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Evaluation of cytokines expression in the phytoflavonoids treated epileptic rats: An *in vivo* study

Hammad Ahmed[1] and Mahtab Ahmad Khan[1,2]*
[1]Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[2]Faculty of Pharmacy, University of Central Punjab, Lahore, Pakistan

Abstract: Seizure are basic characteristic of epilepsy; these initiate due to irregular, excessive and synchronous electrical discharges. We aimed to determine the antiepileptic potential of phyto-flavonoids on the evaluation of the mRNA expression of TNF-α, IL-6, IL-1 Beta, IL-10, IL-1Ra, IL 4 and NF-κB. We induced chronic epilepsy in rats by administering sub-convulsive dose of pentylenetetrazole (25 mg/kg/day for 28 days). We observed intensity and frequency of seizures. Levetiracetam was used as a standard drug and divided all the subjects into 6 groups. All experimental animals were trained to acclimatize the behaviour tests. Food and water intake, changes in the body weight and WBC levels were assessed. Using qPCR, we assessed mRNA expression levels of, TNF α, IL-6, IL1 beta, NF-κB, IL1Ra, IL-4, and IL-10. qPCR confirmed the down regulation of the pro- and up-regulation of the anti-inflammatory cytokines. Significant reduction in seizure frequency, neutrophil counts and improvement in behaviour were observed when compared treatment group to control groups. PF exerts their antiepileptic activity by the downregulation of pro- and upregulation of anti-inflammatory cytokines respectively.

Keywords: Quercetin, catechin, kaempferol, pentylenetetrazole, anti-epileptic flavonoids, levetiracetam.

INTRODUCTION

Epilepsy is characterized by experience of one or more epileptic seizure having a propensity to spawn more seizures. These seizures are accompanied by neuro-pathological, cerebral injury, mental, and social disturbances (Tiwari *et al.*, 2019). Epilepsy is a debilitating brain disease, approximately one out of three epileptic patients are resistant to the existing anti-epileptic drugs (AED), and none of the modern pharmacological options are disease modifying (Zhang *et al.*, 2019). Imbalance between excitatory and inhibitory neurotransmission prompts epilepsy, major neurotransmitters involve are; Glycine Amino Butyric Acid (GABA) and glutamate.

Natural derivatives are gaining importance for the central nervous ailments including epilepsy. For the treatment of neurodegenerative disease some flavonoids are used. We aimed not only to explore the anti-epileptic potential of understudied PF, but also tried to understand the underlying mechanisms by using different molecular techniques. Levetiracetam (LEV) exhibits its antiepileptic effects via inhibition of excessive synchronized activity between neurons. LEV employs its therapeutic effect after binding with the SV2A (Lynch *et al.*, 2019).

Advanced studies confirmed that the inflammatory processes in the brain contribute to the etiopathogenesis of seizures and to the establishment of a chronic epilepsy (Ambrogini *et al.*, 2019). Inflammatory (IL-1β, TNF-α, and IL-6) and non-inflammatory cytokines (IL1Ra, IL-10 and IL-4) overexpressed and/or under expressed respectively, in different areas of brain which are involved in seizure generation and propagation. Similarly, NF-κB a transcription factor is up regulated in seizures (Vezzani *et al.*, 2019). The interactions between cytokines and neurotransmitters e.g., glutamate and GABA, suggest the possibility that these interactions neuronal excitability is cytokine-mediated ameliorate the disease conditions (Li *et al.*, 2020).

Phytoflavonoids (PF) that include kaempferol, quercetin and catechins are the constituents of number of plants which possess anti-epileptic potentials (Moghbelinejad *et al.*, 2016) (Turan *et al.*, 2019). These PF have potential to treat certain neurological disorders (Chauhan *et al.*, 2018), gout (Sharifi-Rad *et al.*, 2018), hepatic carcinomas, cardiovascular disorders and metabolic disorders. The aim of this study was to identify the anti-epileptic potential of the PF and their effects on the different cytokines associated with epilepsy.

MATERIALS AND METHODS

Experimental animals

The male rats weighing (180-220g) obtained from animal house, University of Lahore. Rats were kept in clean, polycarbonate cages under standard lab conditions (Temperature 25±3° C, Humidity 55±5%). We kept the animals in 12 h light and dark cycle with free access to the standard diet and water. The study was approved by Institutional Research Ethical Committee (IREC) and allotted a number IREC-19-112.

*Corresponding author: e-mail: raomahtab@yahoo.com

We divided the rats into six groups (6 rats in each group). Normal (Group A), diseased (Group B, PTZ 25mg/kg i.p.), standard drug (Group C, LEV 21mg/kg p.o.), kaempferol (Group D, 100 mg/kg p.o.), querecetin (Group E, 100mg/kg p.o.) and catechin (Group F, 100mg/kg p.o.) treated groups. The most effective doses of the PF were used in the study (Akinmoladun *et al.*, 2018) (Santangelo *et al.*, 2019). The duration of treatment was 14 days after the induction of epilepsy.

Chemicals

Chemicals of analytical grade were used for research purpose including, LEV (China), Quercetin (China), Kaempferol (China), Catechin (Sigma Aldrich) and PTZ (Sigma Aldrich), Trizol,

Disease induction

To induce chronic epilepsy, sub-convulsive dose of PTZ (25 mg/kg) was injected i.p. in rats for 28 days (Bagheri *et al.*, 2019). Recorded the maximum response for each animal. Only those animals were included in study that showed seizures in three consecutive episodes, and were categorized as kindled rats. Those animals were included in the study that shows appropriate intensity and number of seizures. Each animal housed in an individual plastic cage during the study period. First day after disease induction was considered as Day 0, and so on till day 14.

Food Intake

Provided pre-weighed food to each animal in the hopper of the cage and monitored cumulative food intake (g) daily. After every 24 h, we removed rats briefly from their cages and weighed each rat, and the amount of food remaining, including any on the bottom of the cages or any that had spilled cage. Intake was calculated as the weight (in grams) of food provided less that recovered (Imaoka *et al.*, 2019).

Water Intake

Made available each animal with pre-measured water. Cumulative water intake (ml) calculated every day. Carefully closed all the water bottles tightly so that water could not spill out into cage. The water intake was calculated by subtracting pre-volume with the current volume (Yoo *et al.*, 2017).

Change in the body weight

Noted the percentage change in body weight at days 0, 7 and 14 by using following formula:

Body weight after treatment / body weight before treatment × 100 as reported earlier (Bloch-Shilderman *et al.*, 2018)

White blood cells (WBCs) number

WBC count, neutrophils, eosinophils, lymphocytes, were calculated by haematology analyser (Liu *et al.*, 2016).

mRNA expression of pro and anti-inflammatory cytokines via qPCR

After the 14 days of treatment, sacrificed the animals and we took blood by cardiac puncture. The blood was stored in -70° C prior to the isolation of mRNA. GAPDH was used as a house-keeping gene (El-Missiry *et al.*, 2019).

RNA Extraction

RNA was collected and obtained from blood using TRIzol method. Blood (200µL) and TRIzol reagent (600µL) was vigorously vortexes and homogenised in an Eppendroff by vortex mixer. It was incubated for 5 mins (Sun *et al.*, 2019). Instilled ice-cold chloroform (200µL) in each tube and then vortex it for 15-30 seconds. Mixture was set aside at room temperature for 2mins. Centrifuged these samples at 12000 RPM for 15 mins at 4°C. The obtained mixture was divided in three phases. The uppermost transparent layer containing RNA. Prudently separate it. Separated layer carefully transferred in the new-labelled Eppendroff. Added equal amount of isopropanol in it, kept these tubes at room temperature for 10 mins. Then, centrifuged samples for ten mins at 12000 RPM at 4°C. Noted that RNA pellet was precipitated at the bottom of the Eppendorf. In order to wash the RNA pellet, add 75% ethanol (1 ml in each tube) and centrifuged it for brief period. Withdrawn surplus ethanol, and let RNA pellets air-dried. Re-suspend the RNA pellet in 20 µL of RNAase free water. RNA was stored at -80°C in tubes. Nanodrop spectrophotometric (2000/C Thermo Fisher Scientific) was used to quantify the total RNA. A 260/280 ratio was calculated. Only those samples were proceeded that have the ratio near to 2.0 (Landolt *et al.*, 2016).

Primer designing

The primers of the understudied cytokines were designed by the following steps (Uttra *et al.*, 2018, Shabbir *et al.*, 2016). Ensemble Genome browser was referred to find out gene sequences of studied genes. Select the mRNA sequences. Physical characteristics of all primers were checked using online primer 3 plus. Annealing temperatures of all primers were kept at 55-60^{o}C. GC content of primers was selected between 50-55%. Confirm the absence of Hairpin formation and self-annealing. Lastly, we conducted nucleotide blast to check the specificity. Following primers were designed and tabulated in table 1

cDNA synthesis

cDNA synthesis kit (Thermo Scientific, America) was used to synthesize cDNA by using reverse transcription technique according to the manufacturer guid[elines.

Working solution of primers

Stock solution of primers was prepared by mixing 25 nm primers in 250µL nuclease free water. Resultant concentration of stock solutions was 100 µM. In order to make 10 µM working solution 15 µL of stock solution was further diluted with nuclease free water (135 µL).

Table 1: Primers are tabulated in the table

Molecule	Forward Primer	Reverse primer
Interleukin 1 Beta	CCACTACAAAATCTGGGCGATGCA	CAGAAGAAGAGGATGTCACTCCTCG
TNF-α	CCTCTTCTCATTCCTGCTCGT	TGAGATCCATGCCATTGGCC
NF-κB	AGCTGAGCATGAAGGTGGATG	CAAGGAAGAGGATGTGGGGTT
IL 6	CCCACCAAGAACGATAGTCA	CTCCGACTTGTGAAGTGGTA
IL-4	ACCGAGAACCCCAGACTTGT	GGATGTAACGACAGCCCTCT
IL-10	TCTAGGCCTGTACGGAAGTGTTACT	AGCAGTGCTGAGCTGTGCAT
IL1Ra	GGCCTTTCTCAGAGCGGATGAAGG	TCACCCATGGCTTCAGAGGCAGCC
GAPDH	CTTGCCGTGGGTAGAGTCAT	TCTCTGCTCCTCCCTGTTCT

All the primers were optimized prior it use for final reaction (Bars-Cortina *et al.*, 2019)

Table 1: Expression levels of pro and anti-inflammatory cytokines in blood

Groups	WBC (10^9/ L)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophil (%)
A	$12 \pm 2.0^{**}$	75 ± 3	20 ± 5	3 ± 1	3 ± 1
B	19 ± 2.0	90 ± 5	5 ± 2	3 ± 1	3 ± 1
C	$14 \pm 0.8^{**}$	80 ± 3	10 ± 3	5 ± 1	5 ± 1
D	$13 \pm 0.5^{*}$	82 ± 3	12 ± 3	6 ± 1	6 ± 1
E	$13 \pm 1^{*}$	85 ± 3	10 ± 5	5 ± 1	5 ± 1
F	$13 \pm 0.4^{*}$	83 ± 3	12 ± 3	5 ± 1	5 ± 1

Polymerase chain reaction (PCR)

Take cDNA (2µL) of each sample in each PCR tube, then add forward primer (1µL) and reverse primer (1µL) than add PCR Master Mix (SYBR Green) (6µL) and nuclease free water (3 µL). We placed PCR tubes in thermal cycler. For denaturation, 95°C temperature was set for 10 seconds. For annealing, we adjust the temperature at 58-62° C for 20 seconds and extension was performed at 72° C for 30 seconds. 35 cycles were adjusted as per the manufacturer guidelines.

STATISTICAL ANALYSIS

The result was analyzed by using Graph Pad Prism Version 7. Assigned groups as independent variables and neurological tests' parameters of behavioural modelling were consider as dependent variables. Both one way and two-way Analysis of variance (ANOVA) test was applied with Tukey range test and Newman-Keuls to measure the level of significance among groups accordingly. The level of significance, $p< 0.05$ was considered as significant.

RESULTS

Change in the body weight

In neuropsychological and degenerative diseases, the body weight of the victim varies. However, we have noticed a significant ($P<0.0001$) reduction in the body weight of the diseased rats. Moreover, after the treatment with PF and LEV for 14 days, there was a gradual increase in body weight. PF treated groups showed significant improvement in their body weights at day 7 vs day 14 ($P<0.0001$). However, kaempferol treated group showed better results than the standard treatment groups as shown in fig. 1.

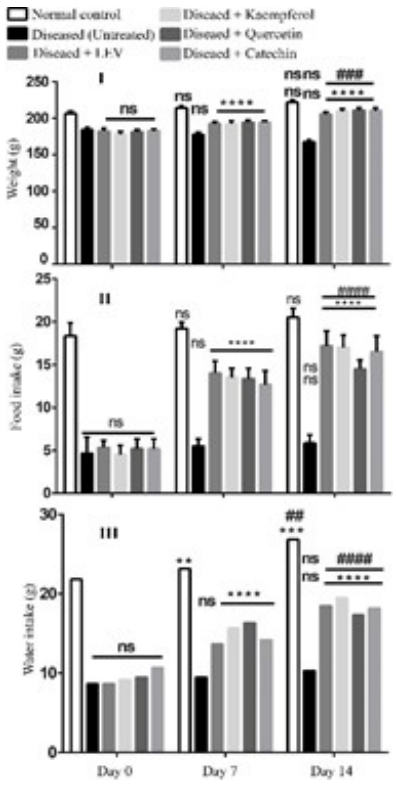

Fig. 1: Body weight (1) Food intake (2) Water intake (3). Day 0: Diseased rats were non-significant in all the groups confirming disease induction. Day 7 and 14 treatment in both Standard treated (LEV) and others PF significantly enhanced the body weight, food and water intake. Data were expressed as

mean ± S.E.M. for 6 rats in each group. ANOVA, confirmed significant improvement in the body weight, food and water intake behaviour at day 14 among the groups and also between two time point. *P<0.01 **P<0.05, ***P<0.0005, **** P<0.0001 (Newman-Keuls Multiple comparison Post-test Day 0 vs Day 7 * and Day 0 vs Day 14*)

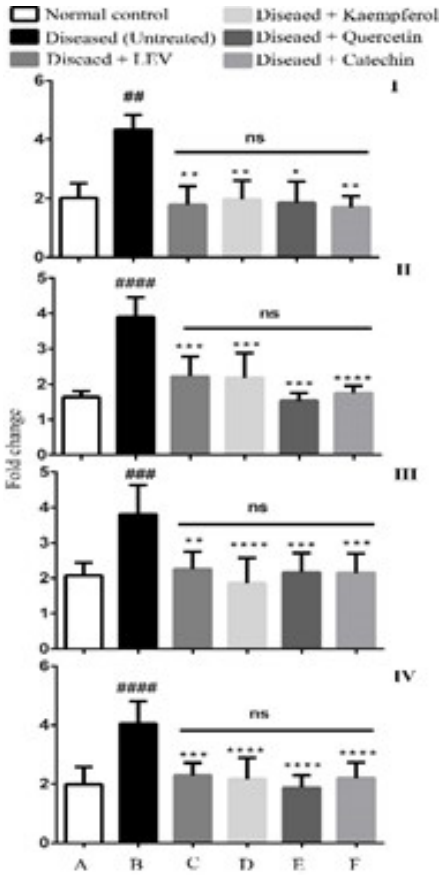

Fig. 2: Relative expression levels of pro-inflammatory cytokines Graphical representation of mean ± SD relative expression levels of TNF-α (I) IL-6, (II) IL1β, (III) NF-κB (IV). The treatment of PF significantly supresses the m RNA expression of the cytokines. The data was analysed by one-way ANOVA followed by the Tuckey's posthoc test. Comparison of Group A vs B, C, D, E & F denoted by (#) while Group B vs C, D, E and F is denoted (*). *P<0.05. **P<0.01, ***P<0.005, ****P<0.0001

Food intake

Food consumption behaviours have extensively highlighted that cognitive systems interact with the metabolic system in driving food intake. Food intake was significantly (P<0.0001) reduced in diseased groups. However, after the treatment of 14 days with PF and LEV groups showed better food intake. On comparison among the groups day 7 vs day 14 also showed significant (P<0.0001) improvement in the food intake, there was significant improvement in all the PF treated group however kaempferol treated group showed better result as compared to the standard treated groups as shown in as shown in fig. 2.

Water intake

Water intake was significantly (P<0.0001) reduced in diseased groups. However, after treatment of 14 days with PF and LEV water intake improved. There was less significant (P<0.01) increase in the water intake among the normal control and diseased groups. On comparison among the groups at day 7 vs day 14 also showed significant (P<0.0001) improvement in the water intake, among all the PF treated group kaempferol displayed better result even better than standard treated groups as shown in fig 3.

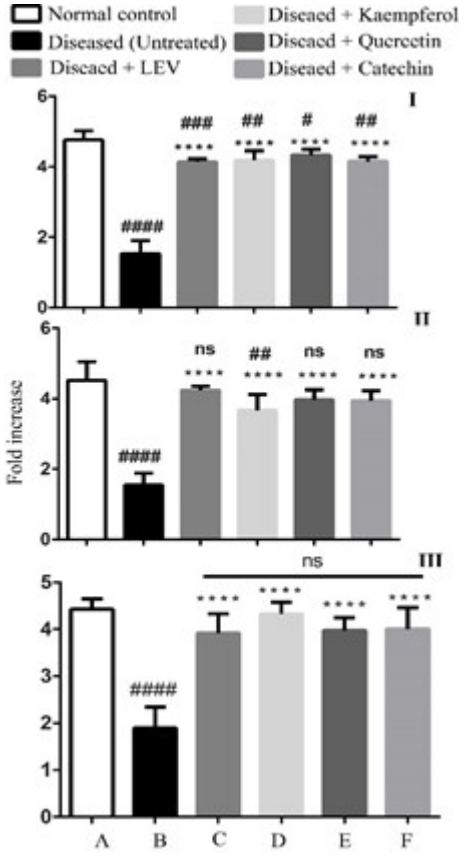

Fig. 3: Relative expression levels of anti-inflammatory cytokines.

Mean ± SD relative expression levels of IL-1Ra (I) IL-4, (II) IL-10 (III). The treatment of PF significantly enhances the mRNA expression of the cytokines. The data was analysed by one-way ANOVA followed by the Tuckey's posthoc test. Comparison of Group A vs B, C, D, E & F denoted by (#) while Group B vs C, D, E and F is denoted (*). *P<0.05. ** P<0.01, *** P<0.005, **** P<0.0001

Levels of WBCs

The results showed the rise in the WBC levels in the diseased group B. After the treatment of 14 days with PF, there is significant improvement in the WBC levels (group C, D, E and F) as shown in table 2. Due to the infiltration of neutrophils at the site of inflammation their levels will be augmented Group A (Normal saline treated), Group B (PTZ 25 mg/kg), Group C (LEV 21

mg/kg), Group D (Kaempferol 21mg/kg), Group E (Quercetin 100mg/kg) and Group F (Catechin 100 mg/kg). Statistical analysis was done through one-way analysis of variance (ANOVA) trailed by Tuckey's post hoc test for WBC. The results are considered significant * if $p<0.005$, ** $p<0.05$, *** $p<0.05$. Results were compared in a column with the respective diseased groups (table 2).

Pro-inflammatory cytokines

In the diseased groups B expression levels of pro inflammatory cytokines TNF-α, IL-6, and IL 1 Beta significantly up regulated as compared normal group A. The treatment with Lev and PF Group (C, D, E and F) caused significant reduction in expression levels. Similarly, comparing with group A non-significant differences were observed in the Group C, D, E and F.

Anti-inflammatory cytokines

IL-10, IL1Ra and IL-4 levels were found significantly down regulated in diseased group B as compared to normal group A (44.49±6.39). These reduced levels were restored after treatment with LEV and PF. By comparing normal group, A with treated groups (C, D, E and F). IL-10 was non- significant however in case of cytokine IL 1 Ra ($P<0.05$) significance was observed likewise in IL- 4 group C, E and F were non-significant while in group D ($P<0.05$) significance was observed.

DISCUSSION

Diseased rats observed generalized seizures. This is due to excessive neuronal firing. Oxidative stress is a foremost mechanism in several neurological disorders (Pearson-Smith *et al.*, 2017). The mechanism of action of LEV is unlike that of conventionally used AEDs (Yu *et al.*, 2018). LEV and its congeners have the binding affinity for the brain based SV2A receptors, and retain the anti-seizure potential (Stout *et al.*, 2019). LEV also reduces cerebral inflammation (Itoh *et al.*, 2016). Flavonoids may cause the facilitation of the GABAergic and glutamatergic neurotransmission. Flavonoids are the antioxidants; these can all cross the blood brain barrier (Nassiri-Asl *et al.*, 2016).

Epilepsy exerts a significant influence on eating behaviours, including food consumption and metabolism which influences the regulation of body weight. The precise mechanisms relating to food intake and body weight have not been subject to extensive research. Several research studies have determined numerous perspectives, highlighting such factors as changes in energy requirements, impeded homeostatic regulation and the influence of certain treatments on the hippocampal neurons and effects on GIT (Yaghouby *et al.*, 2019). In the diseased rats, there was significant reduction in the food intake. While in the treated rats, the food intake was increased. High dietary fat intake relieves the epileptic symptoms. Lipids have been seemed to have modifying and neuro-protective properties, by diminishing the oxidative stress and delaying neuronal apoptosis (D'Andrea-Meira *et al.*, 2019). Both reduction in the food intake and the pathogenesis of the epilepsy causes the significantly reduces the body weight of the epileptic rats. However, treatment with the PF like the LEV there was significant improvement in the body weight along with the other observed parameters.

The major signalling process of TNF-α follows the NF-κB and microtubule associated protein kinase (MAPK) pathways, that leads to cellular killing (Zaidi *et al.*, 2020). It is secreted by neurons, activated microglia and astrocytes (Tang *et al.*, 2020). Pathological activation of TNF-α characterized by significantly diminish the BBB steadiness by changing the cellular morphology, expression of protein, and serine protease production. (Bennett *et al.*, 2019). IL-1β induces expression of IL-1 downstream target genes. During epileptic episodes IL-1β level augmented. This cytokine is primarily produced in microglia, astrocytes and neurons (Zhu *et al.*, 2019). Increased levels of IL-6 are associated with epilepsy in the peripheral circulation system. In diseased circumstances, IL-6 generates in huge extents by neuronal neurons, microglia, and especially astrocytes (Casella *et al.*, 2018). In the epileptic animal models, neurological scarcities amended the amplified IL-4 levels while the early phases of epilepsy. Henceforth, IL-4 can uphold microglial and macrophage differentiation. IL-4 known to participates as defensive role in initiating seizures (Ul-Haq *et al.*, 2016). IL-10 controls the inflammation due to the cerebral injury. Furthermore, it can block NF-κB activity and reduce pro-inflammatory cytokine production by inflammatory cells. The blood levels of IL-10 increase after hippocampal damage (Liu *et al.*, 2019).

The immune-components are involved cerebral inflammation, pathogenesis of epilepsy and seizure-induced cerebral injury (Browning *et al.*, 2016). Pro-inflammatory and anti-inflammatory cytokines are highly expressed in the hippocampus. In the epileptic rats' hippocampal foci were formed because of PTZ induced epilepsy. The hippocampal lesions cause the up regulation and down regulation of pro-inflammatory cytokines and anti-inflammatory cytokines expression respectively. Pro-inflammatory cytokines stimulate the chronic release of excitable neurotransmitters, inhibit the uptake of these neurotransmitters (de Albuquerque Oliveira *et al.*, 2016). The LEV and PF down and up regulate the pro-inflammatory and anti-inflammatory cytokines respectively.

CONCLUSION

Based on current observation, we can conclude that, these PF can significantly attenuate the chronic PTZ induced seizures. Moreover, PF exerts their anti-epileptic potential by modulating the pro-and anti-inflammatory cytokines.

REFERENCES

Akinmoladun AC, Oladejo CO, Josiah SS, Famusiwa CD, Ojo OB and Olaleye MT (2018). Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity? *Pathophysiology,* **25**(4): 365-371.

Ambrogini P, Torquato P, Bartolini D, Albertini MC, Lattanzi D, Di Palma M, Marinelli R, Betti M, Minelli A and Cuppini R (2019). Excitotoxicity, neuroinflammation and oxidant stress as molecular bases of epileptogenesis and epilepsy-derived neurodegeneration: The role of vitamin E. *Biochim. Biophys. Acta Mol. Basis. Dis.*, **1865**(6): 1098-1112.

Bagheri S, Heydari A, Alinaghipour A and Salami M (2019). Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. *Epilepsy Behav.,* **95**(3): 43-50.

Bars-Cortina D, Riera-Escamilla A, Gou G, Piñol-Felis C and Motilva MJ (2019). Design, optimization and validation of genes commonly used in expression studies on DMH/AOM rat colon carcinogenesis model. *Peer. J.,* **7**(4): 6372.

Bennett C, Mohammed F, Alvarez-Ciara A, Nguyen MA, Dietrich WD, Rajguru SM, Streit WJ and Prasad A (2019). Neuroinflammation, oxidative stress and blood-brain barrier (BBB) disruption in acute Utah electrode array implants and the effect of deferoxamine as an iron chelator on acute foreign body response. *Biomaterials,* **188**(3): 144-159.

Bloch-Shilderman E, Rabinovitz I, Egoz I, Yacov G, Allon N and Nili U (2018). Determining a threshold sub-acute dose leading to minimal physiological alterations following prolonged exposure to the nerve agent VX in rats. *Arch. toxicol.,* **92**(2): 873-892.

Browning M, Shear DA, Bramlett HM, Dixon CE, Mondello S, Schmid KE, Poloyac SM, Dietrich WD, Hayes RL and Wang KK (2016). Levetiracetam treatment in traumatic brain injury: Operation brain trauma therapy. *J. Neurotrauma,* **33**(6): 581-594.

Casella G, Colombo F, Finardi A, Descamps H, Ill-Raga G, Spinelli A, Podini P, Bastoni M, Martino G and Muzio L (2018). Extracellular vesicles containing IL-4 modulate neuroinflammation in a mouse model of multiple sclerosis. *Mol. Ther.,* **26**(9): 2107-2118.

Chauhan A, Yu Q, Ray N, Farooqui Z, Huang B, Durbin EB, Tucker T, Evers M, Arnold S and Anthony LB (2018). Global burden of neuroendocrine tumors and changing incidence in Kentucky. *Oncotarget,* **9**(27): 19245.

D'andrea-Meira I, Krüger LT, Romão T, Paiva ME, Pires Do Prado HJ and Da Conceição PO (2019). Ketogenic diet and epilepsy: what we know so far. *Front. Neurosci.,* **13**(5): 5-12.

De Albuquerque Oliveira A, Linhares MI, Chaves Filho AJ, Maia E, Rios ERV, De Carvalho Lima CN, Venancio ET, De Souza AG, De Lima KA, De Sousa FC, Florenço E and Gaspar DM (2016). Antioxidant properties of antiepileptic drugs levetiracetam and clonazepam in mice brain after *in vitro* induced oxidative stress. *Afr. J. Pharm. Pharmacol.,* **10**(14): 278-288.

El-Missiry MA, Othman AI, Amer MA, Sedki M, Ali SM and El-Sherbiny IM (2019). Nanoformulated ellagic acid ameliorates pentylenetetrazol-induced experimental epileptic seizures by modulating oxidative stress, inflammatory cytokines and apoptosis in the brains of male mice. *Metab. Brain Dis.,* **35**(2): 1-15.

Imaoka A, Seki K, Akiyoshi T and Ohtani H (2019). The extent of drug-drug interaction between amlodipine and activated charcoal is attenuated by food intake in rats. *Drug Metab. Pharmacokinet.,* **34**(1): 108-110.

Itoh K, Ishihara Y, Komori R, Nochi H, Taniguchi R, Chiba Y, Ueno M, Takata-Tsuji F, Dohgu S and Kataoka Y (2016). Levetiracetam treatment influences blood-brain barrier failure associated with angiogenesis and inflammatory responses in the acute phase of epileptogenesis in post-status epilepticus mice. *Brain Res.,* **1652**(9): 1-13.

Landolt L, Marti HP, Beisland C, Flatberg A and Eikrem OS (2016). RNA extraction for RNA sequencing of archival renal tissues. *Scand. J. Clin. Lab. Invest.,* **76**(5): 426-434.

Li W, Xing R, Zhu Y, Zhao H and Lv R (2020). Solubility Determination and Preferential Solvation of Diphenoxylate in Aqueous Cosolvent Solutions of Ethanol, Acetonitrile, Methanol and Isopropanol. *J. Chem. Eng. Data,* **65**(7): 3658-3666.

Liu YJ, Chen J, Li X, Zhou X, Hu YM, Chu SF, Peng Y and Chen NH (2019). Research progress on adenosine in central nervous system diseases. *CNS Neurosci. Ther.,* **25**(9): 899-910.

Liu Z, Wang S, Liu J, Wang F, Liu Y and Zhao Y (2016). Leukocyte infiltration triggers seizure recurrence in a rat model of temporal lobe epilepsy. *Inflammation,* **39**(3): 1090-1098.

Lynch MJ, Long MA, Stretesky PB and Barrett KL (2019). Measuring the ecological impact of the wealthy: Excessive consumption, ecological disorganization, green crime and justice. *Social Cur.,* **6**(4): 377-395.

Moghbelinejad S, Rashvand Z, Khodabandehloo F, Mohammadi G and Nassiri-Asl M (2016). Modulation of the expression of the GABAA receptor β1 and β3 subunits by pretreatment with quercetin in the KA model of epilepsy in mice: The effect of quercetin on GABAA receptor Beta subunits. *J. Pharmacopunc.,* **19**(2): 163-174.

Nassiri-Asl M, Hajiali F, Taghiloo M, Abbasi E, Mohseni F and Yousefi F (2016). Comparison between the effects of quercetin on seizure threshold in acute and

chronic seizure models. *Toxicol. Indust. Health,* **32**(5): 936-944.

Pearson-Smith JN, Liang L-P, Rowley SD, Day BJ and Patel M (2017). Oxidative stress contributes to status epilepticus associated mortality. *Neurochem. Res.,* **42**(7): 2024-2032.

Santangelo R, Silvestrini A and Mancuso C (2019). Ginsenosides, catechins, quercetin and gut microbiota: Current evidence of challenging interactions. *Food Chem. Toxicol.,* **123**: 42-49.

Shabbir A, Shahzad M, Ali A and Zia-Ur-Rehman M (2016). Discovery of new benzothiazine derivative as modulator of pro-and anti-inflammatory cytokines in rheumatoid arthritis. *Inflammation,* **39**(6): 1918-1929.

Sharifi-Rad J, Tayeboon GS, Niknam F, Sharifi-Rad M, Mohajeri M, Salehi B, Iriti M and Sharifi-Rad M (2018). *Veronica persica* Poir. extract antibacterial, antifungal and scolicidal activities and inhibitory potential on acetylcholinesterase, tyrosinase, lipoxygenase and xanthine oxidase. *Cell. Mol. Bio.,* **64**(8): 50-56.

Stout KA, Dunn AR, Hoffman C and Miller GW (2019). The Synaptic Vesicle Glycoprotein 2: Structure, Function, and Disease Relevance. *ACS Chem. Neurosci.,* **10**(9): 3927-3938.

Sun Y, Ma J, Li D, Li P, Zhou X, Li Y, He Z, Qin L, Liang L and Luo X (2019). Interleukin-10 inhibits interleukin-1β production and inflammasome activation of microglia in epileptic seizures. *J. Neuroinflam.,* **16**(1): 66-72.

Tang X, Yan K, Wang Y, Wang Y, Chen H, Xu J, Lu Y, Wang X, Liang J and Zhang X (2020). Activation of PPAR-β/δ attenuates brain injury by suppressing inflammation and apoptosis in a collagenase-induced intracerebral hemorrhage mouse model. *Neurochem. Res.,* pp.1-14.

Tiwari MN, Mohan S, Biala Y and Yaari Y (2019). Protein kinase A-mediated suppression of the slow afterhyperpolarizing KCa3. 1 current in temporal lobe epilepsy. *J. Neurosci.,* **39** (50): 9914-9926.

Turan N, Ozkay UD, Can NO and Can OD (2019). Investigating the antidepressant-like effects of some benzimidazole-piperidine derivatives by *in-vivo* experimental methods. *Lett. Drug Des. Discov.,* **16**(3): 341-346.

Ul-Haq Z, Naz S and Mesaik MA (2016). Interleukin-4 receptor signaling and its binding mechanism: A therapeutic insight from inhibitors tool box. *Cytokine Growth Factor Rev.,* **32**(3): 3-15.

Uttra AM, Shahzad M, Shabbir A and Jahan S (2018). *Ephedra gerardiana* aqueous ethanolic extract and fractions attenuate freund complete adjuvant induced arthritis in sprague dawley rats by downregulating PGE2, COX2, IL-1β, IL-6, TNF-α, NF-kB and upregulating IL-4 and IL-10. *J. Ethnopharmacol*., **224**(05): 482-496

Vezzani A, Balosso S and Ravizza T (2019). Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. *Nat. Rev. Neurolo.,* **15**(8): 459-472.

Yaghouby F, Shafer B and Vasudevan S (2019). A rodent model for long-term vagus nerve stimulation experiments. *Bioelectron. Med.,* **2**(2): 73-88.

Yoo S, Ahn H and Park YK (2017). High dietary fructose intake on cardiovascular disease related parameters in growing rats. *Nutrients,* **9**(1): 11.

Yu X, Xu T, Ou S, Yuan J, Deng J, Li R, Yang J, Liu X, Li Q and Chen Y (2018). Endophilin A1 mediates seizure activity via regulation of AMPARs in a PTZ-kindled epileptic mouse model. *Exp. neurol.,* **304**(09): 41-57.

Zaidi AA, Khan MA, Shahreyar ZA and Ahmed H (2020). Lauric acid: Its role in behavioral modulation, neuro-inflammatory and oxidative stress markers in haloperidol induced Parkinson's disease. *Pak. J. Pharm. Sci.,* **33**(2 Suppl.): 755-763.

Zhang YY, Mo XD, Zhang XH, Xu LP, Wang Y, Yan CH, Chen H, Chen YH, Han W and Wang FR (2019). FLT3 internal tandem duplication does not impact prognosis after haploidentical allogeneic hematopoietic stem cell transplantation in AML patients. *Bone Marrow Transplant,* **54**(9): 1462-1470.

Zhu H, Wang Z, Yu J, Yang X, He F, Liu Z, Che F, Chen X, Ren H and Hong M (2019). Role and mechanisms of cytokines in the secondary brain injury after intracerebral hemorrhage. *Prog. Neurobio.,* **178**(5): 101-112.