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Original article

Mitigating effects of *Passiflora incarnata* on oxidative stress and neuroinflammation in case of pilocarpine-Induced status epilepticus model

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ABSTRACT

Virulent epileptogenic insult is still one of the most life-threatening deficits during the clinical consequences and neurological emergencies of epilepsy. Thus, this study is designed to determine the changes in the expression of neurotransmitters, imbalance in blood electrolytes; oxidative stress, levels of pro-/anti-inflammatory cytokines, after treatment with pasipay (*Passiflora incarnata*). The effect of oral administration of 200 mg/kg body weight of *P. incarnata* on pilocarpine-induced seizures was assessed. The correlation between seizure activity and levels of proinflammatory cytokines; anti-inflammatory cytokines; oxidative stress biomarkers, monoamines, neurotransmitters; electrolytes and Th1/Th2 activities was quantified. The present study revealed a highly significant amelioration in amino acids, neurotransmitters, blood electrolytes, antioxidants and inflammatory cytokines in epileptic-treated rats with pasipay (*P. incarnata*). In conclusion, the present study supports further attempts to abrogate the neural dysfunction via antioxidant and anti-inflammatory cascade activities using *P. incarnata*.

1. Introduction

An epileptic seizure is a kind of brain dysfunction caused by over discharge. In the injured brain, endothelial cells release cytokines, activating T cells and triggering acquired immune disorders, thus leading to the pathogenesis of epilepsy (Huang and Zhu, 2018). Damage of blood–brain barriers (BBB) causes entry of inflammatory immune cells. Activation and neutrophil recruitment by kinin lead to mitochondrial alterations, microglial activation and generation of reactive oxygen species (ROS). ROS, in turn, cause oxidative damage, which alters the cellular tight junctions. In addition, other animal studies revealed that breakdown of BBB leads to transcriptional changes in the neurovascular connections and eventual neurodegeneration (Shlosberg et al., 2010). The inflammatory processes represent key contributors to acute and chronic neurological disorders, such as epilepsy (Poole et al., 2000). IL-1β plays a role in the initiation and progression of neuronal dysfunction during epilepsy.

The antiepileptic drugs (AEDs) of epilepsy is associated with dose-related and chronic toxicity, and teratogenic effects (Poole et al., 2000; Ropper, 2005). Drugs based on natural products and folk remedies can offer safe and efficient alternative for AEDs (Raza et al., 2003).

Passiflora species have gained worldwide prominence because of their varied content of phyto-constituents of high therapeutic value that represent potential raw material for the development of new drugs. Amongst the 500 species of the genus Passiflora, *Passiflora incarnata* (*P. incarnate*) is the one that has extensive clinical

Abbreviations: AEDs, Antiepileptic drugs; BBB, Blood-brain barrier; CAT, Catalase; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GABA, gamma-aminobutyric acid; IL4, Interleukin 4; GSH, glutathione; GR, glutathione reductase; GPx, glutathione peroxidase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDA, malondialdehyde; IkB, inhibitor of kB kinase; FAS, Cell surface death receptor; SOD, Superoxide dismutase; Th1/Th2, T helper1/T helper2; TNF-α, Tumor necrosis factor-α; PILO, Pilocarpine hydrochloride; L-DOPA, L-dihydroxyphenyalanine.

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applications throughout the world. *P. incarnata* Linneaus possesses significant CNS depressant properties (Dhawan et al., 2004). The drug is highly effective in convulsions when given prior to an approaching attack. *P. incarnata* is praised for its control over meningeal inflammations and their consequent spasms of childhood (Dhawan et al., 2004).

Various phytoconstituents were reported to be present in *P. incarnata* including carbohydrates)Dhawan et al., 2004), amino acids, and a cyanogenic glycoside gyanocardin. In addition, monoflavonoid of *Passiflora* sp. was reported to reduce morphine withdrawal *in vitro* (Capasso et al., 1998). Chrysin (5,7-dihydroxyflavone), one of the monoflavonoid found in *Passiflora sp*., has been used as an anxiolytic anticonvulsant)Zanoli et al., 2000). Chrysin, a natural product found in a variety of flowers and fruits, has received attention because of its potent antioxidant and anti-inflammatory properties (Ahad et al., 2014; Hajimohammadi and Nosrati, 2018).

Despite the well-documented phytochemical reports on *P. incarnata*, the exact mode of its anti-inflammatory and antioxidant effects and the phyto-constituents responsible for its much-acclaimed effects have never been described clearly. Moreover, only few studies focusing on the potential role of *P. incarnata* as an anti-inflammatory and antioxidant herbal food supplement in epileptic models are available. The study aims at assessing the ameliorative changes after treatment with *P. incarnata* in case of pilocarpine-induced epilepsy in male albino rats.

2. Materials and methods

2.1. Animals and ethics committee approval

White thirty-two Sprague Dawley adult male rats (150–180 g) were obtained from the animal house of the National Research Institute, Eldoki, Giza, Egypt. They were housed in individual cages (23± 1 °C; humidity, 55± 5%, 12-h light/dark cycle). Food and water were available ad libitum. All animal procedures were in accordance with the guidelines for the use of experimental animals by the AEC of Beni-Suef University under an approval number is BSU/FS/020/102.

2.2. Preparation of Passiflora incarnata

An extract of passionflower *Passiflora incarnata* L was as herbal medicine. It was obtained from the dietary supplement manufacturer, Naturactive Co. (Elusanes Passiflore; Lot# PAS K00018; Pierre Fabre Medicament Prod., 45, Place Abel Gance, 92100 Boulogne). According to the entire pharmacopeia leaflet, the active substance for each capsule is *Passiflora incarnata* L. Each capsule contains the dry extract of the aerial parts of *Passiflora incarnata* L. The dose of each capsule was calculated as 200 mg on maltodextrin and colloidal silica hydrate. Extraction Solvent: 70% Ethanol (V/V).

2.3. Induction of epilepsy in the rats using pilocarpine and animal groups

Epilepsy was induced as previously performed (Turski et al. 1989; Abdel-Reheim 2009). Prior to treatment with 300 mg/kg of pilocarpine (New Jersey, USA) hydrochloride injection, the experimental rats were intraperitoneally injected with methylscopolamine (Nanjing, China) (1mg/kg) for 30 min. Then, animal's behavior was evaluated as indicative of seizure activity was assessed. These criteria for successful modeling: epileptic seizure symptoms (sluggishness, salivation, tremors, convulsions, etc.). Such behaviors were observed in model rats for 120 min in comparison to the normal rats. Rats were considered suffer from seizure episodes when generalized seizure activity was continuously observed without normal behavior during each episode. Thus, the attacks occurred every 2–5 min. Seizures were terminated with diazepam (4 mg/kg, i.p.) delivered every 20 min as needed. Control rats were treated with PBS (pH 7.4; 0.2 ml/rat) was injected instead of pilocarpine, followed, 1 h later, by diazepam.

Rats were divide into four groups (n = 8) as follows: (1) Control group (C) received the standard diet, free access to water and orally fed with PBS (pH 7.4; 0.2 ml/rat) using intragastric intubation at intervals parallel to the treated groups. (2) *P. incarnata* group (PI) was orally fed with *Passiflora* extract using intragastric intubation at a dose of 200 mg/kg b.wt/day, continued for four consecutive weeks; (3) the positive epileptic group, epileptic control "E" was treated with 300 mg/kg of pilocarpine hydrochloride injection as described previously; and (4) Epileptic treated with *P. incarnate* (E-PI) (300 mg/kg of pilocarpine hydrochloride injection, then orally fed with *Passiflora* extract at a dose of 200 mg/kg b.wt/day; continued for four weeks).

2.4. Blood, tissue sampling and biochemical measurements

Blood samples were collected in two parts, EDTA-anticoagulated and EDTA-free samples. Plasma were stored at −80 °C for subsequent measurement of cytokines by ELISA in duplicates. Sera samples were separated for electrolytes. Brain tissues were cleaned from the fatty parts and homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.4). The supernatant was separated and lipid peroxidation level (MDA) was estimated according to the methods of Preuss et al. (1998), and glutathione reductase (GR) was estimated as previously mentioned (Kar and Mishra 1976).

On the other hand, MyBioSource Co. assay kits were used for quantitative detection of L-dihydroxyphenyalanine (L-DOPA; μg/ml); serotonin (ng/ml); epinephrine (ng/ml); Norepinephrine (pg/ml); Na+/K+ATPase (mmol/ml); glutamate (μg/ml); aspartic acid (ng/ml); gamma-aminobutyric acid (GABA, pg/ml) and glycine (μg/ml); using kits with catalogue numbers (MBS9357024, MBS775307, MBS031232, MBS269993, MBS8243226, MBS047402, MBS7254596, MBS269152, MBS095285, respectively). Creatine kinase (CPK, U/L) activity was estimated using EnzyChrom™ Assay Kit (Cat. No: ECPK-100). Acetylcholinesterase (U/L) was performed according to the manual of QuantiChrom™ Assay Kit (Cat. No: DACE-100).

According to the instruction manual of MyBioSource; sera concentration of Sodium (Na^+, mmol/L) and Potassium (K^+, mmol/L) were estimated using kits with Cat. No. MBS2540574 and MBS2540590, respectively. On the other hand, Calcium (Ca^{+2}, mg/dL) and Magnesium (Mg^{+2}, mg/dL) levels were quantitatively determined as the manual of QuantiChrom™ Assay (Cat. No: DICA-500 and DIMG-250, respectively).

Level of cytokines were estimated in plasma, according to ELISA quantitative instruction manuals e.g. IL-1β and TNF-α (RayBiotech™; Cat. No: ELR-IL1β & ELR-TNFα, respectively), IL-6 and IL-10 (Quantikine®; Cat. No: R6000B & R1000, respectively), IL-13 and TGF-β (using Picokine™ Cat. No: MBS175932 & MBS175833) and IL-17 (using CUSABIO Cat. No: CSB-E07451r).

Regarding antibodies for Flowy cytometry, cells were stained with mAbs and analyzed using a FACSCalibur (BD, Franklin Lakes, NJ) according to Neu et al. (2013) and Abuelsaad (2014). Briefly, puried lymphocytes from brain tissues (1 x 10^6 cells/50 ml PBS) were washed once with washing buffer (3% (v/v), FBS and 0.1% (w/v) NaN_3 in PBS. Cells were resuspended in blocking buffer (3% (v/v) FBS; 5% (v/v) normal human AB serum (Cat# C11-020; PAA Laboratories, Germany and 0.1% NaN_3 (w/v) in PBS) with puried CD16/CD32 FccII/III mAb; AbD Serotec Co, USA) to prevent non-

specic binding. Cells were incubated with mAb Fluor-conjugated FITC mouse anti-rat antibody purchased from AbD Serotec Co, USA as follows: CD4-FITC (MCA153F, respectively), and PE-conjugated anti-CD8 (CAT# MCA48PE). Subsequently, cells were washed, xed in paraformaldehyde (PFA; 4% (v/v) in PBS; Sigma Aldrich, Germany) and stored at 4 °C in washing buffer until further use. A FACS Calibur flow cytometer was used for data acquisition, with Diva software (BD Biosciences) for data analysis. After gating on viable cells, 10,000 events per sample were analyzed. For each marker, the threshold of positivity was dened beyond the nonspecic binding observed in the presence of a relevant control mAb.

2.5. Statistical analysis

The data were analyzed using Tukey-Kramer method for post-hoc analysis to compare various groups with each other. The results were expressed as mean ± standard deviation (SD). Statistical significance interval is considered as $P < 0.05$ for all data. All results were analyzed using Statistical Package for Social Science (SPSS) version 20 software (IBM Corp., 2011).

3. Results

3.1. Oxidative stress

Table 1 reveals an altered oxidative/antioxidant status and shows a significant increase ($P < 0.001$) in MDA level in the epileptic non-treated group (E) after pilocarpine injection. MDA recorded the highest level in epilepsy group of 579.631 ± 66.56 nmol/g tissue, compared to the control (C), PI, and E-PI groups (324.77 ± 54.21, 252.82 ± 30.05 and 467.66 ± 32.26 nmol/g tissue, respectively). Therefore, treatment with *Passiflora* extract (200 mg/kg) showed ameroliative effects on brain lipid peroxidation and modified the altered level of MDA.

In addition, treatment with *P. incarnata* caused a significant increase ($P < 0.001$) in the antioxidant enzymatic levels of glutathione reductase, GR (57.867 ± 9.84 nm/mg tissue), SOD (8.74 ± 1.44 U/g tissue) and CAT (431.60 ± 11.03 U/g tissue). These values were compared to the dramatic decrease in the epileptic non-treated group which recorded 36.800 ± 3.64 nm/mg tissue, 13.134 ± 2.64 and 477.962 ± 50.20 U/g tissue for GR, SOD and CAT, respectively.

3.2. Changes in brain electrolytes

Regarding the changes in brain electrolytes, pilocarpine injection caused dramatic decrease in K^+, Mg^{++} and Ca^{++} levels in brain tissues, recording 3.05 ± 0.08 mmol/L; 0.29 ± 0.08 mg/dL and 8.00 ± 0.25 mg/dL, respectively (Fig. 1). In addition, Na^+ recorded the highest level in the epileptic non-treated group (189.32 ± 7.62 mmol/L). These imbalanced levels were corrected after treatment with *Passiflora incarnata* extract (200 mg/kg); and recorded 164.66 ± 5.27 mmol/L; 4.07 ± 0.47 mmol/L; 0.698 ± 0.04 mg/dL and 8.63 ± 0.21 mg/dL for Na^+; K^+, Mg^{++} and Ca^{++}, respectively.

In addition, Fig. 2 shows a significant decrease ($P < 0.001$) in the level of Na^+/K^+-ATPase in brain tissues (an electrogenic transmembrane ATPase) after induction of status epilepsy, which recorded 2.11 ± 0.17 mmol/ml. The treatment with *P. incarnata* remodified Na^+/K^+-ATPase to the normal level 4.57 ± 0.34 mmol/ml as recorded in the control group (4.44 ± 0.38 mmol/ml). Moreover, creatine kinase (CK) showed a highly significant increase of 294.35 ± 12.53U/L in the second group (E) and returned to 183.72 ± 5.34 U/L after treatment with *P. incarnata*, though still significantly higher than the control group (111.23 ± 5.92 U/L).

3.3. Changes in neurotransmitters

In addition, the present data revealed significant decrease ($P < 0.001$) in different neurotransmitters and classical monoamines, e.g. norepinephrine (NE) and epinephrine (EP), whereas they decreased in the epileptic non-treated group, recording levels of 43.17 ± 5.75 and 32.10 ± 5.37 Pg/ml, respectively. Treatment with *P. incarnata* mitigated and upregulated them again to the healthy levels of 67.37 ± 4.62 and 48.65 ± 3.59 for norepinephrine and epinephrine, respectively (Fig. 2).

Regarding changes in other monoamines, e.g. dopamine (DOPA) and serotonin (5-HT), the epileptic non-treated group demonstrated a significant increase ($P < 0.001$) in DOPA (57.70 ± 3.77 μg/ml) and a significant decrease ($P < 0.001$) in serotonin level (39.78 ± 2.56 ng/ml), as compared to the control group, which recorded 24.60 ± 2.36 μg/ml and 77.07 ± 7.90 ng/ml for DOPA and serotonin, respectively (Fig. 3). Moreover, the treatment with *P. incarnata* modified both DOPA and serotonin levels, approximating their normal values of 37.43 ± 3.53 μg/ml and 67.72 ± 4.05 ng/ml, respectively (Fig. 3).

The effects of status epilepticus on the brain concentrations of glutamate, gamma-aminobutyric acid (GABA), aspartate and glycine were measured, as illustrated in Fig. 3. As mentioned in the epileptic non-treated group, GABA and glycine showed significant decrease level (13.82 ± 2.17 pg/ml and 4.13 ± 0.88 μg/ml, respectively), whereas glutamate and aspartic acid levels showed significant increase (14.58 ± 1.02 μg/ml and 13.60 ± 0.90 ng/ml, respectively). Treatment with *P. incarnata* modified the imbalanced levels of glutamate, γ-aminobutyric acid (GABA), aspartate and glycine to normal values of 8.165 ± 0.613 μg/ml; 25.338 ± 1.601 pg/ml; 9.022 ± 0.621 ng/ml and 8.000 ± 0.947 μg/ml, respectively.

4. Phenotyping of $CD4^+$ and $CD8^+$ brain infiltrative cells

Meanwhile, phenotyping of $CD4^+$ and $CD8^+$ brain infiltrative cells is illustrated in Table 2 and Fig. 4 by FACS-analysis. Changes

Table 1
Changes in the brain oxidative/antioxidant markers of different groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (E-PI) (200 mg/Kg. b.wt). Groups not sharing common superscripts denote significant differences (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

Groups	MDA (nmol/g tissue)	Glutathione reductase (nm/mg tissue)	SOD (U/g tissue)	Catalase (U/g tissue)
C	324.765 ± 54.208^{a}	47.914 ± 1.380^{bc}	10.286 ± 1.419^{bc}	490.053 ± 20.993^{b}
E	579.631 ± 66.562^{b}	25.543 ± 4.284^{a}	4.599 ± 0.610^{a}	358.342 ± 28.354^{a}
PI	252.822 ± 30.055^{a}	36.800 ± 3.637^{ab}	13.134 ± 2.642^{c}	477.962 ± 50.196^{b}
EP_PI	467.661 ± 32.265^{b}	57.867 ± 9.843^{c}	8.741 ± 1.442^{b}	431.595 ± 11.027^{b}
F value	29.824	16.654	13.733	12.204
P <	0.000	0.001	0.001	0.002

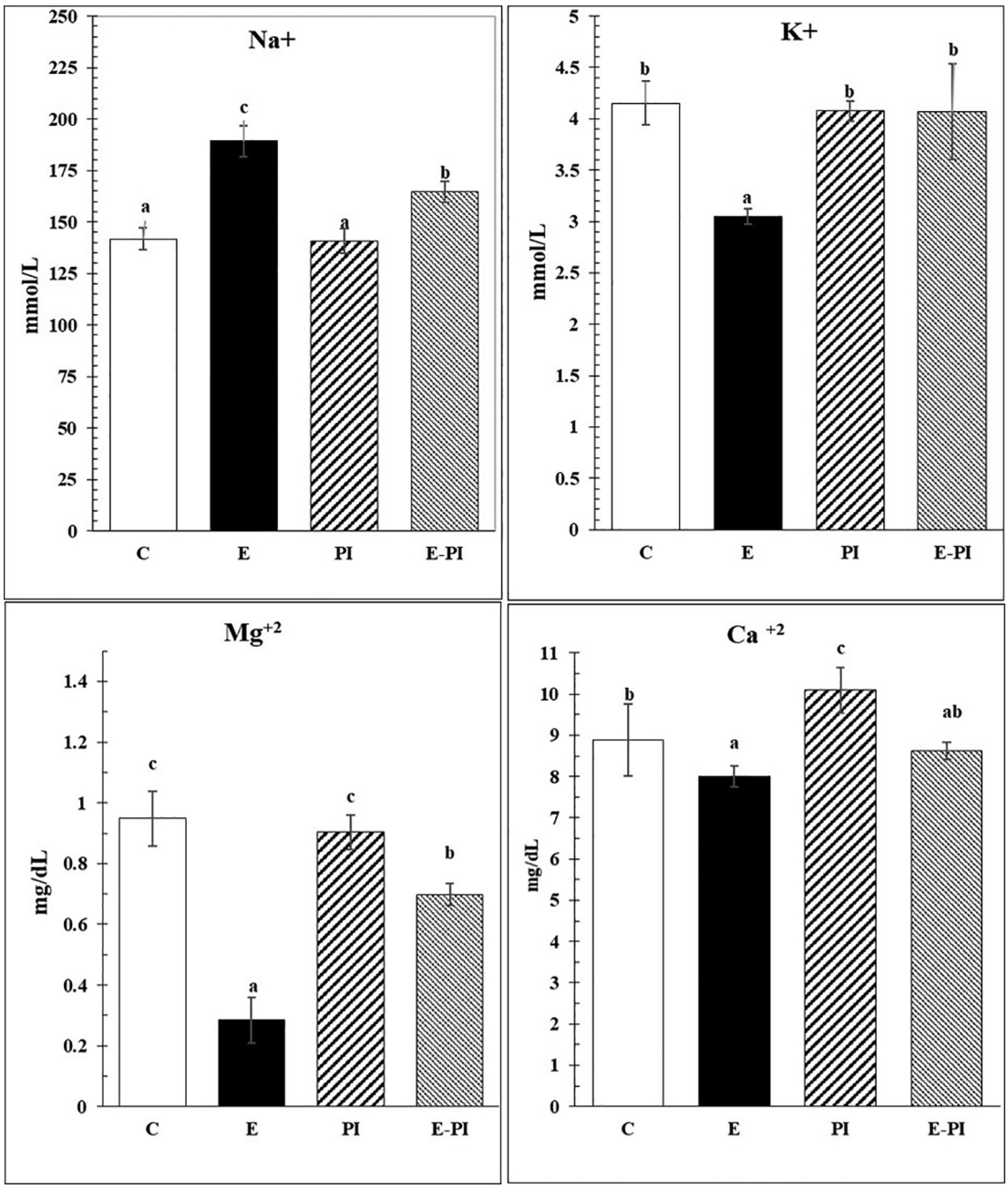

Fig. 1. Changes in sera electrolytes of different groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (E-PI) (200 mg/Kg. b.wt). Groups not sharing common superscripts denote significant differences (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

in $CD4^+$ and $CD8^+$ markers in brain-infiltrating cells showed a highly significant ($P < 0.001$) elevation in both $CD4^+$ and $CD8^+$ expressed cells in the second group (E) ($P < 0.001$), recording values of 54.13 ± 4.54% and 36.52 ± 3.08%, respectively. However, treatment with *P. incarnata* caused a diminution in the subpopulation of CD4 + and CD8 lymphocytes and corrected the above-mentioned activities, recording 27.89 ± 2.31% and 17.31 ± 1.20% for $CD4^+$ and $CD8^+$, respectively. The modified activity was compared to the control group (C) which recorded 17.02 ± 2.63% and 11.55 ± 1.29% for $CD4^+$ and $CD8^+$, respectively.

5. Assessment of the proinflammatory cytokines

Concerning assessment of the proinflammatory cytokines' level, the ameroliative levels of IL-1β, IL-6, IL-17A, TNF-α, and TGF-β were significantly ($P < 0.001$) reduced to 30.03 ± 5.02; 59.72 ± 7.42; 69.42 ± 2.66; 53.37 ± 1.74 and 74.76 ± 5.30 pg/mL, respectively, after treatment with *P. incarnata* (Fig. 5). These ameroliative changes were compared to their level in the epileptic non-treated group (92.40 ± 7.34; 114.68 ± 2.40; 94.03 ± 4.27; 108.00 ± 6.27 and 124.70 ± 3.10 pg/mL, respectively). In addition,

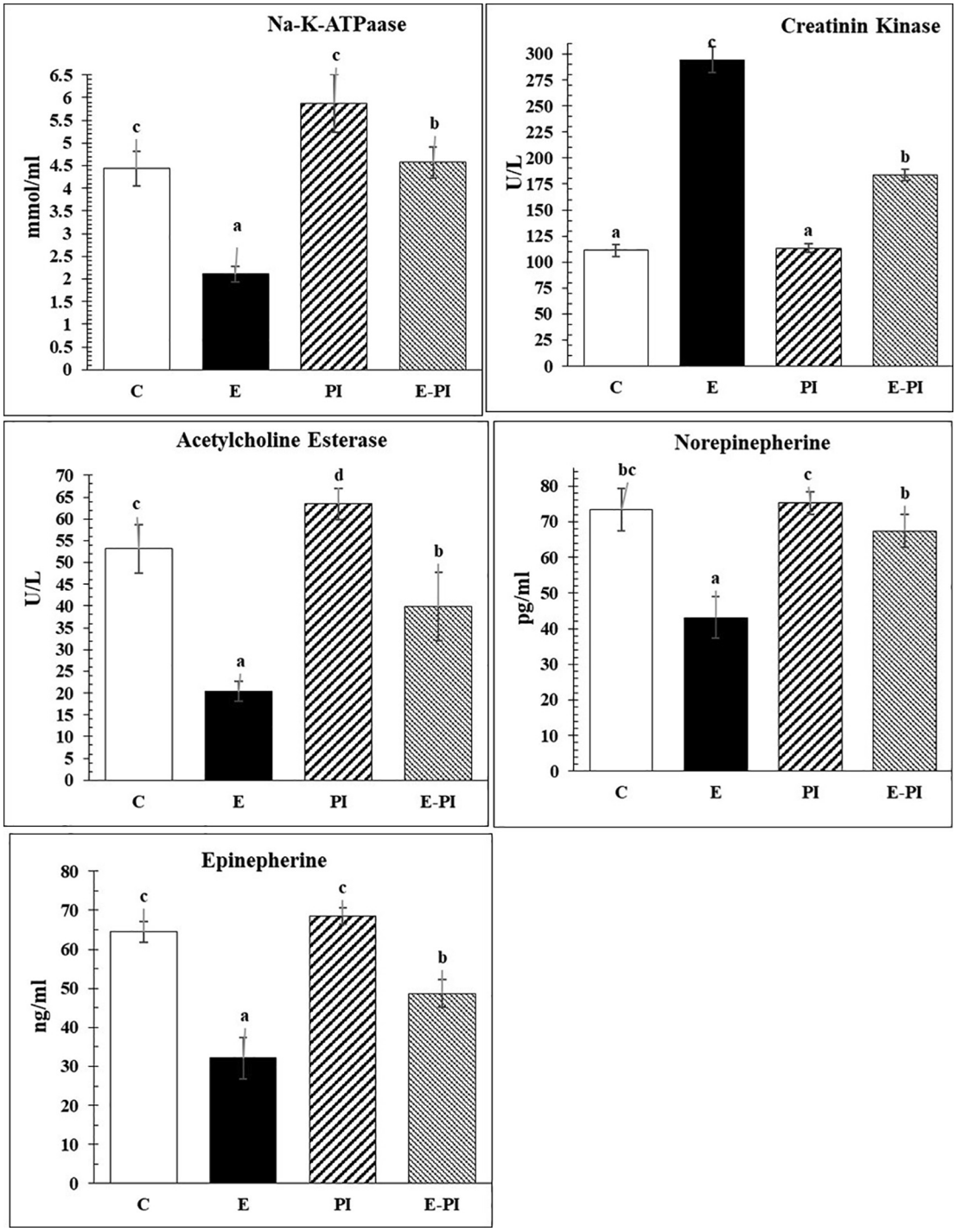

Fig. 2. Changes in brain energy and neurotransmitters of different groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (E-PI) (200 mg/Kg. b.wt). Groups not sharing common superscripts denote significant differences (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

the treatment with *Passiflora* extract (200 mg/kg) showed an ameroliative increase in anti-inflammatory cytokines, e.g. IL-10 and IL-13, which re-increased significantly ($P < 0.001$) in the epileptic-treated group with *P. incarnata*, recording 67.17 ± 4.43 and 93.88 ± 9.27 pg/mL, respectively.

6. Discussion

The present results showed that the antioxidant effect of *P. incarnata* treatment came in agreement with many reports (de Carvalho et al., 2011; Elsas et al., 2010; Sena et al., 2009). It is note-

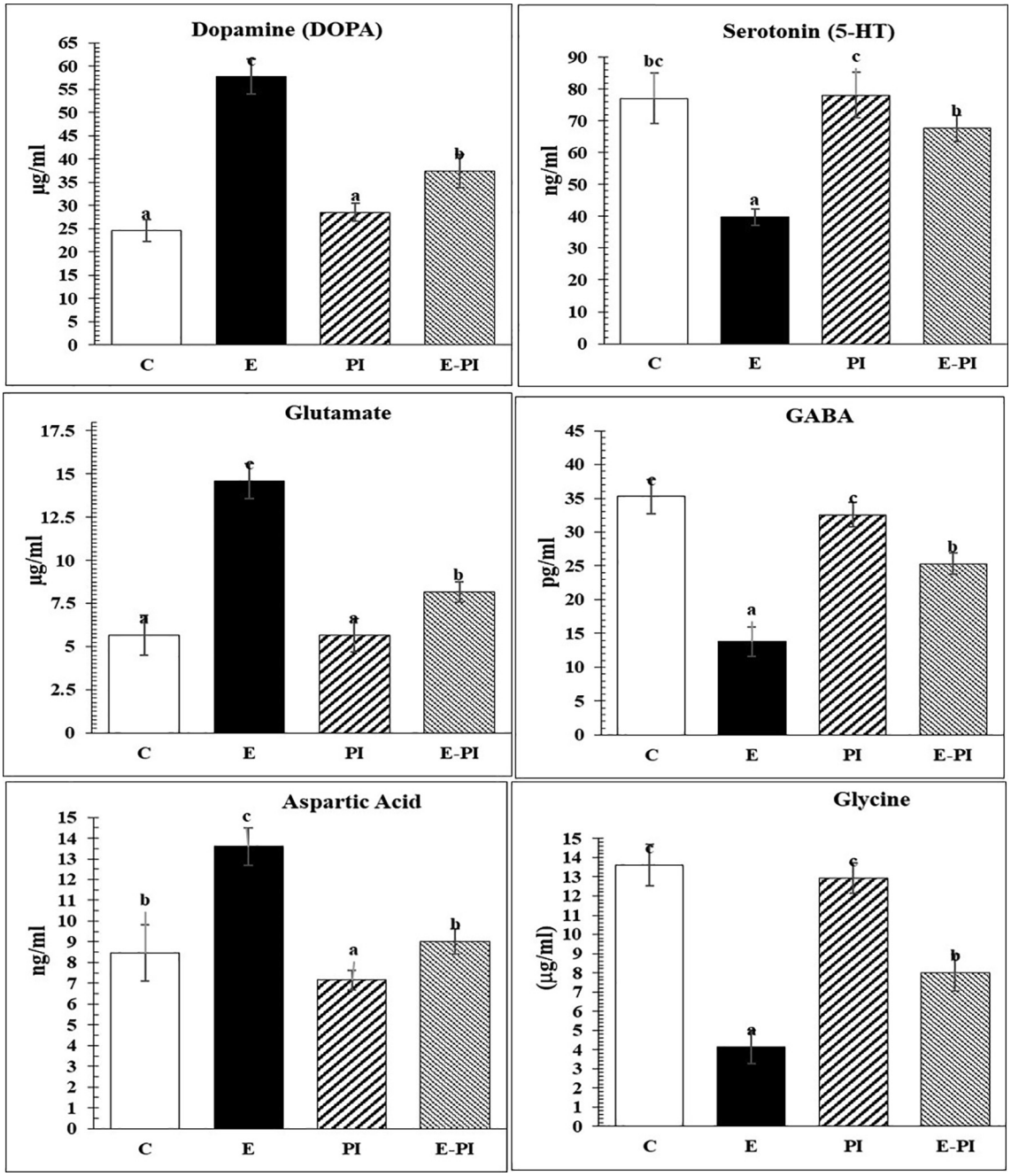

Fig. 3. Changes in neurotransmitters (monoamines) and some amino acids of different groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (E-PI) (200 mg/Kg. b.wt). Groups not sharing common superscripts denote significant differences (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

Table 2
FACS-analysis showing changes in $CD4^+$ and $CD8^+$ brain-infiltrating lymphocytes in different animal groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (**E-PI)** (200 mg/kg. b.wt). Groups not sharing common superscripts denote significant differences ($P < 0.05$, $P < 0.01$ and $P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

Groups	$CD4^+$ cells	$CD8^+$ cells
C	17.017 ± 2.626^{a}	11.550 ± 1.290^{a}
E	54.133 ± 4.539^{c}	36.517 ± 3.076^{c}
PI	15.167 ± 1.595^{a}	11.800 ± 1.203^{a}
EP_PI	27.885 ± 2.314^{b}	17.312 ± 1.198^{b}
F value	218.563	237.969
P <	0.000	0.000

worthy that the antioxidant activities of *Passiflora* spp. can be traced to its nutritional components of flavonoids and phenols, as well as cysteine, glutathione, ascorbic acid, tocopherol, tannins, and aromatic amines (Saravanan et al., 2014). Generally, flavonoids can be oxidized by radicals, resulting in more stable, less-reactive radicals. Some flavonoids can scavenge superoxides, while others can scavenge the highly reactive oxygen-derived radical peroxynitrite (Esposito et al., 2002; Nabavi et al., 2015). In addition, Ingale and Kasture (2017) referred to the antioxidant potential of *Passiflora* via significant DPPH (2,2-diphenyl-1-picrylhydrazyl) and H_2O_2 scavenging ability due to the content of phenolic compounds, mainly flavonoids.

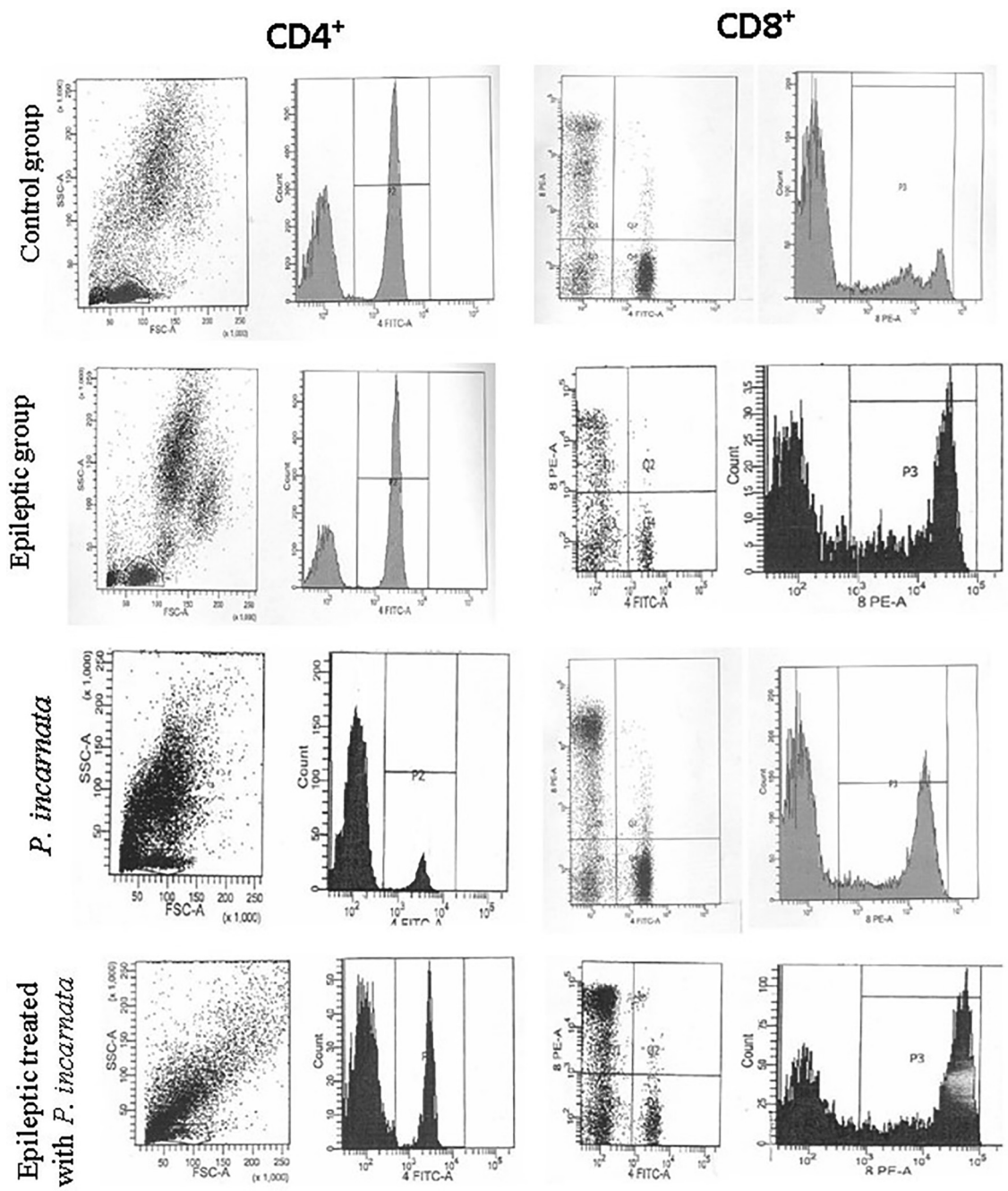

Fig. 4. Representative dot plots of FACS-analysis showing changes in CD4 + and CD8 + brain infiltrating lymphocytes in different animal groups.

Moreover, Sasikala et al. (2014) observed a highly antioxidant scavenging capacity of *Passiflora* extracts spp. towards free radicals. These properties can reduce and donate hydrogen ions and mitigate side effects of free radicals released during lipid peroxidation and cell damage. In addition, Saravanan et al. (2014) confirmed that extracts of *Passiflora* spp. could donate electrons to the released free radicals in biological tissues and reduce its inflammatory stress. Also, Nabavi et al. (2015) mentioned that chrysin, as one of the flavonoids component of *P. incranata*, can reduce nerve cell damage via preventing the release of nitric oxide and inflammatory cytokines from activated microglia, hence preventing neurodegeneration events.

Furthermore, Colomeu et al. (2017) reported that phenolic compounds are ubiquitously present in plants with antioxidant properties. These activities may be attributed to their redox properties, e.g. hydrogen donors, reducing agents, singlet oxygen quenchers and metal chelators.

The significant increment ($P < 0.001$) in the levels of electrolytes recorded throughout the present data could be explained by Liu et al. (2008) who reported significant content of electrolytes and minerals in pasipay (*P. incarnata*), e.g. Na^{+}, K^{+}, Mg^{+2} and Ca^{+2}. Therefore, they suggested such herbal drug as supplement food source in pharmaceutical and industrial fields.

In accordance with our data, during epileptic episodes, a massive increase of amino acids and neurotransmitters is observed (Bramlett and Dietrich 2004). These molecules lead to increased Ca^{2+}, Na^{+} and K^{+} fluxes via stimulation of glutamate receptors. As a result, catabolic processes will be activated, resulting in break-

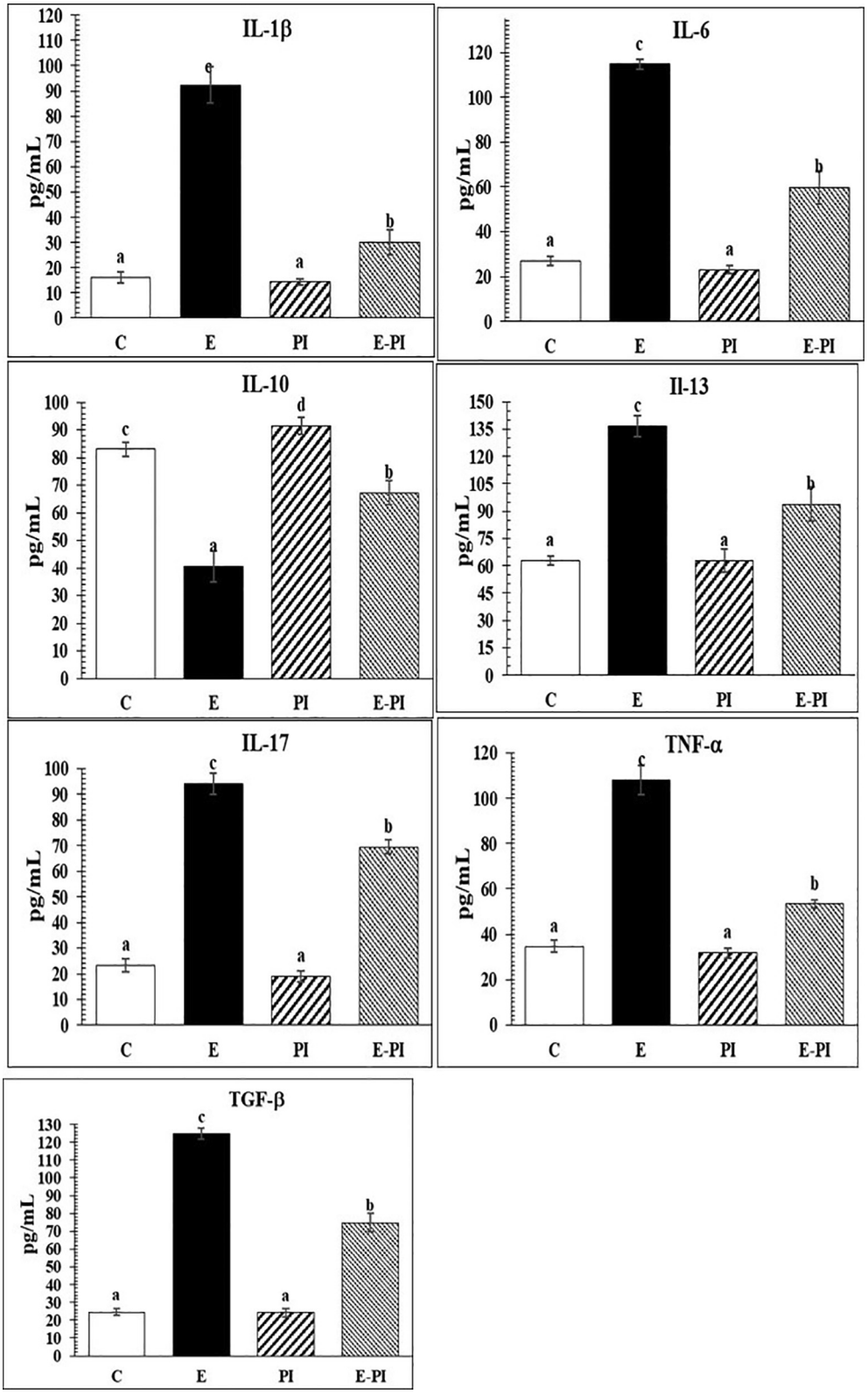

Fig. 5. Changes in plasma pro and anti-inflammatory cytokines of different groups: Control (C); Epileptic-non treated group (E); *Passiflora incarnata* group (PI) and Epileptic-treated with *P. incarnata;* (E-PI) (200 mg/Kg. b.wt). Groups not sharing common superscripts denote significant differences ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$). Values were represented as Mean ± SD; n = 8 rats.

down of the blood–brain barriers (BBB) (Werner and Engelhard 2007). Furthermore, chrysin, as the main plant extract and bioactive component content of *P. incarnata*, can modify Na^+, K^+-ATPase activity in the prefrontal cortex and hippocampus of mice (Borges et al., 2016).

Previously, Singh et al. (2012) demonstrated that rats treated with extracts of *P. incarnata* showed a significant treatment of seizure severity and immobility period in a dose- and time-dependent manner. These effects may be traced to the retained levels of serotonin and noradrenaline in the brain.

The recorded changes in brain amino acid and neurotransmitters levels are strikingly similar to those observed in other studies. The current anticonvulsant effects of pasipay (*P. incarnata*) may be related to its activation for benzodiazepine receptor (BZD), GABAergic and opioid systems (Fernández et al., 2006). Nassiri-Asl et al. (2007) revealed that a 100% protection for seizure episodes and mortality occurs after treatment with a dose of 0.4 mg/kg of *P. incarnata*.

Because GABA is a major inhibitory neurotransmitter, the present data revealed that *P. incarnata* increased the level of GABA. This is in accordance with the findings of Appel et al. (2011) and Guerrero and Medina (2017) who noticed that *P. incarnata* bioactivities may be mediated by GABA modulation by activation of the kappa opioid receptor (KOPr) that leads to support of GABAergic activity or attenuation of glutamatergic activity. This may confirm that *P. incarnata* could activate KOPr and result in protective effects against pentylentetrazole (PTZ)-induced seizure (Nassiri-Asl et al., 2007). The presence of GABA has been reported in *P. incarnata* and has also been suggested to elicit GABA currents in hippocampal neurons (Elsas et al., 2010).

The flavonoids content of *P. incarnate* exerted the modulation of the GABA system, e.g. chrysin and/or homoorientin, orientin, vitexin, and isovitexin (Mani and Natesan 2018; Mark Jr et al., 2008; Ropper 2005). In addition, Borges et al. (2016) and Souza et al. (2015) confirmed that chrysin flavonoids can improve the brain (hippocampal) dysfunction and reduce depressive behavior via elevation of brain-derived neurotrophic factor (BDNF). Furthermore, apigenin, the main flavonoid of *P. quadragularis* extract, can enhance GABAergic system, leading to a sedative activity (Gazola et al., 2015). In addition, Gazola et al. (2018) results showed that the GABAA/benzodiazepine receptors are involved in the sedative activity of vitexin-2″-O-xyloside (V2OX), the main flavonoid of *P. quadrangularis'* extract.

Our results revealed a significant decrease in noradrenaline and serotonin in the epileptic brain, which came in parallel with the findings of other studies, e.g. Madhyastha et al. (2005). In addition, Singh et al. (2012) recorded a retention of serotonin and noradrenaline levels of the brain after treatment with *P. incarnata*. They referred such retention to the presence of alkaloids contents, e.g. harmala alkaloids. These alkaloids are reversible monoamine oxidase (MAO)-A inhibitor, thereby prevent the degradation of serotonin and noradrenaline (Abourashed et al., 2003). Therefore, the recorded ameliorative increased levels of serotonin and noradrenaline in the present study might be due to MAO-A inhibitory action of harmala alkaloids content in *P. incarnata*. In conclusion, the bioactive metabolites of *P. incarnata* might ameliorate the different consequences of epilepsy.

The inflammatory response that occurs in brain tissues during epilepsy involves MHC class I and II proteins, and macrophages activation, and $CD4^+$ and $CD8^+$ lymphocytes (Atkinson and Eisenbarth, 2001). The cytokine is a biomarker for inflammation and predict the next seizure episodes (Wang et al., 2015). The present data revealed a significant elevation for different proinflammatory cytokines e.g. IL-1, Il-6, TNF-α and IL-17 in epileptic non-treated group. These findings came in parallel with Wang et al. (2015) and Huang and Zhu (2018). Some studies reported that the level of serum IL-1β, IL-6 and IL-17 were significantly elevated in CSF patients with epilepsy (Mao et al., 2013; Peltola et al., 2000; Pernhorst et al., 2013; Sinha et al., 2008; Uludag et al., 2013). Other studies reported that after injury and breakdown of blood–brain barriers (BBB), elevated TGF-β expression resulted from the activated astrocytes and microglia. These events came in correlation with the neural dysfunction resulting in elevation of IL-1, IL-6, IL-8, IL-10, G-CSF, TNFα and MCP-1 (Ajmo Jr et al., 2008; Das et al., 2012; Morganti-Kossmann et al., 2010; Walker et al., 2010).

Regarding IL-6, it was elevated after focal and generalized seizures in epileptic patients (Alapirtti et al., 2009; Bauer et al., 2009; Lehtimäki et al., 2004; Lehtimäki et al., 2007). It was similarly increased in many neurological diseases such as trauma, Alzheimer's disease and meningitis (Bauer et al., 2009; Frei et al., 1988; Woodroofe et al., 1991). Wang et al. (2015) reported that IL-6 was a unique cytokine associated with seizure severity. Therefore, IL-17 (Mao et al., 2013) and IL-6 (Wang et al., 2015) may be proposed as candidates to indicate seizure severity in general.

The present data came in parallel with others studies, e.g. Dinarello (2011); Choi et al. (2011); Zurolo et al. (2011) and Wang et al. (2015). The maximal increase in proinflammatory cytokines was reaching a high peak after six hours; IL-1 (445%), IL-6 (405%), and TNF- α (264%). They confirmed such high fold increment in glial cells within areas of intense microglia activation. The elevated proinflammatory cytokines may be due to the activated microglia cells, astrocytes, and cytokine production in late phases after seizures.

Strongly correlated with the recorded level of GABA, Prud'homme et al. (2013) reported that GABA suppresses both T cells and macrophages; suppresses production of the inflammatory cytokines; and increases TGF-beta production and regulatory T cells (Treg; IL-17). The recorded decreased level of GABA in epileptic group came in parallel with the significant high levels of IL-1β, IL-6, TNF-α and Il-17, released from the activated astrocytes and microglia (Su et al., 2015; Wang et al., 2000). Taken together, these events might contribute to the neuronal excitability via inhibition of the GABA system during epileptogenesis.

The present data revealed a strong correlation between neurotransmitters and oxidative stress imbalance and the proinflammatory cytokines levels in epilepsy group. The present data demonstrated that treatment with *P. incarnata* significantly mitigates the neuroinflammation events. In this context, chrysin as a monoflavonide of *P. incranata* is known as a potent antioxidant and anti-inflammatory agent Lee and Park 2015). In addition, chrysin could be a potential prophylactic agent for cerebral ischemia/reperfusion injury (Yao et al., 2014). Mani and Natesan's (2018) study confirmed that chrysin prevents cellular membrane damage, protein damage, and the imbalance of cellular functions, via limiting the lipid peroxidation. They added that chrysin could bind to COX-2 and limit inflammatory cytokines via blocking NF-kB activation. Chrysin also significantly suppresses the serum levels of proinflammatory cytokines, IL-1β, IL-6, TNF-α and TGF-β expression and inhibits NF-κB activation (Ahad et al., 2014). Moreover, chrysin is reported as an inhibitory agent for TNF-α and IL-1β (Bai et al., 2013). The anti-inflammatory activity of chrysin was reported via blocking histamine release and pro-inflammatory cytokine expression (Bae et al., 2011). Therefore, Bae et al. (2011) confirmed a molecular basis for the neuroprotective effects of chrysin in ischemia/reperfusion injury.

Furthermore, the present results recorded a significantly high level of TNF-α in sera of epileptic untreated rats. TNF-α induces the recruitment of inflammatory cells and promotes ROS generation. Therefore, chrysin flavonoid contents may significantly reduce the expression of TNF-α in epileptic treated rats. This finding demonstrates the previously-reported efficacy of chrysin as a potent inhibitor of the TNF-α pathway (Lee and Park, 2015).

However, the present data showed more efficiency of *P. incarnata* to decrease $CD4^+$ and $CD8^+$ activities. These differences on T lymphocytes proliferation may be related to the different types of the phenolic compounds content (Atoui et al., 2005; Colomeu et al., 2017; Zucolotto et al., 2012). In addition, the anti-inflammatory activities of *P. incarnata* may be related to their polyphenols contents, e.g. Rutin, Isoorientin, Vitexin, and Catechin; due to their capability to decrease the inflammatory cells by anti-proliferative action (Colomeu et al., 2017; Tang et al., 2014).

In conclusion, the anticonvulsant effects of *P. incarnata* may be due to its antioxidant and anti-inflammatory actions, as well as reduction in IL-1β; Il-6, IL-17, TNF-α and TGF-β levels and inhibition in amino acids and GABAergic. However, unlike other natural products, the therapeutic benefits of *P. incarnata* remain nascent in current literature. Taken together, the present work tries to support further attempts to modulate seizures and epilepsy manifestation by means of modulating the inflammatory cascades using *P. incarnata*.

7. Availability of data and materials

The data used to support the findings of this study are included in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Compliance with ethical standards

Ethical approval and consent to participate

All animal procedures were conducted in accordance with the standards set in the guidelines for the care and use of experimental animals by the Animal Ethics Committee of Beni-Suef University under an approval number is BSU/FS/020/102.

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