Biochemical role of curcumin on kainic acid-induced epilepsy in male swiss albino mice

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A B S T R A C T

Oxidative stress resulting from excessive free-radical release is likely implicated in the initiation and progression of epilepsy. The potential protective and treatment effect of curcumin (CUR) administration on KA-induced epilepsy in mice was evaluated. 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+ CUR protected group) mice received CUR (200 mg/kg b.wt/day/orally) for 7 days before KA administration. Group IV:(epilepsy+ CUR treated group): mice first injected with KA(10 mg/kg b.wt/i.p.) then after 15 min. CUR was administered as in group III for 3 consecutive days. Blood samples and brain tissue specimens were collected after 12 hours and 3 days from the onset of KA administration for determination of serum sialic acid (SA) and tumor necrosis factor alpha (TNF-α), brain tissue superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), reduced glutathione (GSH), L-Malondialdehyde (L-MDA), nitric oxide (NO), caspase-3 and DNA-fragmentation. The obtained results showed that, KA-induced epilepsy in mice caused significant decrease in serum SA, and brain tissue SOD, CAT, GPX activities and GSH concentration. However, serum TNF-α and brain tissue NO, L-MDA levels, caspase-3 activity and DNA-fragmentation were significantly increased. Administration of CUR was able to mitigate KA- induced epilepsy through rising of serum SA and brain tissue SOD, CAT, GPX activities and GSH in addition to declining NO, L-MDA, caspase-3 and DNA-fragmentation in brain tissue. These results suggest that, CUR may be successful in the treatment of epilepsy by its radical scavenging, anti-inflammatory and antiapoptotic activities and regenerating endogenous antioxidant mechanism. In addition, curcumin protects mice brain against KA induced neuronal damage, decrease the severity of epilepsy and attenuated kainite induced inflammation and apoptosis.

KeyWords: Curcumin, kainic acid, epilepsy, apoptosis; antioxidant enzymes, caspase-3.

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1. INTRODUCTION

Epilepsy is a chronic condition characterized by recurrent unprovoked seizures. It affects about 3 million people in the United States and approximately 65 million people worldwide. Epilepsy affects people of all ages and both genders. Every year, nearly 150,000 new cases of epilepsy are diagnosed in the United States. A seizure is an abnormal electrical discharge in the brain that causes alteration in consciousness, sensation, and behavior. When the risk of spontaneous seizures is sufficiently high, generally after two spontaneous seizures, the patient is diagnosed with epilepsy. Epilepsy is a disorder with many possible causes. Epilepsy may develop because of an abnormality in brain wiring, an imbalance in inhibitory and excitatory neurotransmitters, or some combination of these factors. Primary epilepsy (50%) is idiopathic (unknown cause). In secondary epilepsy (50%), referred as acquired epilepsy, seizures may result from a variety of conditions including trauma, anoxia, metabolic imbalances, tumors, encephalitis,

drug withdrawal seizures, or neurotoxicity. The most common risk factors for epilepsy are cerebrovascular disease, brain tumors, alcohol, traumatic head injuries, malformations of cortical development, genetic inheritance, and infections of the central nervous system (Doodipala and Ramkumar, 2013). A relationship between status epilepticus (SE) and oxidative stress has recently begun 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 (Martinc *et al.*, 2012). Curcumin has been reported to act as a free radical scavenger and an antioxidant, thus inhibiting lipid peroxidation and oxidative DNA damage (Shukla, 2003). Furthermore, both experimental and epidemiological evidence have shown beneficial influence of curcumin on seizures, oxidative stress and cognitive impairment (Morimoto, 2004). Some studies reported that co-administration of curcumin with antiepileptic drugs like valproic acid, phenytoin, phenobarbitone and carbamazepine in sub-therapeutic doses increased latency to seizures and reduced oxidative stress without altering their pharmacokinetics (Reeta *et al.*, 2011). Moreover, Peng *et al.* (2009) showed that, curcumin has anticonvulsant effects against seizures induced by kainic acid (KA). Accordingly, the present study was designed to evaluated the beneficial and the potential protective effect of curcumin against kainic acid-induced epilepsy in Swiss albino mice.

2. MATERIALS AND METHOD

2.1. Experimental animals:

Twenty four male Swiss albino mice of 6-8 weeks old and weighting 25-30 g 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. Curcumin (purity ~99%) was manufactured by Fluka Co. for chemicals and purchased from Elgoumhouria Co. for Trading Chemicals Medicines and Medical Appliances, Egypt.

2.2. Preparation and dosage of Curcumin:

Curcumin was freshly prepared by dissolved in 7% DMSO solution then complete to 100 ml distilled water, and was administered every day orally at a dose of (200 mg/kg b.wt) (Agarwal *et al*., 2003).

2.3. Induction of Brain 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 (Gupta *et al.*, 2002).

2.4. Experimental design:

Mice were randomly divided into four main equal groups, 6 animal each, placed in

individual cages and classified as follow: Group (1): Control normal group: Mice received no drugs, served as untreated control for all experimental groups. Group Π : (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 + CUR protected group): Mice received CUR (200 mg/kg b.wt./orally) and daily for 7 successive days prior to KA injection (10 mg/kg b.wt./i.p). Group IV : (epilepsy + CUR treated group): Mice injected with KA (10 mg/ kg b.wt,/ i.p) after 15 min. mice were treated with CUR (200 mg/kg b.wt/day, orally) for three days. *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:

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°C until used for subsequent biochemical analysis. All sera were analyzed for the determination of sialic acid and TNF-alpha

2.4.2. Tissue samples (Brain tissue):

The skull was opened carefully and the brain was quickly 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, and DNA fragmentation

2.5.Biochemical analysis:

Serum sialic acid and TNF-α were determined using human sialic acid ELISA kit (Cat.No.CSB-E09605h) and Beyaert and Fiers, (1998), respectively. Moreover, brain tissues SOD, CAT, GPx, GSH, L-MDA, NO, caspase-3 and DNA-fragmentation were determined according to the methods described by Kakkar *et al.* (1984), Luck (1974), Gross *et al.* (1967), Moron *et al.* (1979), Mesbah *et al.* 2004), rat caspase-3 ELISA Kit (CUSABIO BIOTECH CO., LTD) Cat.No.CSB-E08857r) and Shi *et al.*, (1996), 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.

3. RESULTS

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

The obtained data demonstrated in table (1) revealed that, a significant decrease in serum SA level and brain tissue SOD, CAT and GPx activities were observed 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 curcumin 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.

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

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

4. DISCUSSION

Epilepsy is one of the leading neurological disorders affecting 50 million of worlds total population, requiring long term antiepileptic drug (AED) therapy (Lowenstein, 2008). Despite treatment with AED, epilepsy remains refractory in one third of patient. Generation of ROS in brain is considered as one of the leading causes of generalized epilepsy associated with recurrent seizures. Some studies have demonstrated neuroprotective effect of curcumin against cerebral ischemic injury or traumatic brain injury (Balosso *et al.*, 2006). Sng *et al.* (2006) reported that, pretreatment with curcumin attenuates histone modifications in kainate- induced status epilepticus. 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) found that, sialic acids play an important role in many neuronal processes including axonal growth plasticity**.** Moreover, Johnson *et al.* (2004) 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

Table (1): Protective and treatment effect of curcumin on serum sialic acid and TNF-α concentrations and brain tissue SOD, CAT and GPx activities of 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 ±4.19^{a}	48.36 ±4.01^{a}	33.59 ±3.79^{c}	33.34 ±2.29^{b}	38.31 ±5.06^{a}	25.16 ±2.22^{a}	60.12 ±2.99^{a}	53.44 ±2.10^{a}	46.66 ±1.80^{a}	41.70 ±1.48^{a}
KA (epilepsy)	19.31 ±3.27^{b}	17.75 ±2.14^{c}	57.64 ±2.85^{a}	81.74 ±10.90^{a}	6.81 ±1.99^{c}	9.36 ±0.82^{c}	16.55 ±5.64^{d}	23.60 ±4.27^{c}	10.90 ±2.25^{e}	9.93 ±2.88^{e}
Curcumim protected	36.58 ±1.75^{a}	26.19 ±1.39^{c}	45.39 ±2.25^{b}	47.12 ±2.97^{b}	14.71 ±1.00^{c}	12.72 ±1.17^{bc}	36.91 ±2.39^{c}	34.55 ±6.68^{b}	26.06 ±0.98^{d}	28.59 ±2.03^{cd}
Curcumim treated	42.54 ±3.4^{a}	35.65 ±2.34^{b}	45.64 ±0.49^{b}	41.74 ±2.89^{b}	33.94 ±3.31^{a}	24.84 ±3.52^{a}	48.76 ±3.35^{abc}	57.77 ±1.83^{a}	10.90 ±2.25^{e}	34.05 ±1.62^{bc}

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≤0.05).

Table (2): Protective and treatment effect of curcumin on brain tissue L-MDA, GSH and NO concentrations, caspase-3 activity and DNA fragmentation percent of 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}
Curcumin protected	87.30 ±8.05^{b}	82.41 ±9.37^{bc}	2.35 ±0.26^{ab}	3.31 ±0.50^{bc}	49.41 ±6.00^{b}	57.51 ±9.66^{b}	1.83 ±0.17^{ab}	1.53 ±0.12^{bc}	730.77 ±70.72^{bc}	664.61 ±43.16^{b}
Curcumin treated	58.40 ±9.84^{cd}	52.08 ±6.39^{c}	3.11 ±1.18^{ab}	2.56 ±0.55^{c}	81.46 ±4.43^{a}	94.25 ±2.83^{a}	1.56 ±0.06^{bc}	1.07 ±0.17^{cd}	449.23 ±58.70^{cd}	296.92 ±65.29^{cd}

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≤0.05).

Dmytro et al., 2007). Curcumin administration in KA induced epilepsy in mice resulted in 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 (Sumangala *et al.*, 1998). In the present study CUR may 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) who reported that, the brain level of TNF-alpha was significantly raised after KA-administration in rats**.** Also, Kerschensteiner *et al.* (2009) 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 (Bezzi *et al.*, 2001). 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 neuroprotective 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 (Lu *et al.,* 2008). However, (Zhu *et al.,* 2010) suggested that, TNF-alpha derived from KA-activated microglia can increase the excitotoxicity 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 (Chaparro-Huerta *et al.,* 2008).Protection and treatment with curcumin administration in KA induced epilepsy in mice resulted in significant decrease in serum TNF-alpha level when compared with KA- non treated group. Similarly, Wang *et al.* (2010) revealed that, curcumin reduces the amyloid-β-stimulated inflammatory responses in primary astrocytes. Also, Lim *et al.* (2001) reported that, low and high doses of curcumin significantly lowered oxidized proteins and interleukin-1 beta (IL-1β), a pro-inflammatory cytokine elevated in the brains of these mice. Who demonstrated the beneficial effects of curcumin on oxidative damage and amyloid β pathology in a transgenic mouse model of Alzheimer disease (AD). Moreover, curcumin administration has been reported to attenuate cognitive deficits, neuroinflammation, and plaque pathology in AD models (Yang *et al.*, 2005). In the present study CUR may be help in protection of brain tissue from KA-induced epilepsy due to its positive effect on decreasing serum TNF-alpa concentrations in both protective and treatment periods.

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) 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) 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 electroconvulsive shock. In patients with progressive myolonic epilepsy, the activity of the cytosolic superoxide dismutase (SOD1) was reported to be low (Ben-Ari *et al.*, 2000). 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 (Eun *et al.*, 2013). 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 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 (Patel, 2002). Curcumin 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, Wang *et al.* (2012) demonstrated that, pretreatment with curcumin at doses of 100 and 300 mg/kg significantly delayed the onset of pilocarpine-induced limbic seizures and status epilepticus. These doses of curcumin also counteracted pilocarpine-induced changes in hippocampal NOS, SOD, and LDH activities and GSH content. Taken together, these results indicate that the anticonvulsant properties of curcumin may at least in part be mediated by the central nitric oxide system and free radical production. In present study CUR may be help in protection of brain tissue from KA-induced epilepsy due to its positive effect on increasing enzymatic antioxidants in both protective and treatment periods.

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 (Liang and Patel, 2006). Whereas lipid peroxidation level increases in brain during epileptic seizures (Sudha *et al.*, 2001). The increase in superoxide production and oxidative DNA damage following KA administration are indications of KA-induced mitochondrial and oxidative damage (Yoneda *et al.*, 2001). Similarly, Parihar and Hemnani (2003) demonstrated that, hippocampal neurons are susceptible to oxidative attack by free radicals. A 3-fold increase in lipid peroxidation were observed after administration of KA. Also, Huang *et al.* (2004) 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 (Korkmaz and Kolankaya, 2010). Furthermore, MDA was increase 2 h post-pilocarpine-induced status epilepsy(SE) in the cortex (Tejada *et al.*, 2007). 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 (Ueda *et al.*, 2002)**.** Curcumin 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 Browse *et al.* (2011) who recorded that, different doses of curcumin (50, 100 and 200 mg/kg, p.o.) administration in pentylene tetrazole (PTZ)-treated rats significantly decreases MDA and increase glutathione levels. Moreover, repeated PTZ administration has significantly increased the free radical generation as indicated by increased MDA and decreased the GSH level in the rat brain. Curcumin administration with dose dependent manner, significantly decreases MDA and increases GSH levels (two oxidative stress markers) in the brain – tissue of PTZ-kindled mice (Ono, 2000). Curcumin has been reported to act as a free radical scavenger and an antioxidant, thus inhibiting lipid peroxidation and oxidative DNA damage (Shukla, 2003). The decrease in MDA level in the groups treated with curcumin as compared to the vehicle treated KA group indicates attenuation of lipid peroxidation.

Kainic acid- induced epilepsy in mice exhibited a significant decrease in brain tissue GSH level when compared with normal control group. Similarly, Shin *et al.* (2009) 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 (Yoneda *et al.*, 2001). Moreover, Ong *et al.* (2006) demonstrated that, even through GSH decreases in neurons after KA injection, there is an up-regulation of GSH synthesis in reactive astrocytes 3 days to 6 weeks after kinate injection. It has been reported that, KA-induced seizure activity impairs glutathione homeostasis and negatively correlates with the GSH/ GSSG ratio or GPx activity. The decrease in GSH is associated with increased levels of GSSG and therefore, a lower ratio of GSH/ GSSG usually evident after KA (Shin *et al.,* 2009). Kainic acid - induced neurotoxicity involves the peroxidation of lipids, a decrease in glutathione content and an accumulation of 4-hydroxzynonenal an especially neurotoxic end product of lipid peroxide decomposition and direct treatment with GSH have been shown to protect a gainst KA - induced neurotoxicity (Duffy *et al.,* 2008). Curcumin treatment in epilepsy-induced in mice significantly increased GSH level in brain tissues when compared with KA non-treated group. Similarly, Piper *et al.* (1998) reported that, increase in the levels of glutathione by curcumin indicates its antioxidant property possibly by increasing the endogenous defense of the brain to combat oxidative stress induced by KA. Also, there was a simultaneous significant increase in the glutathione levels in the curcumin (100 and 200 mg/ kg, i.p) of PTZ treated mice as compared to control group. Glutathione is the most abundant intracellular thiol and low molecular weight tripeptide found in living cells. It reacts with

the free radicals and can protect cells from singlet oxygen, hydroxyl radical and superoxide radical damage. The increase in levels of glutathione by curcumin indicates its antioxidant property possibly by increasing the endogenous defense of the brain to combat oxidative stress induced by KA (Yogendra *et al.*, 2009).

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 (Dzhala *et al.*, 2008). 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 (Zsurka and Kunz, 2010). Also, KA administration increases the generation of ROS and RNS by neuroglia, Microglia can produce large a mounts of soluble factors like NO (Hanisch 2002). Elevated production of NO by increased activity of iNOS is thought to contribute to KA-induced neuronal damage (Amor *et al.*, 2010). Moreover, Yoshida *et al.* (2002) 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. Curcumin administration in epilepsy-induced mice markedly decrease and attenuate the increased of NO concentration in brain tissues when compared with KA 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 (Procházková *et al.*, 2011). Similarly, He *et al.*, (2010) reported that, curcumin significantly inhibits the apoptosis of pre-oligodendrocytes and expression of either iNOS or NOX in the LPS-activated microglia. In *in vivo* studies, curcumin decreases activated microglia and inhibits microglial expression of iNOS and translocation of p67-phox and gp91-phox to microglial cell membranes in neonatal rat brains following LPS injection. Moreover, Hana *et al.,* (2006) showed that, treatment of diabetic rats with curcumin reduced eNOS and iNOS levels in association with reduced oxidative DNA and protein damage. Curcumin has the ability to inhibit iNOS induction by LPS in the mammary glands and to scavenge NO radicals and reduce TNF-α, which might explain, at least partly, its therapeutic properties in inflammation, which curcumin has shown potential antioxidant, anti-inflammatory and cytokine-inhibitory effects (Sharma *et al.,* 2006).

A significant increase in brain tissue Caspase 3 activity and DNA fragmentation 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 (Akbar *et al.*, 2003). These results are nearly similar to those reported by Henshall *et al.* (2001) 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, Henshall *et al.* (2000) 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. Mouser *et al.* (2006) 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 (Ben-Ari *et al.*, 2000). Narkilahti *et al.*, (2003a) 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 (Aras *et al.*, 2012). 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 (Kohman *et al.*, 2013). David *et al.* (2000) 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 (Wijsman *et al.*,1993). 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(Schauwecker and Stewart, 1997). Curcumin administration in epilepsy-induced mice markedly decrease and attenuate the increased of caspase-3 activity and DNA fragmentation in brain tissues when compared with KA non-treated group. 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 (Tomasetti *et al.*, 2001). In addition, Ibrahim *et al.*, (2006) proved that, treatment of rats with either curcumin or chlorophyllin revealed lower DNA fragmentation percentages. These results coincide with that of Siddique *et al.*, (2010) who stated that, curcumin inhibits the generation of ROS that are responsible for the DNA damage. Also, this action of curcumin was explained by Piwocka *et al.*, (2001) who stated that curcumin leads to attenuated DNA fragmentation due to the elevation of GSH. Furthermore, Madkour, (2012) showed that, oral intake of curcumin to lambda cyhalothrin (LCT)-intoxicated rats exhibited significant decrease in liver enzymes activities and lipid peroxidation, significant increase in antioxidant enzymes activities and partial inhibition in DNA fragmentation. Caspase-3 is a key executioner of apoptosis, which is activated by an initiator caspase such as caspase-9. These activated caspases cleave many cellular substrates, ultimately leading to cell death. In addition, Song *et al.,* (2005) found that, curcumin could down-regulate pro-caspase-9 and pro-caspase-3 expression in a time-dependent manner on the HT-29 cells. Furthermore, curcumin was shown to activate caspases 9, 3, and 8 in the colon cancer cell lines SW480 and SW620 (Rashmi *et al.,* 2003). Additionally, David *et al.* (2000) shows that, caspase-3 is cleaved and becomes active within brain

regions exhibiting cell death following seizures induced by intra amygdaloid KA. These events occurred in a sequential manner over a time course compatible with downstream consequences of caspase-3 activation, such as DNA fragmentation. Furthermore, caspase-3 protein likely translocates to the nucleus where it is co-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.

CONCLUSION: the present study demonstrated that, CUR. possesses significantly neuroprotection and treatment effects against epilepsy and oxidative damage in brain tissue induced by KA in mice.

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الدور الكيميائى الحيوي للكركمين على الصرع المحدث بحمض الكينيك في ذكور الفئران

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قسم الكيمياء الحيوية -كلية الطب البيطري بمشتهر-جامعة بنها

الملخص العربي

في هذه الدراسة تم تقييم التأثير *الوقائي* والعلاجي للكركمين على التغيرات في مستوى حمض السياليك، عامل تنخر الورم الفا ، الإنزيمات المضادة للأكسدة ، تركيز إل-مالون داى ألدهيد، انزيم ،الجلوتاثيون المختزل، مستوى اوكسيد النيترك، الكاسبيس 3 وتفتييت الدى ان ايه في دم وأنسجة الفئران المستحدث فيها الصرع فى المخ بحمض الكينيك . هذا وقد أستخدم لأجراء هذه الدراسة عدد24 من الفئران البيضاء أعمارهم تتراوح من 6-8 أسبوع وأوزانها من 25-30جرام وقد قسمت إلي اربعة مجموعات وتم توزيعها كالآتي: المجموعة الأولى: (المجموعة الضابطة): اشتملت على 6 فأر لم تعطى أي أدوية واستخدمت كمجموعة ضابطة للمجموعات الأخرى. المجموعة الثانية: (المجموعة المحدث بها الصرع): تكونت من 6 فأر تم اعطاؤها جرعة واحدة فقط من حمض الكينيك عن طريق الحقن تحت الجلد بجرعة 10ملى جرام /كيلوجرام من وزن الجسم. المجموعة الثالثة:(مجموعة الكركومين الوقائية والمحدث بها الصرع): اشتملت على 6 فأر تم تجريعها بجرعة مقدراها (200 مللى جرام/ كيلوجرام من وزن الجسم) لمدة 7 ايام وفى اليوم الثامن تم حقنها بحمض الكينيك تحت الجلد بجرعة 10ملى جرام /كيلوجرام من وزن الجسم لاحداث الصرع وتكملة العلاج بالكركومين لمدة 3 ايام.. المجموعة الرابعة (مجموعة الكركومين العلاجيه والمحدث بها الصرع):اشتملت على 6 فأر تم تجريعها بجرعة مقدراها (200 مللى جرام/ كيلوجرام من وزن الجسم) لمدة 3 ايام بعد احداث الصرع. هذا وقد تم تجميع عينات الدم والانسجه بعد 24,12ساعه من من حدوث الصرع والعلاج. وقد أسفرت نتائج التحليل البيوكيميائى عن وجود انخفاض معنوى فى حمض السياليك بالمصل بالاضافة إلى نقص معنوى فى نشاط نشاط سوبر أكسيد ديسميوتيز والكتاليز وانزيم الجلوتاثايون ريدكتاز والجلوتاثيون فى انسجة المخ مع حدوث زيادة معنوية فى مصل عامل تنخر الورم الفا بالاضافة الى زيادة معنوية فى تركيز إل-مالون داى ألدهيد، النيترك اوكسيد، كاسبيس-3 وتجزئة الحمض النووي دى ان ايه فى المجموعه المحدث بها الصرع. كما أوضحت النتائج أن مجموعتى الفئران المحدث بها الصرع والتي تم وقايتها وعلاجها بالكركومين عن وجود زيادة في مصل حمض السيالك بالدم بالأضافه الى نشاط نشاط سوبر أكسيد ديسميوتيز والكتاليز وانزيم الجلوتاثايون ريدكتاز والجلوتاثيون فى انسجة المخ فى حين انخفض مستوى عامل تنخر الورم الفا فى المصل بالاضافة الى وجود نقص معنوى فى مستوى النيترك اوكسيد وتركيز إل-مالون داى ألدهيد، كاسبيس-3 وتجزئة الحمض النووي دى ان ايه فى انسجة المخ. وأوضحت الدراسة أن استخدام الكرومين كان له دور فعال في حماية وعلاج انسجة وخلايا المخ من الصرع المحدث باستخدام حمض الكينيك وأدى استخدامه كذلك الى الحفاظ على نسب القياسات البيوكيميائية فى الدم والأنسجة لما يقارب النسب الطبيعية ، ويرجع ذلك إلى نشاط الكركومين الوقائى والعلاجى والمضاد للأكسدة والألتهابات والمضاد لتكسير الدى ان ايه والموت الخلوى للخلايا. لذلك توصى الدراسة بضرورة استغلال تلك المزايا الهائلة للكركومين كماده وقائية وعلاجية مضادة للأكسدة وإدخاله كماده فعالة فى صناعة العقاقير الطبية المستخدمة فى وقاية وعلاج انسجة وخلايا المخ من الصرع.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(2):225–240, ديسمبر 2014)