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Preventive Effect of Resveratrol against Brain Mitochondria DNA Damage, Lipid Peroxidation, Inflammation and Seizures Induced by Kainic Acid in Mice

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ABSTRACT

Epilepsy is a highly prevalent serious brain disorder, and oxidative stress is regarded as a possible mechanism involved in epileptogenesis. The present study was designed to evaluate the potential protective and beneficial effect of resveratrol (RESV) on kainic acid (KA)-induced epilepsy in mice. Twenty four male Swiss Albino mice were divided into four groups. Group I: (Control group) mice received no drugs. Group Π: (epilepsy-induced group): mice administered with a single dose of KA (10 mg/kg b.wt) intraperitoneally (i.p). Group III: (epilepsy+RESV protected group) mice received RESV (10 mg/kg b.wt/day/i.p.) for 7 days before KA administration. Group IV: (epilepsy+RESV treated group): mice first injected with KA (10 mg/kg b.wt/i.p.) then after 15 min. RESV was administered as in group III for 3 consecutive days. The obtained results showed that, KA-induced epilepsy in mice caused significant decrease in serum sialic acid (SA) and brain tissue SOD, CAT, GPX activities and GSH concentration. However, serum TNF-α, interleukin-1 beta (IL-1β) and brain tissue nitric oxide (NO), L-MDA level, caspase-3 activity, DNA-fragmentation, 8-hydroxy-2-deoxyguanosine (8-OHdG), activator protein-1 (AP-1) and Myeloperoxidase (MPO) were significantly increased. Administration of RESV was able to mitigate epilepsy induced by KA through increasing of SA and brain tissue SOD, CAT, GPX activities and GSH in addition to declining NO, L-MDA, caspase-3 , DNA-fragmentation, 8-OHdG, AP-1 and MPO in brain tissue. The histopathological examination of brain tissues obtained from rats injected with KA showed variable pathological changes. Meanwhile, resveratrol injection was able to reduce the severity of these alterations especially in group IV (epilepsy+RESV treated group).The present study demonstrated that, RESV.possesses significantly neuroprotection and treatment effects against epilepsy and oxidative damage in brain tissue induced by KA in mice.

Keywords: Resveratrol, kainic acid, epilepsy, histopathology, Oxidative stress.

1. INTRODUCTION

Epilepsy is a serious neurological disorder while anticonvulsant therapies are limited and unable to control seizures in all patients. The Kainic acid (KA) seizure model is particularly useful for the study of the evolution, propagation, and pathological consequences of epileptic discharge in the limbic system. Activation of the KA subtype of ionotropic gluatamate receptors results in sustained epileptic activity in the hippocampus, followed by a selective pattern of neuropathology that is similar to human temporal lobe epilepsy (TLE) [1].

A relationship between status epilepticus (SE) and oxidative stress has recently begin to be recognized both in animal models. It has been established that blood flow, energy and oxygen are increased during

seizure and that SE induces the production of redundant reactive oxygen species (ROS). Compared with other organs, the brain uses the highest amount of oxygen and contains a high concentration of polyunsaturated fatty acids that are easily peroxidated, which makes it particularly susceptible to oxidative stress. Similarly, increased oxidative stress contributes to seizure-induced brain injury and subsequently results in epilepsy. In turn, ROS may be a contributing factor in the generation of epileptic seizures in animal models and in patients [2].

Flavonoids present a variety of beneficial health effects including regulation of oxidative stress. Resveratrol decreased the frequency of spontaneous seizures and inhibited the epileptiform discharges induced by kainate in rats. Importantly, resveratrol prevented the kainate-induced hippocampal cell death and reduced mossy fiber sprouting, which are thought to be histological markers of epileptogenesis in this model of temporal lobe epilepsy. Studies on mice revealed that, regular exercise and resveratrol administration (40 mg/kg b.wt./day) for 6 weeks inhibited kainate-induced seizure activity, mortality and oxidative stress in those animals. The synergic effect of regular exercise and resveratrol suggests its potential usefulness for the prevention of seizure development. However, in contrast to adult rats, repeated resveratrol administration did not attenuate kainate-induced seizures, and had only modest effect on preventing hippocampal cell death and lipid peroxidation in young rats[3]. Thus, RESV can potently suppress pro-inflammatory responses of microglia. Considering these, it appears that RESV administration would beneficial for curtailing the inflammatory reaction in neurodegenerative diseases. This is particularly applicable to conditions where significant microglial activation is one of the pathological changes such as after acute seizure or SE induced brain injury Brain [4]. Accordingly, the present study was designed to evaluate the beneficial and the potential protective effect of resveratrol against kainic acid-induced epilepsy in Swiss albino mice by determination of various parameters including serum sialic acid (SA), tumor necrosis factor alpha(TNF-α) and interleukin- 1 beta (IL-1β) and brain tissue superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), reduced glutathione (GSH), L-Malondialdehyde (L-MDA), nitric oxide (NO), caspase-3, DNA-fragmentation 8-hydroxy-2-deoxyguanosine (8-OHdG), activator protein-1 (AP-1) and Myeloperoxidase (MPO). Moreover, the histopathological examination of brain tissues under all treatments was also performed.

2. MATERIALS AND METHODS

2.1 Experimental animals

Twenty four male Swiss albino mice of 6-8 weeks old and weighting 25-30 gm were used in this study. Mice were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. The animals were fed on constant ration and water was supplied ad-libitum. The mice were left 14 days for acclimatization before the beginning of the experiment.

Resveratrol (purity~99%) was manufactured by Sigma Chemical Co.(St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt. Resveratrol was freshly prepared in normal saline and administered to mice at a dose level of (10 mg/kg b.wt/day, i.p).

2.2 Induction of epilepsy

Epilepsy was induced in mice by a single intraperitoneal injection of kainic acid at a dose of (10 mg⁄kg body weight). kainic acid has been purchased by Sigma Chemical Co. (St. Louis, Mo, USA) and purchased from Schnelldorf, Germany through the Egyptian International Center for Import Cairo, Egypt. KA was dissolved in normal saline and the PH of KA solution was adjusted to 7.2±0.1. Following administration of KA all mice were observed for behavioral alteration (groom in, rearing, wt dog shakes, jam movement, hind limb scratching, urination, defection, salivation, head nodding, incidence and latency of convulsions and mortality over a period of 4 hours [5].

2.3 Experimental design

Mice were randomly divided into four main equal groups, 6 animals each, placed in individual cages and classified as follows:

Group I: Control Normal Group: Mice received no drugs, served as untreated control for all experimental groups.
Group П: (epilepsy- induced group): Mice administered with a single dose of KA (10 mg/kg b.wt, intraperitoneally), served as epilepsy non treated group.
Group III: (epilepsy + RESV protected group): Mice received RESV (10 mg/kg b.wt, i.p) daily for 7 successive days prior to KA injection (10 mg/kg b.wt, intraperitoneally).
Group IV: (epilepsy + RESV treated group): Mice injected with KA (10 mg/kg b.wt, intraperitoneally) after 15 min. mice were treated with RESV (10 mg/kg b.wt/day, i.p) for three days.

2.4 Sampling

Blood samples and tissue specimens (brain tissues) were collected after 12 hours and 3 days from the onset of KA administration.

2.4.1 Blood samples

Blood samples for serum separation were collected by ocular vein puncture at the end of each experimental period in dry, clean, and screw capped tubes and serum was separated by centrifugation at 2500 r.p.m for 15 minutes. The clean, clear serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20℃ until used for subsequent biochemical analysis. All sera were

analyzed for the determination of sialic acid, TNF-alpha and (IL-1β).

2.4.2 Tissue samples (Brain tissue)

a-For biochemical analysis:

The skull was opened carefully and the brain wasquickly removed, cleaned by rinsing with ice-cold isotonic saline, cleared off blood, then blotted between 2 filter papers. The brain tissue samples were quickly frozen in a deep freeze at -20°C for consequent biochemical analysis.

Briefly, 0.5 gm from each brain tissues were minced into small pieces, homogenized with ice cold phosphate buffer saline (PBS) (i.e., 50 mM potassium phosphate, PH 7.5, 0.1 mM EDTA) to make 10% homogenates using tissue homogenizer. The homogenates were centrifuged at 6,000 r.p.m. for 15 minute at 4°C. The resulting supernatant was directly used for determination of the following biochemical parameters: SOD, CAT, GPx, GSH, L-MDA, NO, caspase-3, DNA fragmentation, 8-OHdG, activator protein-1 (AP-1) and Myeloperoxidase (MPO).

b- For histopathological examination:

Specimens of mice were carefully examined by naked eyes for detection of any abnormalities. The specimens were preserved in 25% neutral buffered formalin solution and subjected for histopathological examination as follows***:*** Brain samples were fixed in 25 % neutral formalin for twenty four hours; and then washed by running water over night. The washed samples were dehydrated by using graded ascending concentrations of ethyl alcohol starting with 50% and ending with absolute alcohol. The dehydrated samples were cleared in xylol for 6 hours. The samples were placed in a crucible containing soft paraffin and kept in an oven at 56°C for 12 hours. The samples were then blocked in hard paraffin and cut into sections of about 5 microns in thickness***.*** Then sections were stained with Harris haematoxylin and eosin for microscopically examination according to the technique described by (Bancroft and Stevens, 1996)[6].

2.5 Biochemical analysis

Serum sialic acid, TNF-α and IL-1β were determined using human sialic acid ELISA kit (Cat.No.CSB-E09605h), Beyaert and Fiers, (1998) [7] and Rat IL-1 beta ELISA (RayBiotech, Inc Company, Cat#: ELR-IL1b), respectively. Moreover, brain tissues SOD, CAT, GPx, GSH, L-MDA, NO, caspase-3, DNA-fragmentation, 8-OHdG, AP-1 and MPO were determined according to the methods described by Kakkar et al., (1984) [8]; Luck, (1974) [9]; Gross et al., (1967) [10]; Moron et al., (1979) [11]; Mesbah et al., (2004) [12]; Vodovotz, (1996) [13]; Rat Caspase 3 (Casp-3) ELISA Kit (CUSABIO BIOTECH CO., LTD) Cat.No.CSB-E08857r); Shi et al., (1996) [14]; StressMarq Biosciences Inc.'s StressXpress® EIA Kits (Cat# SKT-120-96 (96 well kit); mice activator protein-1 ELISA kit (MBS Company, Cat. No. KT-702740) and Rats Myeloperoxidase ELISA kit (Kamiya Biomedical Company, Cat. No.KT-60345) according to the manufacturer's instruction, respectively.

2.6 Statistical analysis

The obtained data were analyzed and graphically represented using the statistical package for social science (SPSS, 13.0 software, 2009), for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

Table 1: Protective and treatment effect of resveratrol on serum sialic acid and TNF-α concentrations and brain tissue SOD, CAT and GPx activities in kainic acid-induced epilepsy in mice.

Groups	Serum sialic acid(mg/ml)		Serum TNF-α (pg/ml)		Brain SOD (U/g.tissue)		Brain CAT (mmol/g.tissue)		Brain GPx (ng/g.tissue)	
	12 hours	3 days	12 hours	3 days	12 hours	3 days	12 hours	3 days	12 hours	3 days
Control	41.23 $\pm 4.19^{a}$	48.36 $\pm 4.01^{a}$	33.59 $\pm 3.79^{c}$	33.34 $\pm 2.29^{b}$	38.31 $\pm 5.06^{a}$	25.16 $\pm 2.22^{a}$	60.12 $\pm 2.99^{a}$	53.44 $\pm 2.10^{a}$	46.66 $\pm 1.80^{a}$	41.70 $\pm 1.48^{a}$
KA (epilepsy)	19.31 $\pm 3.27^{b}$	17.75 $\pm 2.14^{c}$	57.64 $\pm 2.85^{a}$	81.74 $\pm 10.90^{a}$	6.81 $\pm 1.99^{c}$	9.36 $\pm 0.82^{c}$	16.55 $\pm 5.64^{d}$	23.60 $\pm 4.27^{c}$	10.90 $\pm 2.25^{e}$	9.93 $\pm 2.88^{e}$
Resveratrol protected	26.87 $\pm 1.73^{b}$	22.28 $\pm 0.85^{c}$	41.13 $\pm 2.53^{bc}$	50.11 $\pm 2.25^{b}$	12.84 $\pm 0.29^{c}$	10.86 $\pm 1.43^{c}$	46.14 $\pm 4.50^{bc}$	39.35 $\pm 1.49^{b}$	26.25 $\pm 2.17^{d}$	26.89 $\pm 0.68^{d}$
Resveratrol treated	42.22 $\pm 1.41^{a}$	45.64 $\pm 4.17^{a}$	46.37 $\pm 2.84^{b}$	37.38 $\pm 3.94^{b}$	23.57 $\pm 1.35^{b}$	19.30 $\pm 3.08^{ab}$	52.79 $\pm 3.11^{ab}$	52.52 $\pm 0.81^{a}$	40.38 $\pm 2.39^{b}$	36.58 $\pm 2.29^{ab}$

Data are presented as (Mean±S.E) S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Protective and treatment effect of resveratrol on serum sialic acid and TNF-α concentrations and brain tissue SOD, CAT and GPx activities in kainic acid-induced epilepsy in mice:

The results presented in table (1) showed a significant decrease in serum SA level and brain tissue SOD, CAT and GPx activities in KA-induced epilepsy in male mice group. However, serum TNF-α level was significantly increased when compared with normal control group. On the other hand, protection and treatment with resveratrol administration in KA induced epilepsy in mice resulted in a significant increase in serum SA level and brain tissue SOD, CAT and GPx activities with significant decrease in serum TNF- α level when compared with epilepsy-induced non treated group.

Table 2: Protective and treatment effect of resveratrol on brain tissue L-MDA, GSH and NO concentrations, caspase-3 activity and DNA fragmentation percent in kainic acid-induced epilepsy in mice.

Groups	Brain L-MDA(mmol/g.tissue)		Brain GSH(ng/g.tissue)		Brain Nitric Oxide(mmol/g.tissue)		Brain Caspase-3(ng/g.tissue)		Brain DNA fragmentation %	
	12 hours	**3 days**	**12 hours**	**3 days**	**12 hours**	**3 days**	**12 hours**	**3 days**	**12 hours**	**3 days**
Control	49.21 ±12.33^{d}	52.14 ±15.59^{c}	4.57 ±0.57^{a}	4.79 ±0.22^{a}	29.63 ±4.35^{c}	32.58 ±6.07^{c}	0.56 ±0.13^{d}	0.46 ±0.21^{e}	235.34 ±80.00^{d}	185.19 ±31.66^{d}
KA (epilepsy)	115.91 ±0.87^{a}	139.39 ±10.02^{a}	1.78 ±0.64^{b}	2.27 ±0.34^{c}	86.03 ±5.59^{a}	100.24 ±5.27^{a}	2.26 ±0.19^{a}	2.36 ±0.15^{a}	1394.42 ±222.26^{a}	1207.50 ±229.71^{a}
Resveratrol protected	81.48 ±4.59^{bc}	98.62 ±2.60^{b}	3.02 ±0.47^{ab}	4.49 ±0.49^{ab}	44.83 ±5.52^{b}	66.90 ±2.75^{b}	1.61 ±0.19^{bc}	1.76 ±0.07^{b}	856.92 ±58.44^{b}	640.00 ±111.38^{bc}
Resveratrol treated	39.85 ±9.10^{d}	51.41 ±10.81^{c}	3.23 ±0.46^{ab}	2.71 ±0.33^{c}	75.47 ±2.45^{a}	99.41 ±3.71^{a}	1.25 ±0.21^{c}	0.98 ±0.25^{de}	208.27 ±37.66^{d}	272.46 ±71.53^{d}

Data are presented as (Mean±S.E) S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

3.2 Protective and treatment effect of resveratrol on brain tissue L-MDA, GSH and NO concentrations, caspase-3 activity and DNA fragmentation percent in kainic acid-induced epilepsy in mice:

The obtained results demonstrated in table (2) revealed that, administration of KA induced epilepsy in mice exhibited a significant decrease in brain tissue GSH level and significantly increased L-MDA, NO, Caspase 3, and DNA fragmentation when compared with normal control group. Meanwhile, protection and treatment with resveratrol administration in epilepsy-induced in mice significantly increased GSH level and markedly decrease and attenuate the increased of NO and L-MDA concentrations, Caspase 3 activity and DNA fragmentation in brain tissues when compared with KA-induced epilepsy non-treated group.

3.3 Protective and treatment effect of resveratrol on serum IL-1β concentration and brain tissue 8-OHdG, AP-1 and MPO in kainic acid-induced epilepsy in mice.

The obtained data demonstrated in table (3) revealed that, a significant increase in serum IL-1β level and brain tissue 8-OHdG, AP-1 and MPO activities were observed in KA-induced epilepsy in male mice group. On the other hand, protection and treatment with resveratrol administration in KA induced epilepsy in mice resulted in a significant decrease in serum (IL-1β) level and brain tissue 8-OHdG, AP-1 and MPO activity when compared with epilepsy-induced non treated group.

Table 3: Protective and treatment effect of resveratrol on serum IL-1β concentration and brain tissue 8-OHdG, AP-1 and MPO in kainic acid-induced epilepsy in mice.

Groups	serum IL-1 β (Pg/ml)		Brain(8-OHdG) (ng/g)		Brain (AP-1) (Pg/g)		Brain (MPO) (ng/g)	
	12 hours	**3 days**	**12 hours**	**3 days**	**12 hours**	**3 days**	**12 hours**	**3 days**
Control	32.68 ±5.36^{d}	52.49 ±4.07^{c}	3.40 ±0.36^{d}	3.83 ±0.56^{d}	56.42 ±20.07^{e}	142.19 ±8.26^{c}	4.77 ±0.58^{c}	7.40 ±0.67^{bc}
KA (epilepsy)	307.62 ±36.71^{a}	310.86 ±46.89^{a}	6.99 ±0.95^{a}	7.24 ±0.74^{a}	6.81 ±1.99^{c}	9.36 ±0.82^{c}	13.43 ±0.92^{a}	12.79 ±1.07^{a}
Resveratrol protected	127.46 ±28.13^{bc}	124.30 ±24.61^{bc}	4.81 ±0.23^{bcd}	5.35 ±0.31^{c}	132.68 ±8.9^{d}	164.73 ±3.98^{bc}	7.37 ±0.46^{b}	6.63 ±0.89^{bc}
Resveratrol treated	116.80 ±23.52^{cd}	99.42 ±21.35^{bc}	5.75 ±0.47^{abc}	6.54 ±0.21^{ab}	225 ±12.42^{ab}	242.14 ±4.33^{a}	11.45 ±0.35^{a}	9.15 ±0.70^{b}

Data are presented as (Mean±S.E) S.E = Standard error.
Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

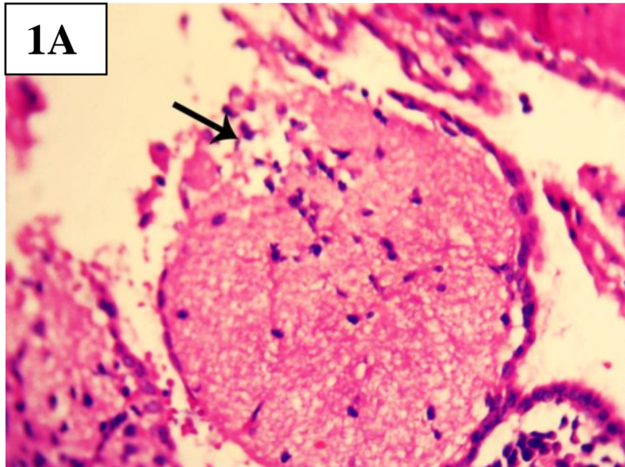
3.4 Histopathological findings:

The microscopical examination of the brain of mice of control untreated group showing normal histopathological structure. The brain of mice received Kainic acid-induced epilepsy show marked congestion of the meningeal blood vessels with degeneration of the endothelial cells lining of these blood vessels (fig. 1A). Additionally, congestion of cerebral and cerebellar blood vessels with perivascular hemorrhage was seen. Multifocal hemorrhages were also noticed in different sites particularly brain stem (fig. 1B). Proliferation of ependymal cells with degenerative changes of some of these cells (fig. 1C) was the most common findings in this group. Furthermore, diffuse vacuolation with the presence of multiple areas of malacia was also observed with glial cells degenerations (fig. 1D). Moreover, the neuron in the brain of these mice showing different degenerative changes mainly tygrolysis as most of neurons became rounded, swollen and more eosinophilic. Neurophagia was characterized by engulfing of microglia cells to the necrotic neurons. Furthermore, the brain showed numerous microglia cells around the degenerated neurons manifested by microgliosis. Perineuronal edema and vacuolations were also noticed around the degenerated neurons.

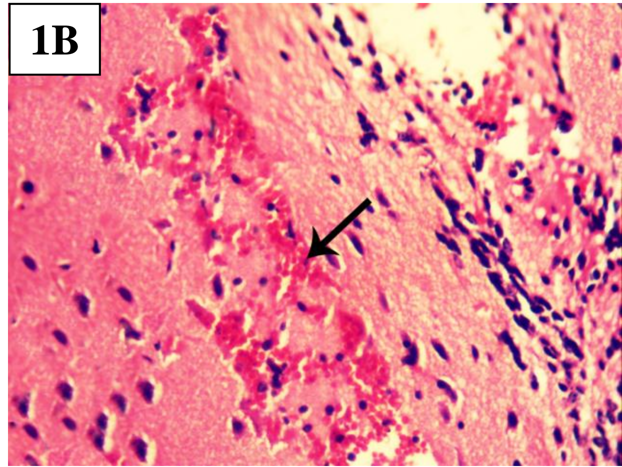
In the same time, mild congestion of the meningeal and brain blood vessels was observed in the brain of mice received Kainic acid plus RESV (protected group III). Moreover, mild vacuolization of the brain substances with mild edema in the glial cells were also detected. Additionally, mild degeneration of the neuron of the

cerebellum (fig. 1E) was demonstrated. The degenerated neurons become more eosinophilic, rounded and contain pyknotic nuclei complete in association with disappearance of large number of neurons.

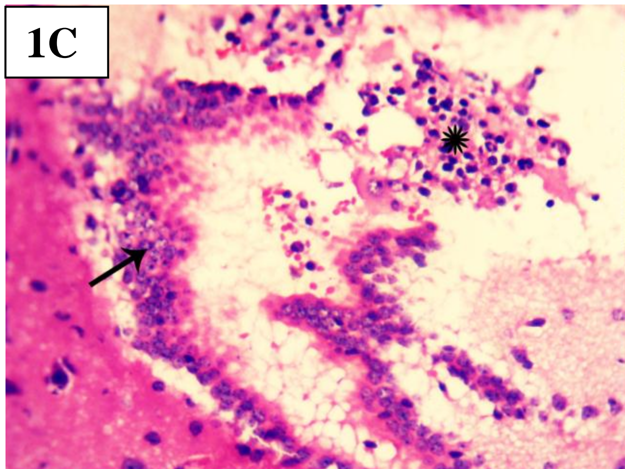
However, mild congestion of the brain blood vessels (fig. 1F) was observed in the brain of mice treated with RESV (group IV). Additionally, some of neuron of few treated rats exhibited mild vacuolization.



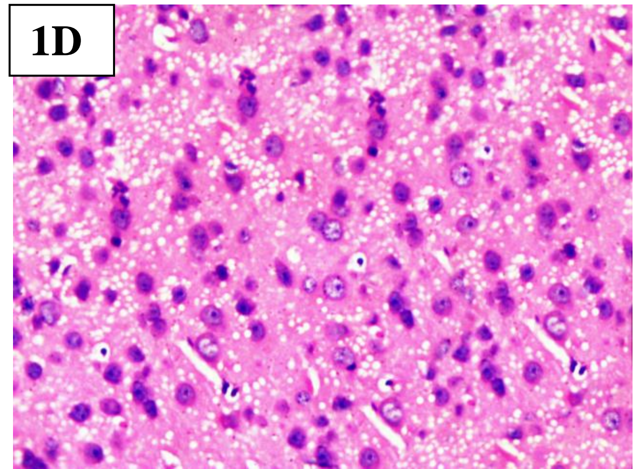
Brain of mice (induction of epilepsy by KA) showing severe congestion of meningeal blood vessels with rupture of blood vessels wall (arrow). H&E, x 400



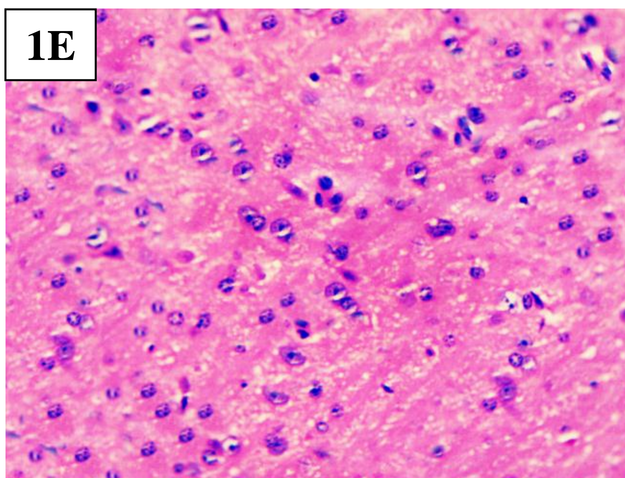
Brain of mice (induction of epilepsy by KA) showing multiple areas of hemorrhage in the brain substances (arrow). H&E, x 200



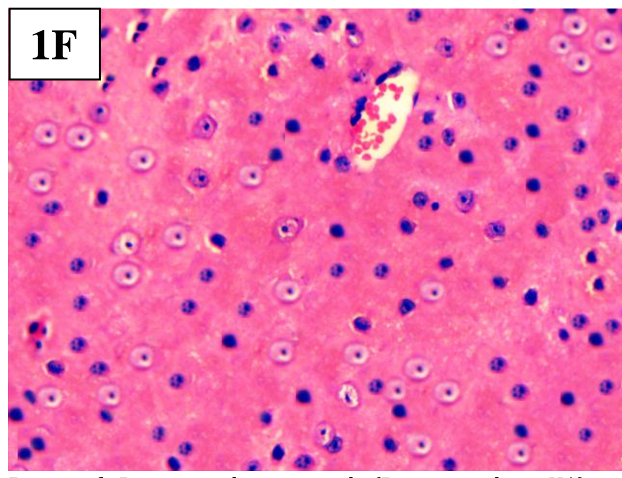
Brain of mice (induction of epilepsy by KA) showing hyperplasia of the lining ependymal cells with degeneration of the lining epithelium (arrow) and infiltration by mononuclear leukocytes (asterisk). H&E, x 400



Brain of mice (induction of epilepsy by KA) showing vacuolization of the brain substances with glial cell degeneration. H&E, x 200



Brain of Resveratrol protected (Resveratrol + KA) rats showing mild degeneration of neurons of cerebellum. H&E, x 200



Brain of Resveratrol protected (Resveratrol + KA) rats showing mild congestion of cerebral blood vessels with nearly normal appearance of glial cells. H&E, x 200

Epilepsy is a chronic neurological disorder characterized by recurrent unprovoked seizures. In rodents, systemic administration of KA leads to a well-characterized seizure syndrome. One hour after KA administration, the animals start to present with recurrent limbic motor seizures. The limbic seizures then develop into status epilepticus and lasted 1-2 hours [15]. The obtained results revealed that, a significant decrease in serum SA concentration was observed after 12 hours and 3 days in KA-induced epilepsy group. Bonfanti (2006) [16] establish that, sialic acids play an important role in many neuronal processes including axonal growth plasticity. Moreover, Johnson *et al.,* (2004) [17] indicated that, the glycosidic linkage of sialic acid is a potential target for superoxide and other related ROS. Charged sialic acid residues have also been proposed to be the moieties responsible for the effects of divalent ions on channel gating behavior. The extracellular membrane surface contains a substantial amount of negatively charged sialic acid residues. Some of the sialic acids are located close to the pore of voltage-gated channel, substantially influencing their gating properties. However, the role of sialylation of the extracellular membrane in modulation of neuronal and network activity remains primarily unknown. The level of sialylation is controlled by neuraminidase (NEU), the key enzyme that cleaves sialic acids. Who showed that, NEU treatment causes a large depolarizing shift of voltage-gated sodium channel activation/inactivation and action potential (AP) threshold without any change in the resting membrane potential of hippocampal CA3 pyramidal neurons. Cleavage of sialic acids by NEU also reduced sensitivity of sodium channel gating and AP threshold to extracellular calcium. At the network level, exogenous NEU exerted powerful anticonvulsive action both in vitro and in acute and chronic in vivo models of epilepsy. In contrast, a NEU blocker (N-acetyl-2,3-dehydro-2-deoxyneuraminicacid) dramatically reduced seizure threshold and aggravated hippocampal seizures. Thus, sialylation appears to be a powerful mechanism to control neuronal and network excitability. Who propose that, decreasing the amount of extracellular sialic acid residues can be a useful approach to reduce neuronal excitability and serve as a novel therapeutic approach in the treatment of seizures [18]. On another hand, Ratajczak *et al.,* (2011) [19] showed that, inflammation in the CNS results in increased amounts of sialic acids which are key determinants of degenerative processes in the brain. The importance of cell surface glycosylation in the brain, changes in the composition of sugar chains of glycoproteins and glycolipids can be crucial for the processes of repair and regeneration of CNS after injury and exposure to degenerative factors [20].

Protection and treatment with resveratrol administration in KA induced epilepsy in mice resulted in a significant increase in serum SA level when compared with epilepsy-induced non treated group. Sialic acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipids oligosaccharide side-chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction, contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions, and affecting the function, stability and survival of glycoproteins in blood circulation[21]. In the present study RES may be help in protection of brain tissue from KA-induced epilepsy due to its constructive effect on rising serum sialic acid level in both protecting and treatment periods.

A significant increase in serum TNF- α concentration was observed in KA-induced epilepsy in mice. These results are nearly similar to those reported by Mahaveer *et al.,* (2011) [22] who reported that, the brain level of TNF-alpha was significantly raised after KA-administration in rats. Also, Kerschensteiner *et al.,* (2009) [23] showed that, activated microglia and astrocytes after KA treatment release a large amount of inflammatory mediators such as NO, TNF-alpha, and IL-Iβ. Seizures and status epilepticus induced by chemical or electrical means stimulates a massive inflammatory response in the brain that consists of increased levels of cytokines, including IL-1β. In addition, IL-1β inhibits glutamate reuptake by astrocytes and enhances its astrocytic release via tumor necrosis factor-alpha (TNF-α) induction [24]. TNF-alpha is mainly produced by microglia and astrocytes in the CNS. KA-activiated microglia expressed high levels of TNF- α mRNA and protein. As with many other cytokines, TNF-α bears neuro-protective properties in contrast to its well-known deleterious role as a pro-inflammatory cytokine, which implies an intricate biological balance in immune and inflammatory responses mediate by TNF-α[25]. However, Zhu *et al.*, (2010) [26] suggested that, TNF-alpha derived from KA-activated microglia can increase the excite-toxicity of hippocampal neurons and can induce neuronal apoptosis in vitro and *in vivo*. Pro-inflammatory cytokine TNF-α has been implicated in playing an important role in the neuronal apoptosis caused by a variety of brain insults as well as the neurodegenerative disorders [27].

Protection and treatment with resveratrol administration in KA induced epilepsy in mice resulted in significant decrease in serum TNF-alpha level when compared with epilepsy-induced non treated group. Resveratrol was very effective in attenuating NO and TNF-α production from LPS-activated primary microglia cultures. Thus, RESV can potentially suppress pro-inflammatory responses of microglia. Considering these, it appears that RESV administration would beneficial for curtailing the inflammatory reaction in neurodegenerative diseases. This is particularly applicable to conditions where significant microglial activation is one of the pathological changes such as after acute seizure. RSV is also able to affect survival pathways like NF-ĸB and mitogen activated protein kinases (MAPKs)[28]. This protective activity was

mediated by the inhibition of tumor necrosis factor-α (TNF-α)- induced activation of NADPH oxidase, thus lowering H_2O_2 formation. Furthermore, RSV attenuated both mRNA and protein expression of TNF-α [29]. RSV was very effective in attenuating NO and TNF-α production from LPS-activated primary microglia cultures. This protective effect was characterized by reduced accumulation of reactive oxygen species and a significant increase in cellular glutathione levels [30], and thought to be due to the direct antioxidant and free radical scavenging properties of this dietary compound [31]. Resveratrol acting as an anti-inflammatory dietary phytochemical blocked some catabolic effects of proinflammatory mediators such as IL-1β and TNF-α via the inhibition of NF-κB[32].

The obtained data revealed that, a significant decrease in brain tissue enzymatic antioxidants (SOD, CAT and GPx) activities were observed in KA-induced epilepsy in male mice. Similarly, Bechman *et al.,* (2002) [33] demonstrated that, KA-induced increased seizure susceptibility is associated with mitochondrial oxidative stress in the hippocampus (increased mitochondrial lipid peroxidation and protein oxidation and mitochondrial loss of glutathione homeostasis), that KA-induced mitochondrial dysfunction is attributable to decreased Mn-SOD protein expression, mitochondrial membrane potential, and uncoupling protein (UCP)-2 mRNA expression, and that KA-induced activation of caspase-3 triggered by cytochrome c release potentiates neuronal degeneration. These findings may indicate that, endogenous mitochondrial antioxidant systems do not respond rapidly enough to oxidative stress. Moreover, Erakovic *et al.,* (1997) [34] reported that, an acute decrease in regional brain antioxidant levels was observed following electroconvulsive shock in rats. Who showed reduced SOD and glutathione peroxidase (GPx) activities in the hippocampus and the frontal cortex two hours after a single electro-convulsive shock. In patients with progressive myolonic epilepsy, the activity of the cytosolic superoxide dismutase (SOD1) was reported to be low [35]. Mitochondarial manganese superoxide dismutase (SOD2) was found to be down-regulated in the cerebral cortex of patients with epilepsy in contrast to non epileptic subjects[1]. GPx and CAT levels in neuronal tissue appear too low for the prevention of peroxide-induced lesions. Furthermore, neuronal cell membranes contain high levels of polyunsaturated fatty acids. Studies conducted by modulating the level of SOD in a mouse model of epilepsy have given us insights into the role antioxidant system in the prevenation of oxidative stress and a see mingly causal role of oxidative damage in seizure. It has been shown that over expression of Mn SOD, 0.5-2.0 fold, can attenuate kainite induced seizures, however animals with diminished Mn SOD levels showed an exacerbation of Kainate induced seizure and hippocampal damage, which was attenuated with antioxidant treatment [36].

On the other hand, resveratrol administration in KA induced epilepsy in mice resulted in a significant increase in brain tissue SOD, CAT and GPx activities when compared with epilepsy non treated group. Similarly, Simão *et al.*, (2011) [37] reported that, treatment with RSV markedly reversed the alterations in enzymatic antioxidants status SOD, GPx and CAT brought about by ischemia/reperfusion (I/R). The values were almost restored to near normal levels. Also, resveratrol treatment reversed the decrease of SOD activity and the increase of MDA level caused by spinal cord injury (SCI), suggesting its anti-oxidation role in response to the injury [38].

Administration of KA induced epilepsy in mice significantly increased L-MDA concentration when compared with normal control group. KA exposure can significantly increase the production of malondialdehyde (MDA) and 4-hydroxy-alkenals, suggesting an increase in lipid peroxidation [39]. Whereas, lipid peroxidation level increases in brain during epileptic seizures [40]. The increase in superoxide production and oxidative DNA damage following KA administration are indications of KA-induced mitochondrial and oxidative damage[41]. Similarly, Parihar and Hemnani (2003)[42] demonstrated that, hippocampal neurons are susceptible to oxidative attack by free radicals. A 3-fold increase in lipid peroxidation was observed after administration of KA. Also, Huang e*t al.,* (2004) [43] reported that, elevation of protein oxidation and lipid peroxidation were observed in the hippocampus at early time points (i.e. 4 and 24 h) post-KA administration. The nervous system is more susceptible to the damaging effect of oxidative stress, due to the high content of polyunsaturated fatty acids that are susceptible to lipid peroxidation. Lipid peroxidation, mediated by ROS, is believed to be an important cause of destruction and damage to cell membranes in accordance with the increases in ROS, the MDA level was also significantly increased, indicating the presence of enhanced lipid peroxidation [44]. Furthermore, MDA was increase 2 h post-pilocarpine-induced status epilepsy (SE) in the cortex [45]. Additionally, lipid radicals have been detected in the extracellular space during KA-induced seizure activity using *in vivo* electron spin resonance microdialysis in freely moving rats, suggesting a progression of lipid peroxidation during seizure activity which may lead to neuronal damage in the hippocampus following acute seizure activity [46].

Resveratrol administration in epilepsy-induced in mice markedly decrease and attenuate the increased of L-MDA concentrations in brain tissues when compared with KA group. These results are nearly similar to those reported by Simão *et al.,* (2011) [37] who found that, a single dose of RESV (at 40 mg/kg i.p) five- minutes prior to kA treatment (10 mg/kg i.p) increased the latency to convulsions. However, with multiple doses of RESV treatment (i.e. at 5 min prior to KA injection and

at 30 and 90 min post-KA injection), the incidence of convulsions was significantly reduced. RESV treatment also inhibited the KA-injury related increases in the level of MDA, suggesting that antioxidant function is one of the mechanisms by which RESV mediates neuro-protection against excitoxic injury and acute seizures. The brain MDA levels were found to be significantly attenuated in the trans-resveratrol-treated groups (multiple doses of 20 and 40 mg/kg b.wt) as compared to the kainic acid alone. The protective effect of trans-resveratrol against kainic acid-induced convulsions and the attenuation of raised MDA level suggest the potential use of antioxidants in the prevention of posttraumatic epilepsy [6]. Furthermore, malonyldialdehyde levels were significantly increased in model of epilepsy in rats. Dissimilarity, malondialdehyde levels decreased significantly after treatment with resveratrol in the cortex and hippocampus as compared with the model group [47].

KA-induced epilepsy in mice exhibited a significant decrease in brain tissue GSH level when compared with normal control group. Similarly, Shin *et al.*, (2008) [48] demonstrated that, administration of KA caused a decrease in reduced form of glutathione (GSH) levels in the hippocampus. So that intravenous GSH administration protected against KA-induced neuronal loss in the hippocampus and subsequent development of edema. Therefore, GSH may protect neuronal cells against KA neurotoxicity through a mechanism associated with ROS scavenging [49]. Moreover, Ogata *et al.*, (2001) [41] showed that, prolonged GSH depletion may lead to sensitization of the KA receptor to potentiate AP-1 DNA-binding activity in the murine hippocampus, suggesting that endogenous GSH may be partly involved in the underlying molecular mechanisms of transcription control by KA. Resveratrol treatment in epilepsy-induced in mice is significantly increased GSH level in brain tissues when compared with KA non-treated group. Additionally, Kumar *et al.* (2007) [50] reported that, a significant elevation in brain GSH levels of diabetic rats protected with RSV. In the present study RES, may be help in protection of brain tissue from KA-induced epilepsy due to its helpful effect on increasing GSH level in both protective and treatment periods.

Administration of KA in mice exhibited a significant increase in brain tissue NO level. The increase concentrations of NO and decreased levels of GSH support the role of oxidative stress in KA mediated epilepsy [51]. Systemic or intracerebral KA injections may result in consistent epileptic activity. During an experiment in which KA was injected directly into the CA3 area of the hippocampus, an increase in NO synthesis was demonstrated, contributing to cell death by apoptosis in the CA3 area of the hippocampus after the induction of an status epilepsy (SE) in the experimental temporal lobe [52]. Also, KA administration increases the generation of ROS and RNS by neuroglia, Microglia can produce large a mounts of soluble factors like NO [53]. Elevated production of NO by increased activity of iNOS is thought to contribute to KA-induced neuronal damage [54]. Moreover, Yoshida *et al.,* (2002) [55] demonstrate that, injection of kainate into the hippocampus induces seizure activity and NO synthesis in the contra lateral hippocampus and that both responses are attenuated by the specific neuronal NOS inhibitor.

Resveratrol administration in epilepsy-induced mice is markedly decrease and attenuate the increased of NO concentration in brain tissues when compared with KA group. Similarly, Simão *et al.*, (2011) [37] reported that, administration of RSV to ischemic rats significantly inhibited the increase of NO content in cortex and hippocampus when compared to vehicle-ischemic group. Flavonoids exerted NO production inhibitory activity in several cell lines and cultures (mouse peritoneal macrophages). This effect was probably caused by flavonoid inhibitory effect on expression of inducible NOS but not by the inhibition of its activity. Flavonoids also possess the ability to directly scavenge molecules of NO [56]. The level of NO in the brain was diminished in response to treatment with RSV. Resveratrol is reported to possess significant anti-inflammatory activity in various cells and tissues and is reported to inhibit the production of NO by Kupffer cells in a dose dependent manner that occurred at a post-transcriptional level [57].

A significant increase in brain tissue Caspase 3 activity, DNA fragmentation and 8–OHdG were observed in KA-induced epilepsy in mice. caspases are a family of aspartate-specific cysteine proteases. Caspase-3 is among the most studied regulators of apoptosis in the setting of seizure-induced neuronal death. Induction of caspase-3 mRNA and protein occurs within the hippocampus and extrahippocampal regions after seizures [58]. These results are nearly similar to those reported by Henshall *et al.,* (2001) [59] who reported that, caspase-3- like protease activity was increased within the ipsilateral hippocampus following seizures. A putatively selective caspase-3 inhibitor significantly improved neuronal survival bilaterally within the hippocampal CA3/CA4 subfields following seizures. Also, Kondratyev *et al.,* (2004) [60] found that, caspase-activated DNase, which is activated by caspase-3, is involved in DNA fragmentation and apoptotic neuronal cell death in rhinal cortex and hippocampus following SE. Moreover, Mouser *et al.,* (2006) [61] suggests that, caspase 3 activity is crucial for cellular alterations during epileptogenesis. KA induces different neurodegeneration among CA1, CA3 and the dentate gyrus (DG-hilus) regions which may be due to that the stratum lucidum region of CA3 is highly enriched with high-affinity KA binding sites [35]. Furthermore, Narkilahti *et al.* (2003) [62] suggested that, SE-mediated nuclear caspase 3 activation may activate caspase-activated DNase (CAD) results in DNA fragmentation and apoptosis. The express of active caspase 3 in the glial fibrillary acidic protein (GFAP)-positive radial glial cells was increased after KA-injection, suggests that caspase 3 functions as a

regulatory molecule in neurogenesis [63]. The co-injection of caspase 3 inhibitor prevent KA-mediated increase of radial glial cells, newly born neurons, and activated microglia, but not the astrogliosis, suggesting that astroglial caspase 3 was activated after gross astrogliosis, which then regulate microglial activation and neurogenesis. Microglia has been described to be a mediator of neurogenesis [64]. Likewise, Henshall *et al.,* (2000) [65] showed that, caspase-3 is cleaved and becomes active within brain regions exhibiting cell death following seizures induced by intra amygdaloidal KA. These events occurred in a sequential manner over a time course compatible with downstream consequences of caspase-3 activation, such as DNA fragmentation. Further, caspase-3 protein likely translocates to the nucleus where it is localized with fragmented DNA. Selective inhibition of caspase-3 in vivo may confer significant protection against seizure-induced brain injury, and inhibition of caspase-3 may therefore provide a novel neuroprotective approach as an adjunct to anticonvulsant therapy. Furthermore, systemic administration of kainate results in apparent DNA fragmentation in a precise and predictable anatomical distribution that is correlated with seizure severity. DNA fragmentation is a delayed effect of kainate [66]. Additionally, DNA fragmentation occurs within 24 h of KA administration and is maximal by 72 h. In general DNA fragmentation in mice is transitory, disappearing by 1 week after treatment [67]. These results are nearly similar to, systemic kainic acid, which induces seizures, increased cerebral 8-OHdG levels, up to seven-fold within 72 h. This was compatible with the patient [68]. Oxidative DNA damage could be evoked within a few days after epilepticus status begins, and neuroimaging changes may continue for some time beyond this. The researchers speculate that augmented oxidative stress was associated with refractory epilepticus status in the patient, because 8-OHdG is increased in the brain, including the amygdale and hippocampus, in the seizure-induced rat, and is associated with increased DNA fragmentation that results in neuronal cell death. Hence, serial measurements of oxidative stress markers in acute encephalitis, encephalopathy or status epilepticus could clarify the relationships between acute brain damage and free radicals (FR) in epilepsy [69]. Kainate administration to adult rats produced high levels of 8-OHdG, a marker of oxidative DNA damge. mtDNA was reported to be the major source for 8-OHdG in these experiments. The documented increase of 8-OHdG preceded overt cell death [70].

Acute seizure activity caused extensive 8-OHdG accumulation in the mitochondrial genome, by contrast, no measurable damage to the nuclear DNA was observed. This present study supports a disproportionately greater role of mitochondrial genomic injury following SE. The increased susceptibility of the mitochondrial genome compared to the nuclear genome to oxidative insults has been observed in a wide array of neurodegenerative diseases [71]. In addition, the present study suggests that oxidative stress may have an important role in mtDNA damage following SE. This is evidenced by increased levels of the 8-OHdG/2-dG ratio in conjunction with increased mitochondrial H2O2 production. Furthermore, the activity of the oxidative-sensitive aconitase was markedly decreased during SE, whereas the oxidative-insensitive fumarase was not affected. The mtBER proteins Ogg1 and Pol γ were up-regulated immediately after SE. Presumably; the increased expression of DNA repair proteins is a protective mechanism due to increased levels of mitochondrial oxidative stress, 8-OHdG accumulation and an attempt to repair this lesion. Oxidative stress has been previously shown to modulate DNA repair proteins in a variety of pathologies [72].

Resveratrol administration in epilepsy-induced mice markedly decrease and attenuate the increased of Caspase 3 activity, DNA fragmentation and (8-OHdG) in brain tissues when compared with KA-induced epilepsy non-treated group. polyphenolic compounds were investigated for their protective effects on oxidative DNA damage in a neuronal cell model, with a particular focus on the mechanisms by which they may be acting. Their antioxidant properties have already been well characterized [73]. In neuronal cells, it is described the cumulative effect of DNA damage in human brain over time (especially in mitochondrial DNA), which is supposed to play a critical role in aging and in the pathogenesis of several neurodegenerative diseases[74]. It should be taken into account that the effect of antioxidants on recovery from oxidative DNA damage may be justified by at least two different explanations: 1) by stimulating the activity of repair enzymes or 2) through a direct protection against oxidation [75]. Also, Pervaiz, (2004) [76] recorded that, because the resveratrol (RES) is a robust scavenger of ROS. So, RES has beneficial effects against diabetes-induced systemic and renal oxidative stress. RES treatment is associated with decrease in 8-hydroxy-2- deoxyguanosine. In the present study RES, may be help in protection of brain tissue from KA-induced epilepsy due to its helpful effect on decrease caspase-3 activity, DNA fragmentation and (8-OHdG) in both protective and treatment periods.

A significant increase in brain tissue activator protein-1 was observed in KA-induced epilepsy in mice. These results are nearly similar to those reported by Feng *et al.,* (1997) [77] who shown that, kainate caused increases in AP-1 DNA-binding activity in the hippocampus and that there were differences in the components of AP-1 complexes at different stages after kainite treatment. It is possible that the short-term and long-term AP-1 complexes play different roles in the regulation of neuronal function. The short-term AP-1 complex may mediate the induction of certain genes that respond to stimuli in an acute fashion ranging from minutes to hours. On the other hand, the long-term AP-1 complex may be associated with the changes in neuronal plasticity that often require protracted time periods ranging from days to months [78]. Moreover, this is supported by *in vivo* evidence of increased

hippocampal AP-1 binding within an hour after seizure, just before increased NGF gene expression [79]. However, the chronic increase in hippocampal AP-1 binding activity after kainic acid-induced seizures is not linked to chronically elevated NGF mRNA expression, Similarly, after kainate induced seizures, JunB and JunD are major components of AP-1 binding at early and late time points, respectively [80]. It is generally accepted that oxidative stress upregulates the expression of inflammatory genes via activation of redox-responsive transcription factors. Indeed, activation of activator protein-1 (AP-1) and nuclear factor kB (NF-kB) is considered to contribute to the general regulation of a number of inflammatory genes by cellular oxidative stress and/or intracellular glutathione levels (Arrigo, 1999) [81]. Also, Cowie *et al.,* (1994) [82] suggests that, seizure-induced NGF expression in the brain is predominantly, if not fully, regulated through AP-1 activation. Moreover, Szekely *et al.,* (1990) [83] suggest that, the composition of AP-1 binding changes with time after depolarization and seizures. Likewise, Hsu *et al.,* (1993) [84] shown that, changes in AP-1 composition can increase or suppress transactivation of target genes in a number of systems. Furthermore, Ogata *et al.,*(2001) [41] suggesting that endogenous GSH may be partly involved in the underlying molecular mechanisms of transcription control by KA. Changes in AP-1 DNA-binding activity were qualitatively similar to those observed with NF-kB in the adult rat brain.

Resveratrol administration in epilepsy-induced mice markedly decrease and attenuate the increased of activator protein-1 in brain tissues when compared with KA-induced epilepsy non-treated group. These results are nearly similar to, Activation of AP-1 can be inhibited by numerous natural product polyphenols such as resveratrol [85]. In the present study RES may be help in protection of brain tissue from KA-induced epilepsy due to its helpful effect on decrease activator protein-1 in both protective and treatment periods.

A significant increase in brain tissue MPO were observed in KA-induced epilepsy in mice. These results are nearly similar to those reported by Pattie (2004) [86] suggested that, myeloperoxidase expression was increased in tissue with Alzheimer pathology where it localized to amyloid plaques and, surprisingly, neuronal populations in both the neocortex and the molecular layer of the hippocampus. These observations raise the possibility that the enzyme represents one pathway for promoting oxidative stress in AD. However, Leeuwenburgh *et al.,* (1997) [87] establish that, increased myeloperoxidase expression and associated production of oxidants may contribute to tissue injury during chronic inflammation in both peripheral diseases such as atherosclerosis and diseases of the CNS such as multiple sclerosis and AD. Inhibition of myeloperoxidase activity may prove to be a valuable therapeutic target beyond general antioxidant regimens to slow oxidative mediated damage in these diseases. Also, Barone *et al*. (1995) [88] shown that, at 3 days after ischemia MPO activity increases dramatically, peaking at 5 days, before decreasing to preischemic values by 15 days. The increase and peak in MPO activity at 5 days has previously been demonstrated histologically to be the result of a dramatic increase in monocyte and macrophage accumulation within the ischemic brain, and corresponds to a time when neutrophils were reported to be decreasing. Myeloperoxidase (MPO) is a heme protein expressed at high levels in neutrophils [89], and is a potent pro-inflammatory mediator [90].

Resveratrol administration in epilepsy-induced mice markedly decreases and attenuates the increased of MPO in brain tissues when compared with KA-induced epilepsy non-treated group. These results are nearly similar to those reported by Chi *et al.* (2013) [91] who stated that, resveratrol significantly reduced rotenone-stimulated MPO levels and prevented the accompanying overproduction of NO in microglia, we asked whether resveratrol directly reduced MPO levels in MPO-treated rat primary microglia. Cells were left untreated or pretreated with 10 μM resveratrol for 1 h, and then mock-treated or treated with 100 ng/ml MPO for 24 h. FACS analyses showed that MPO-dependent increases of MPO levels in rat primary microglia were markedly inhibited by pretreatment with resveratrol. Sherer *et al.*, (2003) [92] exhibited the inhibitory effects of resveratrol on MPO levels in rat primary microglia. Similar to the results obtained in microglia, resveratrol also markedly reduced MPO levels in rat primary astrocytes. Microglias were the targets of resveratrol action and resveratrol exerted neuroprotection through the inhibition of microglial activation. Microglial activation leads to reaction of superoxide anion with NO to form highly reactive intermediates which have an important role in neurodegeneration [29]. Additionally, resveratrol-induced neuroprotection is thus mediated by both antioxidant and NO promoting properties [93]. The neuroprotective effects of RESV are likely mediated through multiple mechanisms, which might include the inhibition of the: (1) voltage- gated potassium currents; (ii) electrical activity of CA/neurons and (iii) excitatory synaptic transmission in the hippocampus via inhibition of the post- synaptic glutamate receptors [94].

4. CONCLUSION

In conclusion, the results of the present study suggest that, resveratrol administration attenuate kainate-induced epileptic seizures in mice and may be potential effectiveness for the prevention and control of seizure development, has anticonvulsant therapies of brain epilepsy by its anti-inflammatory effect, radical scavenging and anti-apoptotic activity, inhibited caspase-3 and regenerating endogenous antioxidant mechanisms in brain tissues.

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