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Pinocembrin Ameliorates Neuronal Cell Death by Regulating Autoimmune Signals

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ABSTRACT

Pinocembrin is a type of flavonoid present in honey and propolis. It has high antioxidant and neuroprotective effect. The present study was designed to evaluate the immunomodulatory properties of pinocembrin (PCB) and the anti-apoptosis effects using *in vitro* and *in vivo* mice splenocytes and experimental autoimmune encephalomyelitis (EAE) in C57BL/6j female mice, respectively. The reduction of clinical symptoms and disease index were evaluated by histochemical analysis. T-cell population and cytokines levels in myelin oligodendrocyte glyco-protein (MOG) challenged PCB treated cells were estimated using flow cytometry and ELISA kits. Apoptotic and neuronal markers were evaluated using quantitative real time PCR and protein blots. Results showed that PCB inhibited the infiltration of inflammatory cells and improved the myelin protective proteins. It also positively regulated the antioxidants and apoptotic markers including Caspase-3, TGF-β, SIRT-1, CCL-2 and MBP. Moreover, PCB modulated the levels of inflammatory mediators and TGF-β in splenic cells. The production of myelin basic protein (MBP) increased in PCB treated EAE mice. Taken together, the current finding revealed that, PCB suppressed EAE pathological checkpoints for demyelination and apoptosis in CNS and act as a therapeutic role for treatment of MS in upcoming prospects.

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AAS, HIMI and EAA helped in conceptualization, methodology and formal analysis. AAS acquired funds, wrote discussion and supervised the study. ANAA validated the results. HIMI, ANAA and EAA wrote the manuscript.

Key words
Multiple sclerosis, Demyelination, Inflammation, SIRT-1, Pinocembrin.

INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease characterized by inflammation and demyelination of the central nervous system (CNS) (Compston and Coles, 2008; Huang *et al*., 2019). In patients, MS progresses slowly causing variable symptoms including fatigue, vision problems, numbness and tingling, muscle spasms, stiffness and weakness, pains and mobility problems and subsequently depression and anxiety. At the pathological level, the induced demyelination and the loss of oligodendrocytes besides the inflammation resulted in infiltration of T lymphocytes and macrophages mediated neurodegeneration (Popescu *et al*., 2013; Macrez *et al*., 2016).

The most well characterized experimental model for the human MS disease is the experimental autoimmune encephalomyelitis (EAE) animal model, that provided convincing evidence about cell subtypes involved in the neuro autoimmune process and the pathological features of MS (Constantinescu *et al*., 2011; Chen *et al*., 2014). The EAE model is induced by either passive transfer of activated myelin-specific CD^{4+} T lymphocytes or through active immunization with myelin-derived proteins or peptides (*e.g.,* myelin oligodendrocyte glycoprotein (MOG) in an adjuvant to sensitize the T cells in the peripheral lymphoid tissue (Farooqi *et al*., 2010).

Several immune cells are involved in the induction of MS disorder including autoreactive T cells, infiltrating

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monocytes, local active microglia cells and astrocytes. These cells enhance the production of inflammatory mediators during neuro-inflammation. Moreover, macrophages and crude T cells regulate the cytokine mediators including excessive production of IL-1β and TNF-α that trigger an acute generalized inflammatory response (Bittner *et al*., 2014; Alvaro *et al*., 2020). Classical studies indicated that CD8+ T-cells (interferon producing cells) play a predominant role in processing the MS autoimmune disorder compared to other T-cell subsets (Ying *et al*., 2011). However, nowadays CD^{4+} T cell-mediated autoimmunity is considered as one of the most important preidomenant in MS pathogenesis (Chen *et al*., 2014).

The myelin degradation was linked with sirtuins, it is an NAD+-dependent class III histone deacetylase mediators. Its pathological role in MS and EAE was already discussed in many studies (Gregath and Lu, 2018; Nimmagadda *et al*., 2017). Sirutin family proteins represents therapeutic targets in neurodegenerative diseases, However, SIRT-1 activity and its function on autoimmune mediated myelin damage has partially established.

The differentiation of naïve CD^{4+} T cells into T helper (Th1, INF-γ and TNF producing cells) and Th17 (IL-17 producing cells) enhance autoimmune responses and mediate tissue inflammation (Raphael *et al*., 2014). IL-17 recruits both neutrophils and monocytes, and regulating the release of pro-inflammatory cytokines including TNF-α and IL-1β (Xu *et al*., 2014; Wang *et al*., 2017).

Pinocembrin (PCB) is one of the most important phytochemical flavonoids from propolis exerting several biological functions including anti-inflammatory, anti-microbial, and anti-oxidant properties (Estevinho *et al*., 2008). In various *in vitro* and *in vivo* preclinical investigation, pinocembrin was recently used to treat ischemic injury, Alzheimer's disease, cardiovascular diseases and atherosclerosis (Meng *et al*., 2011; Liu *et al*., 2014; Shen *et al*., 2019; Abdullah *et al*., 2019). Designing new drugs based on the pharmacological effects of PCB could help in treatment many diseases including the autoimmune diseases. For instance, PCB was found to decrease oxidative stress and pro-inflammatory cytokines production to inhibit liver fibrosis via inhibiting the TGF-β1/Smad and activating Nrf2/HO-1 signaling pathways in liver fibrosis mouse model. In the present study, we have tested the anti-inflammatory and immunoregulatory role of PCB in EAE mice model.

MATERIALS AND METHODS

Mice and ethics statement

Animal for this experiment were procured from Animal facility of biology department, college of science, King Faisal University, Saudi Arabia. We used 6-8-week-old C57BL/6J female mice. Animals were maintained in our laboratory under specific pathogen free (SPF) conditions throughout the experimental time span. We carried out all *in vivo* and *in vitro* experiments following the protocols approved by the Research Ethics Committee (KFU-180123) of King Faisal University, Saudi Arabia. The humane endpoints included 25% body weight loss, paralysis or for limb paralysis for 24 h.

Experimental autoimmune encephalomyelitis (EAE) models

MOG_{35-55} peptide was used to immunize 12 female C57Bl/6J mice with a dose of 150 µg (Peptide international) mixed with complete Freud's adjuvant (CFA; Sigma-Aldrich; USA) in concentration of 10 mg/ml heat-killed *Mycobacterium tuberculosis* H37Ra (Difco Laboratories). Pertussis toxin were injected into mice intraperitoneally (List Biological Laboratories; 500 ng) at day 0 and 2. Clinical symptoms were scored for every two days as following: 0 no clinical signs; 1, hind limb weakness of limb tail; 2, paralyzed hind limb; 3, paralyzed for limb; 4 complete paralysis and 5, death. PCB were encountered orally 100 mg/kg/day or vehicle (corn oil-0.1 ml) for 10 days starting one day before the MOG_{35-55} immunization.

Drug preparations

Pinocembrin (PCB) from (Sigma Aldrich, MO, USA). Corn oil 10% was used to dissolve PCB and was fed to mice in the concentration of 100 mg/kg/day. PCB treatment started from 10^{th} day of MOG immunization upto 21^{st} day of sacrificing the treated mice. Treated and control mice group received an equal volume of 10% corn oil for 21 days.

Proliferation assay of splenocytes

PCB effect on lymphocyte proliferation response was measured using cytotoxicity cell kit (CCK)-8 reagent (Roche). Spleen is isolated from naïve mice, cell suspensions was prepared and passed throughout a cell strainer (70 µm) using sterile syringe. The cells were washed with the culture medium (RPMI 1640) (UFG, Saudi Arabia) and splenocytes with minimal viability 90% were cultured in 96-well plate that with flat bottom in concentration of 5 x 10^5 cells/ well in RPMI containing 5 % fetal bovine serum (FBS) (Gibco) and 100 µg/ml penicillin-streptomycin as antibiotics (Gibco). Splenocytes were activated with 10 µg/ml PHA (phytohemagglutinin) (Gibco) with and without 10 µg/ml MOG as an antigen specific stimulator. Stimulated cells were grown in an incubator at 37°C in a condition of 5% CO_2 for 48 h. CCK-8 reagent added to wells (10 µl/well) and the cell viability

was assessed after 2 h using a microplate reader at 450 nm.

Cytokine estimation

Mice cytokines such as IL-17, IL-6, IFN-γ and TGF-B ELISA kit (K4800-100) were purchased from Genway Bioscience (USA). Estimation of different cytokines was investigated according to manufacturer manual with few modifications. The plate was read on a microplate reader at 450 nm to calculate the cytokine levels and values expressed as pg/ml.

Quantitative real-time PCR

TaqMan reverse transcription kit was used for cDNA synthesis . synthesized cDNA was amplified by using a ViiA7 system and TaqMan gene expression assays for target gene primers in Table I for coding genes and microRNA primers. Kits, probes and reagents were obtained from Applied Biosystems. The relative expression of mRNAs and miRNAs were calculated by the $2^{-\Delta\Delta Ct}$ method.

Table I.- Primer used to analyse the demyelination and anti-apoptotic markers.

Name	Sequence	Product size
Caspase-3	F: GGACAGCAGTTACAAAATGGATTA	121
	R: CGGCAGGCCTGAATGATGAAG	
TNF-a	F: TGATACGCCTGAGTGGCTGTCT	156
	R: CACAAGAGCAGTGAGCGCTGAA	
SIRT-1	F: TTGTGAAGCTGTTCGTGGAG	147
	R: GGCGTGGAGGTTTTTCAGTA	
CCL-2	F: GCTACAAGAGGATCACCAGCAG	122
	R: GTCTGGACCCATTCCTTCTTGG	
MBP	F: ATTCACCGAGGAGAGGCTGGAA	106
	R: TGTGTGCTTGGAGTCTGTCACC	
GAPDH	F: GGTGCTGAGTATGTCGTGGAG	160
	R: GGTGCTGAGTATGTCGTGGAG	

Protein quantification

We prepared cell lysate using lysis buffer (RIPA lysis buffer system). Proteins were fractionated using SDS-PAGE. The target proteins in the lysate were immune blotted using semi dry blot systems. The immunoprecipitation elutes was detected using rabbit polyclonal antibodies specific for targets such as TGF-β, SIRT-1, CCL-2, Caspase-3 and MBP (dilution; 1:800). β-actin specific monoclonal antibodies (dilution; 1:1,000) and corresponding horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2,000). Quantification of band intensity was determined by LICOR imaging scanner and it was calculated by ImageJ software (version 1.48; https://imagej.nih.gov/ij/download.html).

Immunohistochemistry

For detection of demyelination and infiltration of T cells in EAE spinal cord, immunohistochemically staining for myelin basic protein (MBP) was performed on lumber spinal cord sections with a few modification; spinal cord sections were passed in routine histological steps. Slides were blocked in 10% normal goat serum and with antibodies against myelin basic protein (1:500; Steinberger Monoclonal). This is followed by washing of sections and its incubation with HRP-conjugated secondary antibodies (1:500, Abcam) followed by color development using DAB substrate solution.

Quantification of T-lymphocyte population using flow cytometry

We stimulated the splenic T cells with phorbol 12-myristate 13-acetate (SigmaAldrich) (50 ng/mL) and ionomycin at 800 ng/mL (Sigma-Aldrich) for 5 h. Two hours with Protein Transport Inhibitor (Invitrogen). CD8 population stained using anti-CD8-FITC conjugatefrom eBioscience were used. The analysis was performed by using a FlowSight system (Amnis, Millipore, Germany).

Statistical analysis

Data were pooled from three independent experiments as the mean ± standard deviation (SD) using three mice per experiment unless otherwise indicated. The mean values were tested for statistical significance by regression plot for viability and biochemical parameters and student T test Post-Hoc test for quantification of slides, biochemical quantification, PCR expression and protein blot expressions. Variations are considered to be significant when $p < 0.05$.

RESULTS

Pinocembrin effects on the clinical signs and myelin protein modifications in vivo

First, we confirmed by the EAE disease severity index and body weight in our MS model. Next, we evaluated the role of PCB in ameliorating the clinical severity of MOG-induced EAE by scoring clinical symptoms everyday on a 0–5 scale. The disease signs treated groups two weeks after immunization (day 0) and at the end of the experiment are summarized in Figure 1A and B. The clinical symptoms score was higher in the EAE mice and significantly reduced in EAE + PCB groups two weeks after the immunization compared to controls (all groups, $p < 0.05$). The symptoms score increased at the end of the experiment in EAE group ($p < 0.05$). The PCB gradually controls the symptoms at the end of the experiment ($p < 0.05$) compared to the beginning of induction (day 0). The mean of disease severity in the un-treated group was higher than PCB

treated groups. Further, administration of PCB apparently decreased disease severity compared to the PBS-treated control group ($p<0.01$; Fig. 1A). At the end of the study, PCB significantly alleviates the disease symptoms and reduces the clinical disease signs compared to untreated EAE group. We examined further relationship between the myelin protein and apoptosis of CNS were recorded.

At the end of experiments, histochemical analysis of PCB treated group was compared with untreated group as shown in Figure 1C. The PCB treated mice showed suppressed infiltration of inflammatory cells. The CD4 and CD8 were also counted. The altered myelination architecture in EAE group and microenvironment was improved in PCB treated group. These results indicated that histopathology of CNS in mice treated with PCB was associated with clinical signs in mice. That was further confirmed by expression of myelin basic protein (Fig. 1D). The PCB group showed upregulated expression of MBP protein and this was confirmed by western blot. It was significantly overexpressed (p>0.05) compared to EAE group and (p>0.02) against untreated PBS group.

Effect of PCB on regulation of inflammatory biochemical mediators and splenocytes

The anti-oxidant and inflammatory biochemical mediators such as melondialdehyde (MDA), Lipid peroxidase (LPO) and superoxide dismutase (SOD) were quantified as activity index and PCB significantly suppress the MDA and LPO activity in CNS and upregulates the SOD level in myelin physiology sites (Fig. 2A). Next the mice spleen mononuclear cells were isolated and cultured for 72 h in the presence of 2ng/ml of MOG35-55.

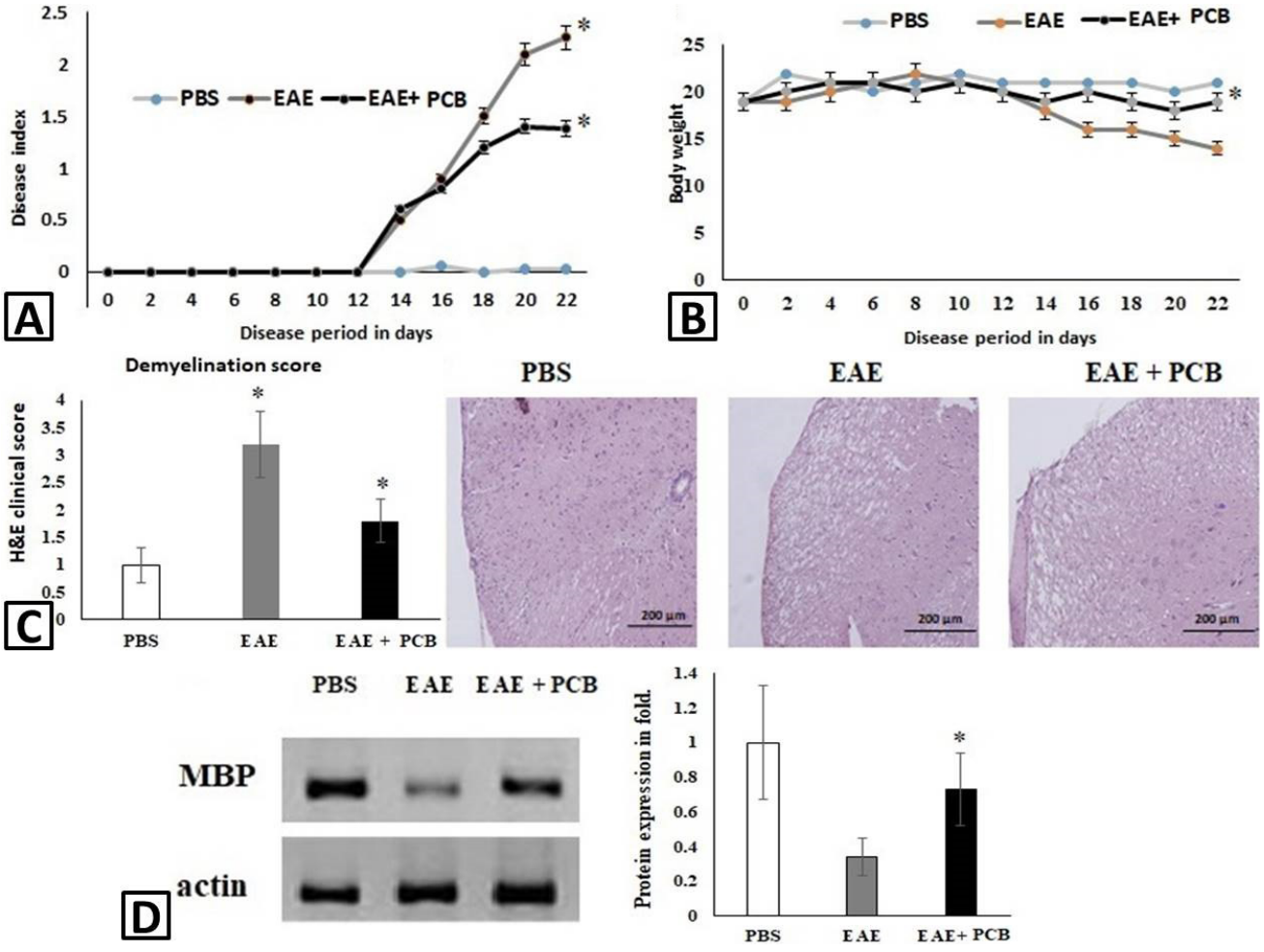

Fig. 1. The effect of PCB (pinocembrin) on EAE (experimental autoimmune encephalomyelitis) mice in C57bl/6j mice model. Mice were immunized with MOG and pertusis toxin. From the day of immunization mice were received PCB 100mg/kg of mice body weight. A, clinical signs were graded as a described in material and methods; B, body weight; C, H&E of spinal cord; D, MBP immunoblot. Values are expressed using student t-test and one-way ANOVA (significant at $p \leq 0.05$).

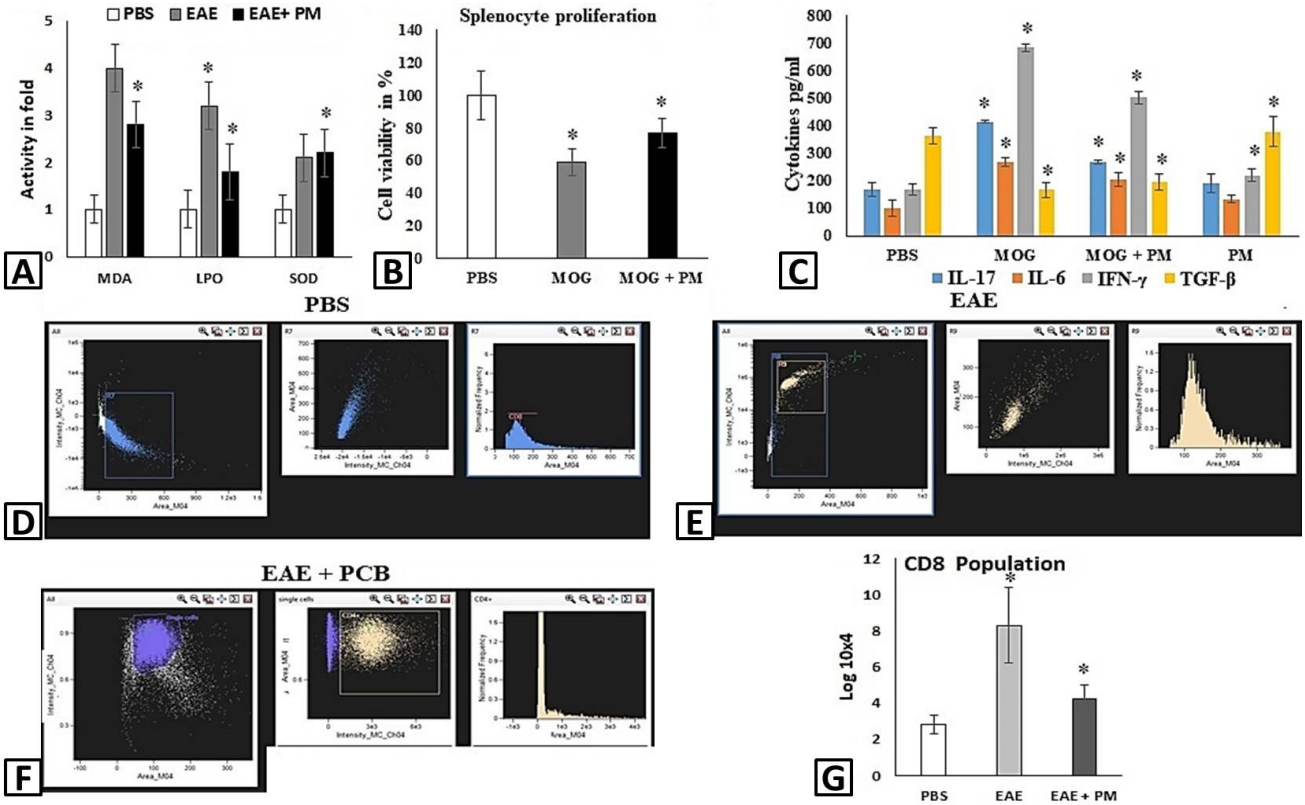

Fig. 2. Biochemical assays in mice brain and CNS. The splenic cells from naïve mice were analyzed for proliferation and MOG challenged cytokines estimation. T-cell population were counted in PCB treated EAE mice. A, MDA, LPO and SOD activity were scored in PCB treated EAE mice; B, effect of PCB on cell viability of mice splenocytes; C, effect of PCB on MOG induced splenocytes cytokines level (IL-17, IL-6 IFN-γ and TGF-β) are quantified. For each group values were presented as mean ± SD. One way ANOVA followed by post hoc t-test were used for statistical analysis (significant at $p \leq 0.05$).

The PCB treated splenocytes showed significant proliferation and improved viability compared to MOG group (Fig. 2B). The MOG treated cells express IFN-ɣ, which was significantly (p value) suppressed in PCB group compared with non-treated group of EAE model mice (Fig. 2C). In addition, the amount of IL-17 secreted from Th17 cells was significantly (p lower in the PCB treated group compared to the non-treated group. In contrast, IL-6 cytokines in the PCB treated group was significantly inhibited compared to the non-treated MOG group. Moreover, the reciprocal regulation of IFN-γ and TGF-β were observed in PCB treated MOG challenged splenocytes (Fig. 2C).

Effect of PCB on CD8 population in spleen of MOG induced EAE mice

The pathogenesis of EAE has revolved primarily T cell mediated activities. The CD8 plays a key role in EAE disease progression and pathological contributions. In the current study, the CD8 population was quantified and it is significantly increased in EAE mice (Fig. 2D). The Total T lymphocytes were isolated and the population was grossly increased in EAE disease group. PCB treatment reduced the severity through the inhibition of CD 8 differentiation of T lymphocytes. The total CD8 population in PCB treated group was 4.1 x 10^4 is 50% population of EAE group ($p > 0.05$).

In silico *docking of PCB ligand against Caspase-3 receptor*

In silico evaluation of apoptogenic marker caspase-3 against PCB was studied using auto dock docking tools. The PCB act as ligand and mouse Caspase-3 act as receptor. The PCB bound with Caspase at active site pocket, it is competitive to substrate binding at a site. Figure 1A showed docking details between PCB with Casp-3 and found two hydrogen bond docked with short Armstrong length (Table II). These results revealed that, the bond was covently formed with binding energy -5.4 against arginine and cysteine amino acid residues (Fig. 3C). PCB binding pockets against caspase-3 observed (Fig. 3D, E).

Table II.- *In silico* docking of Pinocembrin against caspase-3 and sirtuin-1 proteins. Hydrophobic interaction of pinocembrin and amino acid residues of target proteins.

S. No.	Target proteins	Binding energy	Ligand efficiency	Intermole energy	Ligand atoms (ring)	Docked amino acid residue (bond length)
1.	Caspase-3	-4.16	-0.22	-4.77	C-H	ARG164 (1.9 Å)
2.	Sirtuin-1	-4.43	-0.44	-8.87	C1-OH	ALA'262 (2.1 Å)
					C1-NH	SER`442/HN (2.2 Å)
					C1-NH	LYS`444/NH (1.9 Å)

ARG, Arginine; ALA, Alanine; SER, Serine; LYS, Lysine.

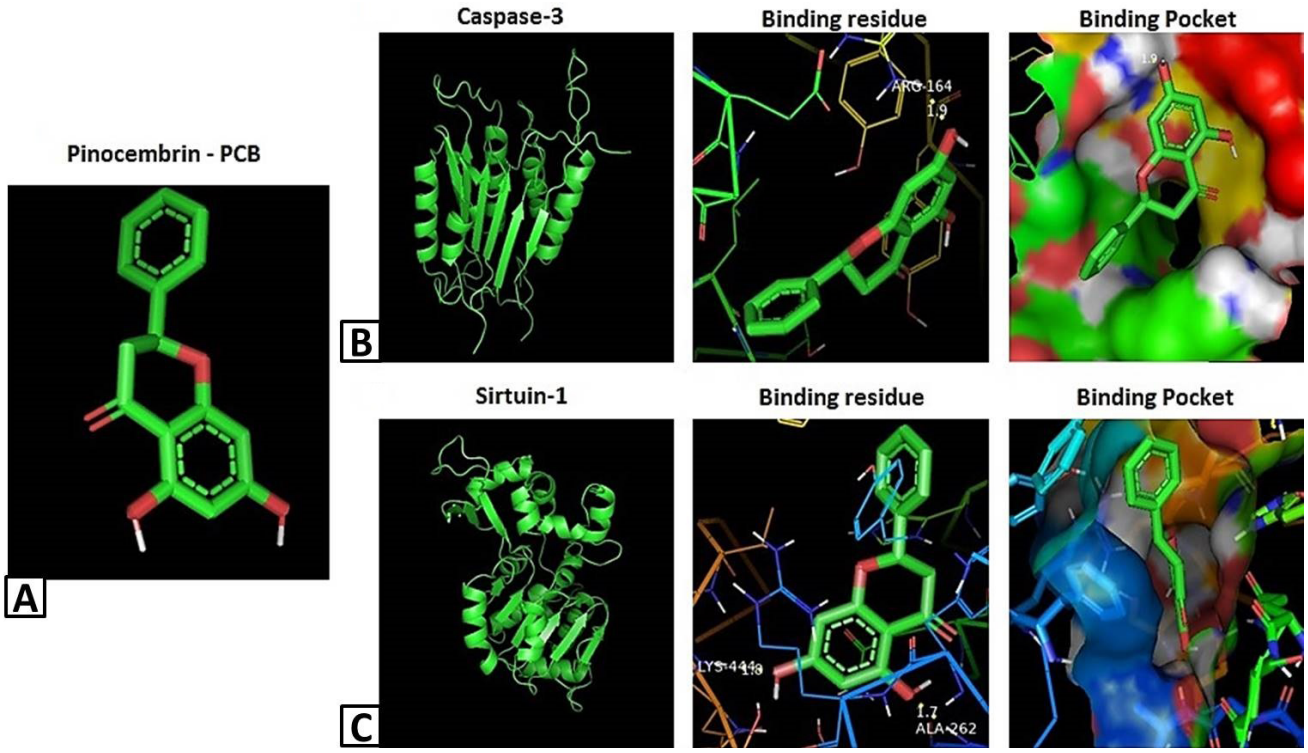

Fig. 3. *In silico* evaluation of apoptogenic marker caspase-3 against PCB. A, 3D structure of PCB; B, 3D structure of caspase-3; C, PCB Docked with caspase 3 by two H-bond; D, arginine with cysteine; E, binding pockets of PCB against caspase 3. Docking was performed using autodoc4.2 software.

Effect of PCB on MBP protein and apoptosis

The MBP plays a key role in central nervous cells and myelin communication. EAE group significantly damaged the monopole of neuronal cells. The PCB increased the expression of MBP protein and it was confirmed in CNS histology (Fig. 4A). The MBP protein reciprocal regulated the apoptosis of myelin cells. That was further confirmed by caspase estimation. The PCB treated groups showed decreased apoptotic bodies in CNS tissue, on the other hand, the caspase-3 level was overexpressed in EAE group (Fig. 4B). PCB treatment improved the MBP protein and it was further confirmed by immunofluorescent system.

PCB regulates the inflammatory markers related to multiple sclerosis

PCB suppresses the expression of immune triggering molecules and augments the inflammatory balancing molecules. The mechanism of inflammatory response regulated by PCB was analyzed and showed inhibitory activity against Caspase-3 and CCL-2 markers ($p>0.05$) compared to control untreated groups (Fig. 5A). PCB negatively regulated the mRNA and protein expression of inflammatory markers. On the other hand, it polarized the TGF-B, SIRT-1 and MBP markers. This was confirmed parallel for the both analysis (Fig. 5A, B).

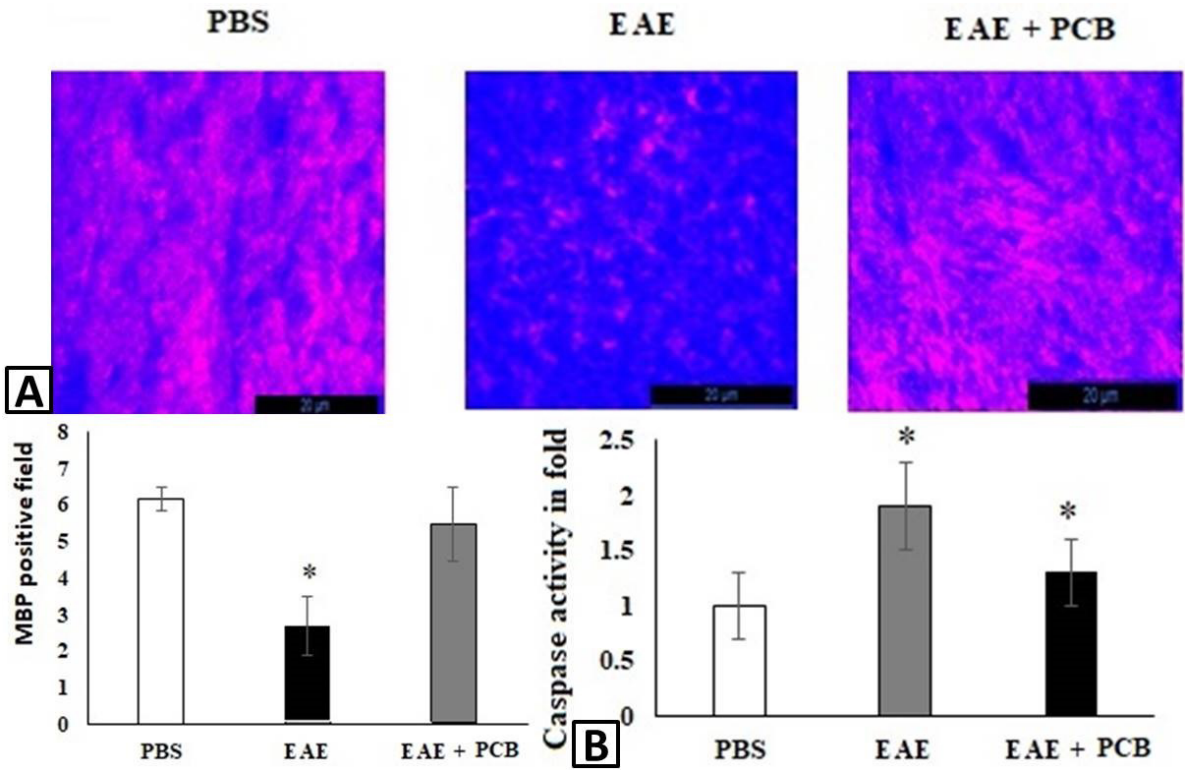

Fig. 4. Immunofluorescence quantification of myelin basic protein to assess myelin sheath damage after 16 days post MOG induction. A, Myelin sheath marker MBP (red). The myelin damage in the subcortical white matter of spinal cord was alleviated in PCB group at 16 days of MOG induction; B, Caspase 3 activity to asses apoptotic marker in CNS. Significant improvement of cell damage observed at PCB treated groups. For each group values were presented as mean ± SD. One way ANOVA followed by post hoc t-test were used for statistical analysis (significant at p. ≤ 0.05).

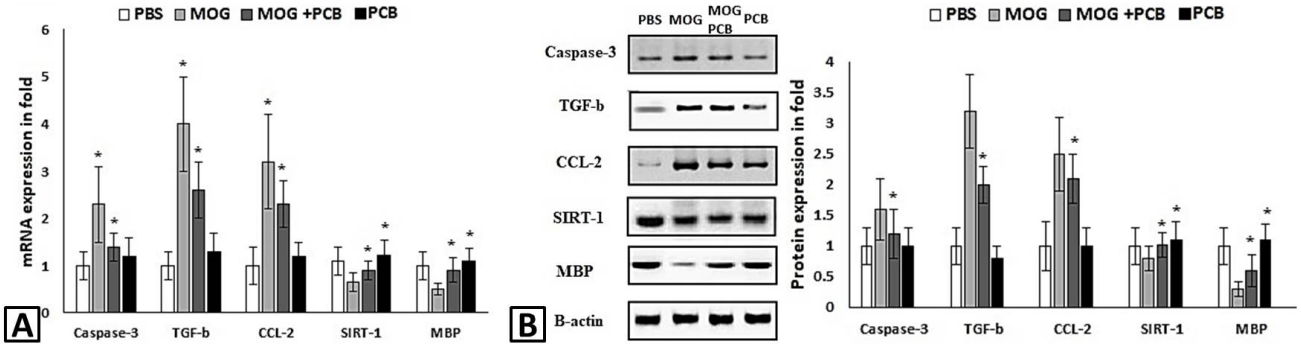

Fig. 5. Expression level of caspase-3 TGF-β, CCL-2, SIRT-1, MBP mRNA and protein *in vivo*. A, mRNA of caspase-3, TGF-β, CCL-2 were downregulated in mouse model after PCB treatment. Whereas, SIRT1 and MBP were upregulated in PCB group compared with untreated EAE group; B, protein expression was observed similar like mRNA expression. GAPDH used as internal control for mRNA. Beta actin used as internal control for protein. For each group values were presented as mean ± SD. One way ANOVA followed by post hoc t-test were used for statistical analysis (significant at p. ≤ 0.05).

The SIRT-1 activation controls the neuronal health and microglia microenvironment. The insignificant activation was observed in protein blot and mRNA showed ($p>0.03$) comparatively significant activation.

DISCUSSION

In the present study, we investigated the role of the antioxidant flavonoid, PCB (Hanieh *et al*., 2017; Ahmed

et al., 2021), in ameliorating the clinical severity of MOG-induced EAE. The severity of disease was scored after two weeks of immunization day (day 0). Signs scoring were evaluated on a 0-5 scale, where MS group showed a significant higher sings compared with MS and PCB groups within two weeks of immunization ($p < 0.05$). Even after 21 days of in immunization EAE group showed a higher scored signs. Disease signs decreased in PCB group indicating an ameliorating role on MOG induced MS conditions. Administration of PCB decreases significantly the disease signs even at the end of the experiment. PCB showed anti-inflammatory activity and neuroprotective characters (Lan *et al*., 2016). Furthermore, PCB found to alleviate the oxidative stress in the brain by reducing pro-inflammatory cytokines (Saad *et al*., 2015). PCB also inhibited the cell apoptosis through decreasing the concentration of ROS and MDA and increasing SOD activity therefore PCB is recommended to be an effective agent in reduction of oxidative stress (Wang *et al*., 2020).

Multiple sclerosis is a rapid progressed neurodegenerative disease in the central nervous system resulting from degeneration of myelin and most of patients succumb for mortality (Steen *et al*., 2015). EAE is a good animal model for MS where it was used to investigate the mechanisms of pathogenesis (immunological and pathological characters) of MS that induced in mice by injection of myelin-derived components like MOG, MBP and PLP (Basler *et al*., 2014). It is used to evaluate the immunosuppressive drugs (Halmer *et al*., 2015) and is mediated by CD^{4+} T cells. MS is a complicated and debilitating disease with high recurrence rate endangering millions of people all over the world (Dendrou *et al*., 2015). Treatment of MS is still limited due to the severe side effects of the chemically synthesized drugs (Bhise and Dhib-Jalbut, 2016). Attention paid towards the natural product due to its effectiveness and safety use. Histological analysis after 22 days showed that the PCB treated group showed less inflammation if compared by MS group. The myelination architecture was altered in EAE group and the myelination was improved (Fig. 2). Myelination is reported to be improved by PCB (Fig. 2). PCB was also found to work as a potent neuroprotective compound against spinal cord injury (Lan *et al*., 2017). The brain hemorrhage was reported to be ameliorated by PCB. Our histological analysis showed a significant protective effect of PCB of the histology of the spinal cord in comparison with the EAE group (Fig. 1C). Scoring of the histology recorded that altered myelination in EAE group is improved using PCB (Fig. 1C). These investigations were recommended by other studies where PCB was found to have a neuroprotective effect against many oxidative stress agents (Zhang *et al*., 2014; Talebi *et al*., 2017). PCB also upregulated the expression of myelin binding protein (Fig. 1D).

The cultured spleen monocytes recorded a high viability and proliferation rate (Fig. 2A, B) in PCB group when compared with that of EAE group. The cytokine profile is improved significantly when compared by the MS one. The pro-inflammatory cytokines such as IL-17 and IL-6 were reduced in the PCB group which improved the oxidative status of CNS. PCB was reported by many studies to significantly ameliorate the pro-inflammatory cytokines and inflammation of the CNS (Lan *et al*., 2017; Soromou *et al*., 2014). The pathogenesis of EAE has revolved primarily T cell mediated activities. The CD8 plays a key role in EAE disease progression and pathological contributions. The results of the current study showed, the cell population of CD8 cells was reduced up to 50% in the PCB treated group when compared by that of MS group (Fig. 2G). This recommended by the histological analysis and qPCR where results revealed that PCB reduction demyelination, CD4, CD8 when compared with EAE group. Th-17 is a subset of CD^{4+} those attracted to the CNS during inflammation. Th-17 is maturated in the presence of both IL-6 and TGF-β cytokines and secretes IL-17 cytokine that play an important role in inflammation (Granados-Pineda *et al*., 2018; Glatigny and Bettelli, 2018). So, the reduction of IL-17, IL-16, IFN-γ and TGF-β happened in results of PCB treated groups (Fig. 2C), prove the protective role of PCB in the alleviation of MS conditions. This finding is supported by many studies those recommend the protective role of PCB in the protection of the CNS from the oxidative molecules (Lan *et al*., 2017).

Prophylactic effect of PCB significantly reduced the disease symptoms and demyelination of central nervous system. PCB treated mice improved spinal cord architecture significantly; the migration and infiltration of inflammatory cells and its mediators were attenuated compared to untreated EAE group. Moreover, PCB pretreatment inhibits the infiltration and polarization of T-lymphocytes. The CD4/IL-17 and IFN-γ positive cells in spinal cord were augmented by PCB treatments. CD8 population count was also suppressed in treated mice.

Regarding the investigation of EAE mice (Prozorovski *et al*., 2019) found that, upregulated SIRT family transcription factors in CNS of disease mice compared to naïve mice. The differentiated cells express upregulated SIRT-1 protein compared to undifferentiated progenitor cells (Papadopoulos *et al*., 2020). Thus, SIRT1 plays a key role in apoptotic activity (Rezaei *et al*., 2019). The epigenetic modification of P53 was inhibited by SIRT-1. The further results indicated that, PCB regulates the mRNA of demyelination mechanisms through

regulation of SIRT-1 and CCL-2 markers. CCL2 markers are important factors for development and progression of neuroinflammation. The lack of CCL-2 signaling receptor in CNS showed resistant to EAE pathogenesis (Noorbakhsh *et al*., 2006), it was correlated in this study. CCL-2 inhibition activated the myelination recovery and enhanced the improvement of microenvironment (Noorbakhah *et al*., 2006). Accumulating results revealed that, PCB treated mice showed reduced inflammatory and demyelination markers and prevent the apoptosis mediated cell damage and alleviate theCD8 population in CNS.

CONCLUSION

The present study clearly demonstrated that consecutive oral administration of PCB improves neurodegenerative symptoms, controls infiltration in spinal cord, suppresses the apoptosis of myelin cells and reciprocal regulation on SIRT-1 and CCL-2 markers, respectively, in MOG induced EAE mice models. Histopathological studies stand as perfect evidence for the above results by reverting the cell damages with oral administration of PCB. The biochemical studies on the Caspase-3 and MBP estimations indicate the neuroprotective effect of pinocembrin on EAE mice. These data support the idea that PCB may be an effective therapeutic approach for treating MS and other autoimmune-based neurodegenerative diseases.

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Ethics approval and consent to participate

No human participants were involved in this study. Animal studies were followed by ethical guidelines of Deanship of scientific research, King Faisal University.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210225080232

Statement of conflict of interest

The authors have declared no conflict of interests.

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