did show an inverse correlation with time since diagnosis but not with age in either patients or controls, suggesting a trend toward RBC vulnerability in MS patients and a similarity to the decreased myelin yield reported in MS patients with normal-appearing white matter [30]. Kocer et al. [36] found non-significantly lower Hb% concentrations in MS patients contrary to healthy controls, which is consistent with our findings. Although immune cells from MS patients had a weak relationship to the outcome of the disease, the neutrophil percentage was higher in patients and had a weakly positive correlation with Cerebellar Functional system scores (FSS). Despite the correlation's lack of statistical significance, it has been demonstrated that an elevation in peripheral leukocyte count is associated with MS patients [54]. It has been suggested that abnormal platelet electrophoretic behavior in MS patients is due to abnormal plasma-phospholipid patterns [14].

Prostaglandin biosynthesis is required for one of the many mechanisms by which platelets assemble because it triggers the release of the fatty acid C20:4n-6 (arachidonic acid) from the membrane and its subsequent conversion to the potent aggregation activator thromboxane A2 [41]. In this context, a reduction in C18:2n-6 and/or C20:4n-6 in the membranes of platelets, RBCs, and WBCs has been reported [18, 31]. Compared to rats injected with MOG, treatment with GTO significantly improved the depletion of Hb%, RBCs, HCT, and lymphocytes and increased platelets, WBCs, and neutrophils in the current study. Our investigations parallel with the previous findings [39, 57]. The significant antioxidant effect of catechins extracted from GT on hematopoietic cells may account for the improvement in hematological markers following GT consumption. In the presence of uncontrolled ROS accumulation, hematopoietic cells appear to be particularly sensitive, as deficiencies in numerous ROS scavengers can lead to severe or even fatal anemia and/or hematopoietic tissue cancers [37]. With ECG and ECGC, Kao et al. [34] observed an increase in PCV, Hb%, and RBC count. In addition, the WBCs, neutrophils, and lymphocyte levels returned to normal after 30 days of GTO treatment. Moreover, catechins in green tea work by altering multiple cellular signaling pathways, resulting in reduced inflammation and platelet aggregation as well

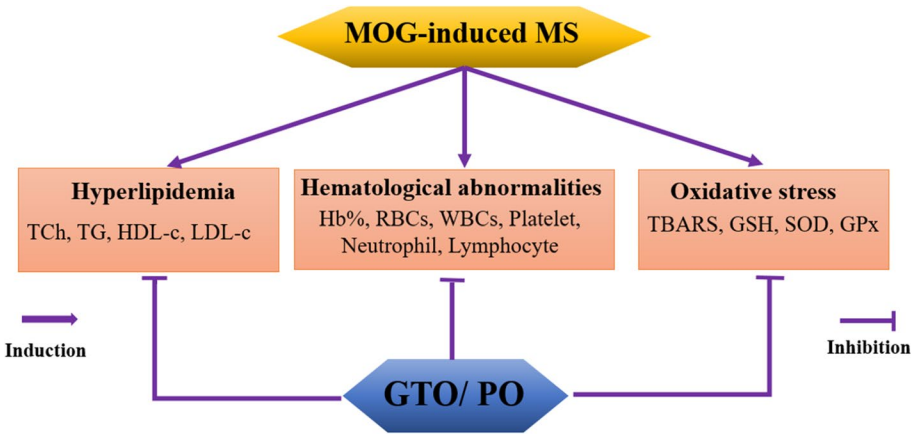

Fig. 5 Work model demonstrating the ameliorative effect of GT and PO on rats injected with MOG

as increased vascular responsiveness [53]. Also, the treatment of MOG-administered rats with PO significantly ameliorated the changes in their hematological profile. This result is consistent with earlier research [1, 4] that discovered increased RBC when consuming large quantities of pumpkin. An increase in RBCs could be attributed to the heightened oxygen-carrying capacity of the blood. Similarly, in a study by Yongabi et al. [68], it was observed that the elevated levels of Hb%, RBC, HCT, and MCHC in the plant's composition demonstrated its medical significance in combating anemia.

Conclusion

Our results concluded that both GTO and PO have the potential to modulate hyperlipidemia, oxidative stress, and hematological abnormalities in MOG-injected rats (Fig. 5). However, extensive chemical and pharmacological research is necessary to determine the precise mechanism by which GOT and PO exert their ameliorative effect and to identify the active constituents responsible for their effect.

Authors' contributions

Hanan A. Soliman and Rasha Rashad Ahmed– conceived and supervised the study; Nahed S. Lamloum– carried out experiments; Nahed S. Lamloum, Osama M. Ahmed, and Mohamed Y. Zaky – discussed the results of experiments with input from all authors; Nahed S. Lamloum – wrote the manuscript; Osama M. Ahmed and Mohamed Y. Zaky – edited the manuscript.

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Declarations

Ethics approval and consent to participate

All procedures performed on animals were in compliance with the ethical standards of the Institutional Animal Care and use Committee (IACUC), Faculty of Science, Beni-Suef University, Egypt (approval number: BSU/FS/2017/12).

Consent to participate

Consent was taken from all participants.

Competing interests

The authors declare no conflict of interest.

Author details

[1]Department of Biochemistry, Faculty of Science, Beni-Suef University, P.O. Box 62521, Beni-Suef, Egypt. [2]Cell Biology, Histology and Genetics Division, Department of Zoology, Faculty of Science, Beni-Suef University, P.O. Box 62521, Beni-Suef, Egypt. [3]Molecular Physiology Division, Zoology Department, Faculty of Science, Beni-Suef University, P.O. Box 62521, Beni-Suef, Egypt.

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