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RESEARCH ARTICLE

Curcubita pepo seed oil ameliorative effects on histological and biochemical changes in rat epileptic model

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Abstract

The objective of this study was to investigate whether pumpkin seed oil would reduce neuronal death and pathophysioloical and histological alterations in pilocarpine-epileptic model compared to topiramate as a common used antiepileptic drug.

60 rats were divided into 4 groups, the 1st and 2nd groups are the control groups (24hrs and 21 days). The3rd and 4th are the treatments, one treated with 20 mg/kg b. wt. topiramate and the other with 40 mg/kg b. wt. pumpkin seed oil for 21 days. Another 90 rats were injected i. p. with 300 mg /kg b. wt. pilocarpine. They were subdivided into 4 groups the 5th & 6th were the epileptic control groups (24 hrs and 21 days). The 7th and 8th, one treated with the same previous dose of topiramate and the other treated with the previously mentioned dose of pumpkin seed oil for 21 days.

After 21 days of pilocarpine injection, some behavioural changes accompanied with mossy fibres sprouting and activation of progenitor cells formation with granular cell loss and apoptosis confirmed by gel electrophoresis and immunohistochemical methods in the dentate gyrus were observed. These changes were associated with Significant decreases in GABA, aspragin, histidine, norepinephrine, sertonin, serum Ca^{2+}, Na^{+} and hippocampal K^{+} concentrations, reduced glutathione content and abated reactivity towards Bcl-2 antibody. In addition, significant increase in glutamate, aspartate, dopamine, Ca^{2+} and Na^{+} in the hippocampus, serum K^{+}, lipid peroxidation, nitric oxide concentrations, glutathione peroxidase activity and reactivity against p53 and PCNA antibodies, were recorded. All the changes reported after 21 days of pilocarpine injection showed similar pattern after 24 hrs, except for decreased DA level, GPx activity and proliferative index and severe mossy fibres sprouting and granular cell loss.

Both pumpkin seed oil and topiramate were able to ameliorate most of the physiologically-altered parameters in epileptic rats and also proved high efficacy towards decreasing the neuronal cell death via interrupting the apoptotic mechanism which was confirmed by DNA fragmentation indicated by gel electrophoresis and immunohistochemical methods. Pumpkin seed oil elicited more pronounced effects. Therefore, it is concluded that natural antioxidants like pumpkin seed oil, could act as promising antiepeleptic drugs of high efficacy in retarding neuronal cell death and pathophysiological and histological complications related to epilepsy.

INTRODUCTION

Epilepsy is a heterogeneous syndrome characterized by recurrent and spontaneous seizures affecting 1% of the population in the world. Though, 20% – 30% of the patients are obstinated to available antiepileptic drugs (AEDs) (Pirttila, 2006). Several factors have been described as triggers for some of the epilepsies like structural abnormality in the brain, brain injury, infection and/or genetic mutations, but most of the epilepsy cases remain of unknown etiology (Lopez-Picon, 2008).

Epilepsy manifests in different ways, from a single seizure to status epilepticus (SE) lasting for more than 30 minutes (Fisahn and Loscher, 2005; Parent *et al*., 2006). Epileptic seizures can be either generalized (generalized epileptic seizure), originating in both hemispheres of the brain simultaneously, or partial (focal seizures) (Isaac, 2005).

Temporal lobe epilepsy (TLE) is one of the most prevalent types of adult human focal epilepsies which starts from temporal lope structures as hippocampus (Engel, 2001). It is thought to be a result of transient and direct events that induce seizures (ictogenesis) due to excessive discharges from groups of neurons initiated by the sequential opening of the voltage-dependent Na^+ channels due to membrane depolarization resulting from K^+ and/or Ca^{2+} channel-mediated events or via neurotransmitters and/or activation of ionic glutamate receptors and epileptogenesis involving long-lasting and prolonged histological and biochemical alterations (Sasa, 2006) which may result in excessive production of reactive oxygen species, a factor believed to be involved in the mechanism leading to cell death and neurodegeneration (Freitas *et al.*, 2004). Though, the processes leading to spontaneous seizures involving the hippocampus have not been yet fully determined (Lopez-Picon *et al.*, 2006) and the mechanism of neuronal death was still unclear (Pavlova *et al*., 2003; Xu *et al*., 2009).

Owing to its drug-resistant nature, the studies tries to focus on the pathophysiological mechanisms underlying this disease and several experimental models that mimic the drug-resistant TLE have been suggested (Pothuizen *et al.*, 2004; Niessen *et al.*, 2005; Curia, *et al.*, 2008).

The pilocarpine seizure model represents a considerable progress in the understanding of the pathophysiology and pharmacotherapy of seizures and epilepsy where it replicates many of the features observed in human temporal lobe epilepsy, including an initial episode of prolonged status epilepticus followed by spontaneous, recurrent seizures, and temporal lobe pathology similar to that seen in the human (Detour *et al*., 2005).

Currently, there is no cure for TLE and the treatment is purely symptomatic directed towards alleviating the seizures (Hauser and Hesdorffer, 2001). Therefore, the period of epileptogenesis provided by a pilocarpine seizure model represents an attractive target for novel therapeutic interventions to prevent the development of epilepsy in patients at risk, where during this period several and molecular alterations take place making the brain more susceptible to seizure generation (Jutila *et al*., 2002). Topiramate (TPM) is a drug that has been used to treat epilepsy in adults and children due to its inhibitory action against excitotoxicity (Guerrini and Pameggiani, 2006).

Pumpkin (*Cucurbita pepo*) is a vegetable cultivated for human consumption and for use in traditional medicine (Caili *et al.*, 2006). Schinas *et al.* (2009) showed that the oil content of pumpkin seeds was remarkably high (45%). The fatty acid profile of the oil showed that it is composed primarily of linoleic, oleic, palmitic and stearic acids which proved efficacy in preventing many chronic diseases (Bombardelli and Morazoni, 1997 and Kumar *et al.*, 2006).

Thus, our study aims to investigate the effect of pumpkin seed oil as a natural product against physiological and histological changes in pilocarpine-epileptic model compared to topiramate as a common used antiepileptic drug.

MATERILAS AND METHODS

1- Animals: 180 male albino rats (*Rattus norvegicus*) weighing about 130-160 g were obtained from the animal house of the Research Institute of Ophthalmology, El-Giza, Egypt. Animals were kept under observation for about one week before the onset of the experiment to exclude any intercurrent infection. They were housed at normal atmospheric temperature (25 ± 5°C) as well as good ventilation and received standard balanced diet and water *ad libtium*. 30 rats from the total number of rats were died as a result of pilocarpine injection.

2- Chemicals: Pilocarpine hydrochloride, a cholinergic agonist (C11H16N2O2. HCl) was purchased from Acros Organics, New Jersy, USA. Topiramate, an antiepileptic drug [2,3:4,5-bis-*O*-(1-methyl-ethylidene-)-β-D-fructopyranose sulfamate] was purchased from Cilag AG, Schaffhausen, Switzerland. Pumpkin (*Curcurbita pepo*) seed oil was purchased from El Captin Company, Cairo, Egypt. The Na^+, K^+ and Ca^{2+} kits were purchased from Spinreact. Glutathione peroxidase and nitric oxide kits were purchased from Biodiagnostic Company. The mouse monoclonal antibody-1 (Clone PC 10), rabbit polyclonal antibody p53 and Bcl-2 and ultravision® detection system were purchased from Thermo Fisher Scientific, Lab Vision Corporation (Thermo fisher scientific, USA). Other non-mentioned chemicals used in this experiment were purchased from Sigma, USA.

3- Experimental Design: Scheme 1 illustrates the animal grouping and treatment schedule.
4- Sampling: Rats were sacrificed under diethyl ether anesthesia at the end of the two examined periods. Blood samples were collected from each rat, allowed to coagulate at room temperature then centrifuged at 3000 r.p.m. for 20 minutes. The clear, non-haemolysed supernatant sera were quickly removed and kept at -20°C for the electrolytes measurements. The hippocampus region was obtained as slices. Some were fixed in 10 % neutral buffered formalin to carry on the histological and immunohistochemical studies. Others were kept on ice and homogenized in 10 % (weigh/volume) 75% TLC methanol for amino acid and neurotransmitters estimation
and the rest slices were homogenized in 0.9 % saline for oxidative stress parameters determination. The homogenate was centrifuged at 20000×g for 10 min at 4°C. The supernatant was collected and preserved at -20°C until used. The 1st for amino acids and monoamines HPLC analysis and the 2nd for antioxidant, oxidative stress and calcium measurements.

5- Physiological Studies: The amino acids were determined by the method of Heinrikson and Meredith (1984) while the neurotransmitters were determined by Pagel *et al.* (2000). Na^+, K^+ and Ca^{2+} were measured according to Young (2001) using reagent kits. Malondialdehyde (MDA) concentration in hippocampus brain region homogenate was determined according to the method described by Albro *et al.* (1986). GSH concentration was assayed according to method of Beutler *et al.* (1963).Glutathione peroxidase was assayed according to Paglia and Valentine (1967), nitric oxide was assayed by measuring of endogenous nitrite concentration according to the method of Montgomery and Dymock (1961) using reagent kits.

6- Histological and Immunohistochemical Studies: The wax-embedded specimens representing the hippocampus regions were sectioned in two slices of 10 µm. Both re-dehydrated with descending series of alcohols after deparaffinization and then one was processed to stain with haematoxylin and eosin for histological study (Bancroft and Gamble, 2002) while the second after mounting on positive slides was prepared according to the method of Hua and Ya-wei, 2005 for immunohistochemical examination. Briefly, before the incubation with antibodies, endogenous peroxidase activity was quenched, slices washed and then incubated in a blocking solution containing 2% bovine serum albumin, 2% horse serum, and 0.1% Triton X-100 in PBS (pH 7.4) for 1 h, and thereafter with the primary antibodies for either PCNA (PC10), p53 or Bcl-2 diluted 1: 200, 1: 100 and 1 : 150 respectively in PBS, were applied for 1 hour at 37ºC. The slices were incubated with the biotin conjugated secondary antibody, rinsed, and incubated with avidin-peroxidase conjugate in blocking solution at room temperature.

7- Gel Eectrophoresis for DNA Fragmentation Detection: Apoptotic DNA fragments were analyzed using gel electrophoresis after DNA extraction using (BDtract™ genomic DNA Isolation kit, Maximum Biotech, San Francisco, USA) following the instruction manual (Everson *et al*., 1993).

8- Statistical Analysis: Data were analyzed using one way analysis of variance (ANOVA) (Rao *et al.*, 1985) followed by LSD analysis to evaluate multiple comparisons between different groups. Results are expressed as mean ± standard error (SE). Values of $P > 0.05$ were considered statistically non significant, while values of $P < 0.05$ were considered statistically significant.

RESULTS

1. Behavioural Changes: For the behavioral changes, there are a number of peripheral cholinergic signs, tremors, sniffing , mouth and facial movements, head nodding, forelimb clonus, and rearing and falling, these all signs were observed during the 2 h after the induction.
2. Physiological Variables: Regarding amino acid and neurotransmitters estimation, the amino acids levels in the brain were expressed in our study by some excitatory and inhibitory amino acids in table (1) and showed increased levels in aspartic, glutamic, serine and glycine in the epileptic groups either after 24 hrs or 21days compared to the control groups. In contrary Asparagin, histidine and GABA were decreased in both the epileptic control groups. Topiramate and pumpkin seed oil treatments ameliorate all the amino acids changes either the increased or the decreased ones except serine which showed a non significant decrease with topiramate treatment and GABA which showed a non significant increase with pumpkin seed oil treatment. Using the treatments only without pilocarpine, there were non significant values with the control group for aspartic and aspargin amino acids while glutamate and glycine levels were significantly decreased compared to the control one. On the other hand, compared to the 21 days control group, serine concentration was increased after topiramate treatment and histidine level was increased after pumpkin seed oil treatment when used without pilocarpine.

Table (2) illustrates the neurotransmitters concentrations in the hippocampus of different groups. All the measured neurotransmitters were decreased in the 24 hrs- epileptic group norepinephrine (NE) and serotonin or 5-Hydroxytryptamine (5-HT) continued its decrease during the experimental period in the 21day-epileptic control group while dopamine (DA) reversed, with an increased measurement in the 21day- epileptic control group in comparison to their control ones. Both treatments showed non significant changes in the three neurotransmitters compared to the control groups except topiramate decreasing effect on NE. Pumpkin seed oil gave significant amelioration in the changed epileptic neurotransmitters level while topiramate ameliorate 5-HT level only.

Regarding the electrolytes levels indicated in table (3), Na^+ and Ca^{2+} levels had an opposite direction to k^+ as they were significantly deceased in the serum and increased in the hippocampus of the epileptic groups compared to their related control ones while, k^+ was significantly increased in the serum and decreased in the hippocampus in comparison to its related control one. These all changes were ameliorated significantly after treatments compared to the epileptic control group. Topiramate and pumpkin seed oil treatments without pilocarpine did not change the electrolytes balance compared to the 21 day control group except for the significant decreased level in serum Ca^{2+} with topiramate treatment.

Concerning oxidative stress, it was found that using the treatments only without pilocarpine; there were non significant changes in MDA and nitric oxide, GSH concentrations and GPx activity in tissues among topiramate-treated group, Pumpkin seed oil-treated group and the control groups as shown in table (4). Regarding the effect of pilocarpine, the results illustrated in table (4) showed a significant increase in values of oxidative damage indicators such as MDA and nitric oxide concentrations, with a significant decrease in GSH concentrations in the hyppocampi of rats in both 24 hrs- and 21 day-epileptic control groups compared to their controls. GPx showed lower activity in 24 hrs- but higher activity in 21 day-epileptic control groups compared to their controls. Administration of topiramate and pumpkin seed oil to epileptic rats had a significant lowering effect on MDA concentration, significant increases in the values of GSH and GPX activity compared to 21 day-epileptic control group. Topiramate had a slight lowering effect on nitric oxide values while Pumpkin seed oil could significantly decrease nitric oxide concentration in tissues compared to 21 day-epileptic control group.

3. Histological Manifestations: Histological examination of the dentate gyrus layers in all groups revealed no histological lesions except as a result of epilepsy. Epileptic rats showed significant granular cell loss in the dentate granular layer after 24 hours (Fig. 3). Though, this loss was significantly reduced after 21 days (Figs. 4) compared to that after 24 hours but it was still higher than the control. Some of the granular cells were observed in the molecular and polymorphonuclear layers (Fig. 4).

In the polymorphonuclear layer, some progenitor cells were observed in the 21-days epileptic group (Figs. 53, 60, 67) while many mossy fibres sprouting with the granular cells were seen in the molecular layer (Figs.31 & 32) in both epileptic groups. This sprouting was much severe after 24 hours (Fig. 31) of epilepsy induction.

4. Immunohistochemical Observations: Both topiramate and pumpkin seed oil administration to normal rats caused no significant changes in the antibody reactivity towards Bcl-2 (Figs. 12, 13, 33, 34, 54, 55), p53 (Figs. 19, 20, 40, 41, 61, 62) or PCNA (Figs. 26, 27, 47, 48, 68, 69) in all layers of dentate gyrus. These parameters, also, showed no significant alteration in control rats after 21days compared to those recorded in rats sacrificed after 24 hours.

Rats in epileptic groups showed similar patterns of reactivity towards Bcl-2 (Figs. 10, 11, 31, 32, 52, 53) and p53 (Figs. 17, 18, 38, 39, 59, 60) antibodies in the dentate gyrus of hippocampus after 24 hours and 21 days where a significant increase in the p53-positive cell number was noticed in all examined layers of the dentate gyrus, accompanied by a significant decrease in Bcl-2 compared to those of the control. However, epileptic rats after 24 hours showed significant decrease in the proliferation index of dentate gyrus cells in all layers (Figs. 24, 45, 66) and a significant increase after 21 days (Figs. 25, 46, 67).

Treatment of epileptic rats for 21 days with topiramate significantly increased the rate of expression of antiapoptotic marker (Bcl-2) in all layers of dentate gyrus (Figs. 14, 35, 56) relevant to the epileptic group but it was still lower than the control. Though, it caused no significant change in p53 (Figs. 21, 42, 63) relative to the epileptic group (i.e. significant increase relevant to the control) and the proliferative index (Figs. 28, 49, 70) relative to the control (i.e. significant decrease relevant to the 21-days epileptic group).

Contrary, adminestration of pumpkin seed oil for 21 days to epileptic rats significantly decreased the rate of expression of p53 (Figs. 22, 43, 64) while it caused no significant change in Bcl-2 (Figs. 15, 36, 57) and proliferative index (Figs. 29, 50, 71) values, relative to 21 days-epileptic group. Though these values were still deviated from the normal.

5. Gel Electrophoresis Analysis: Apoptosis was confirmed by DNA-gel electrophoresis, showing an increased generation of apoptotic DNA fragments in pilocarpine-injected rats after 24 hours and 21 days compared to the control or pumpkin seed oil- and topiramate-treated animals. The fragmentation was highly reduced using pumpkin seed oil while it was still noticed in topiramate-treated group (Fig. 72).

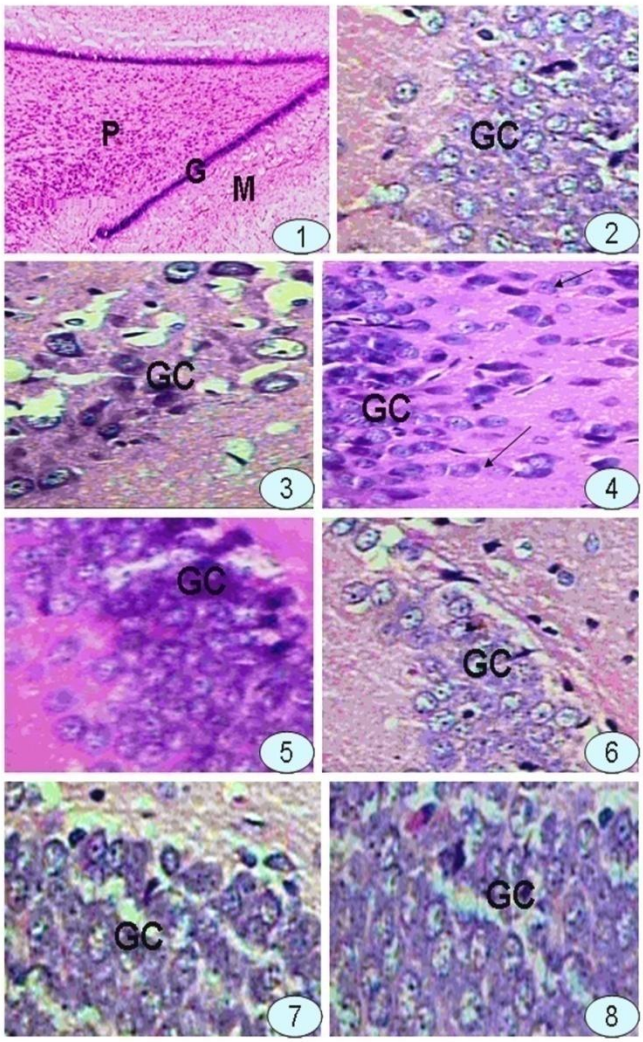
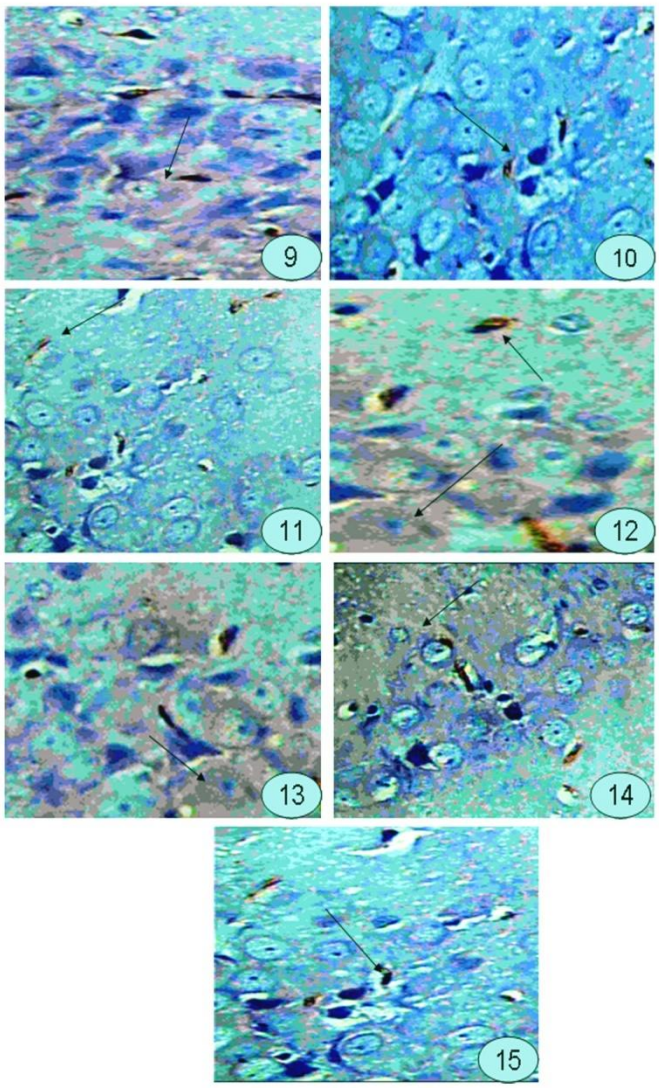
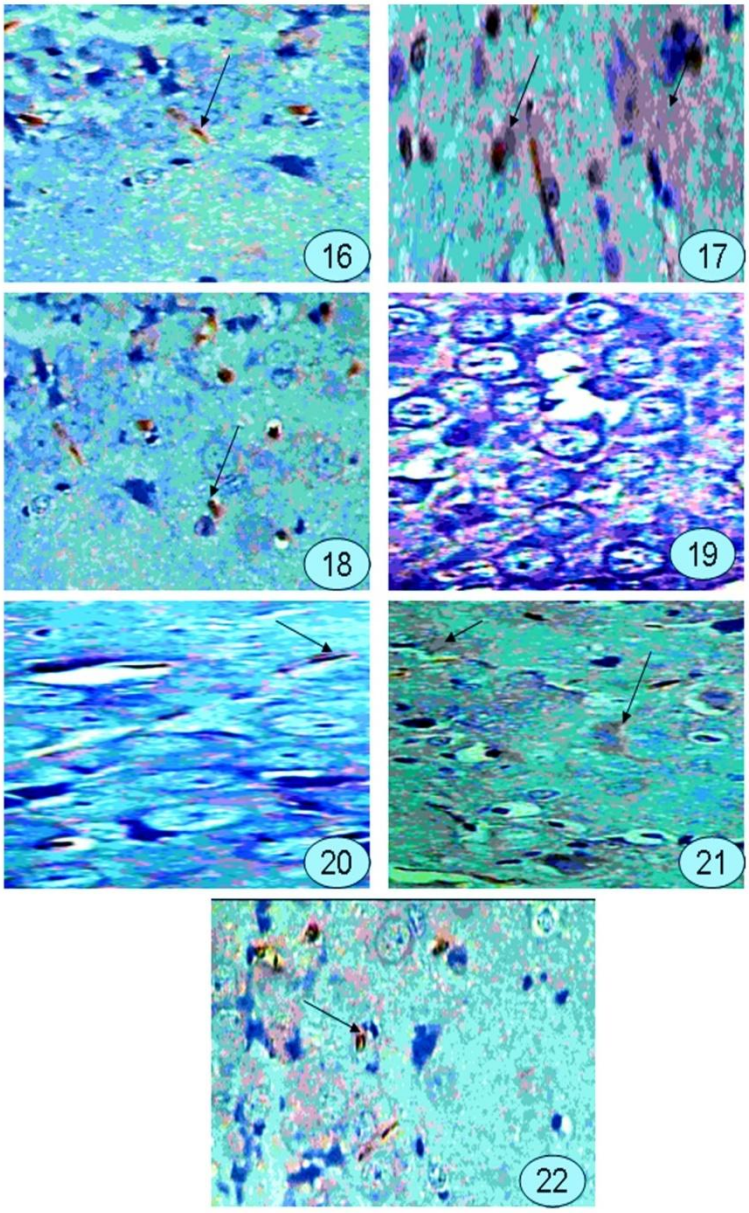


Fig. 1: A photomicrograph of a section of the dentate gyrus region of the hippocampus of a control rat showing its characteristic layers, the granular (G), the polymorphonuclear (P) and the molecular (M). (H & E X 40)

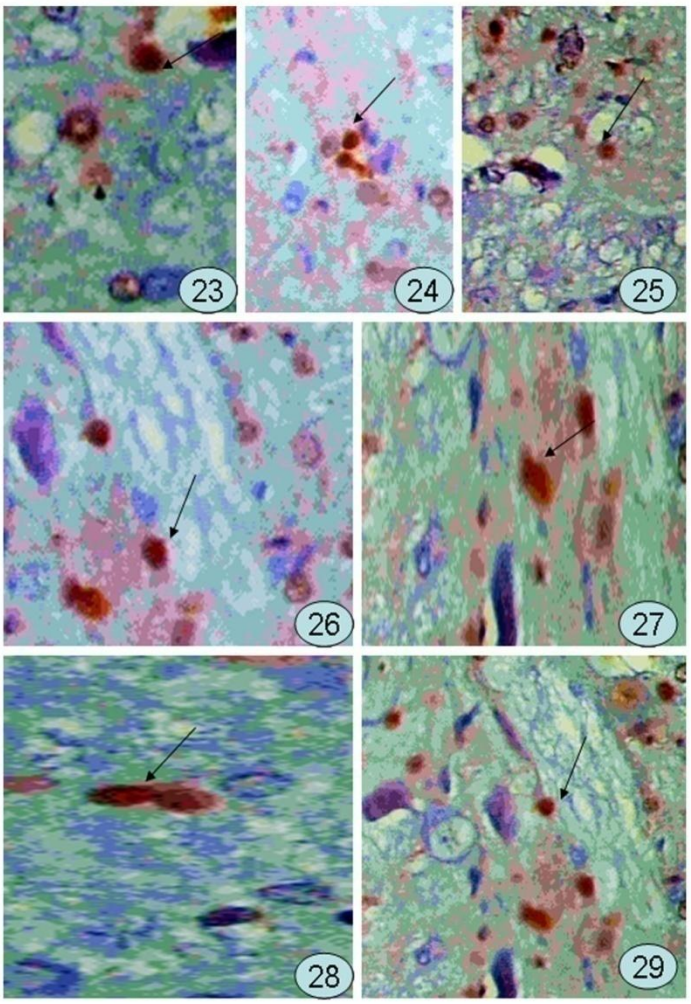
Figs. 2-8: Photomicrographs of transverse sections of the granular layer of the dentate gyrus illustrating the changes in number and distribution (↓) of the granular cells (GC) in different groups; the control (Fig.2), the 24-hours epileptic (Fig.3), the 21-days epileptic (Fig. 4); the topirmate-treated for 21days (Fig. 5), the pumpkin oil-treated for 21days (Fig. 6), the epileptic treated with topirmate for 21 days (Fig. 7) and the epileptic-treated with pumpkin oil for 21 days (Fig. 8). (H & E X 160)



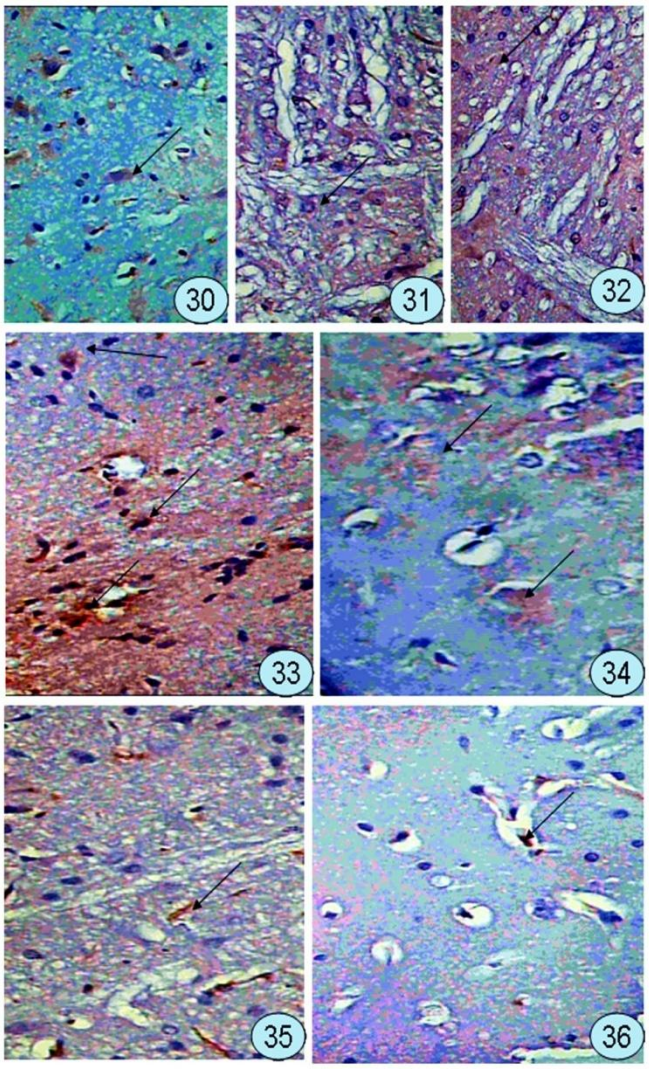
Figs. 9-15: Photomicrographs of transverse sections of the granular layer of the dentate gyrus showing the reactivity (↓) towards Bcl-2 antibody in different groups; the control (Fig.9), the 24-hours epileptic (Fig.10), the 21-days epileptic (Fig. 11); the topirmate-treated for 21days (Fig. 12), the pumpkin oil-treated for 21days (Fig. 13), the epileptic treated with topirmate for 21 days (Fig. 14) and the epileptic-treated with pumpkin oil for 21 days (Fig. 15). (X 160)



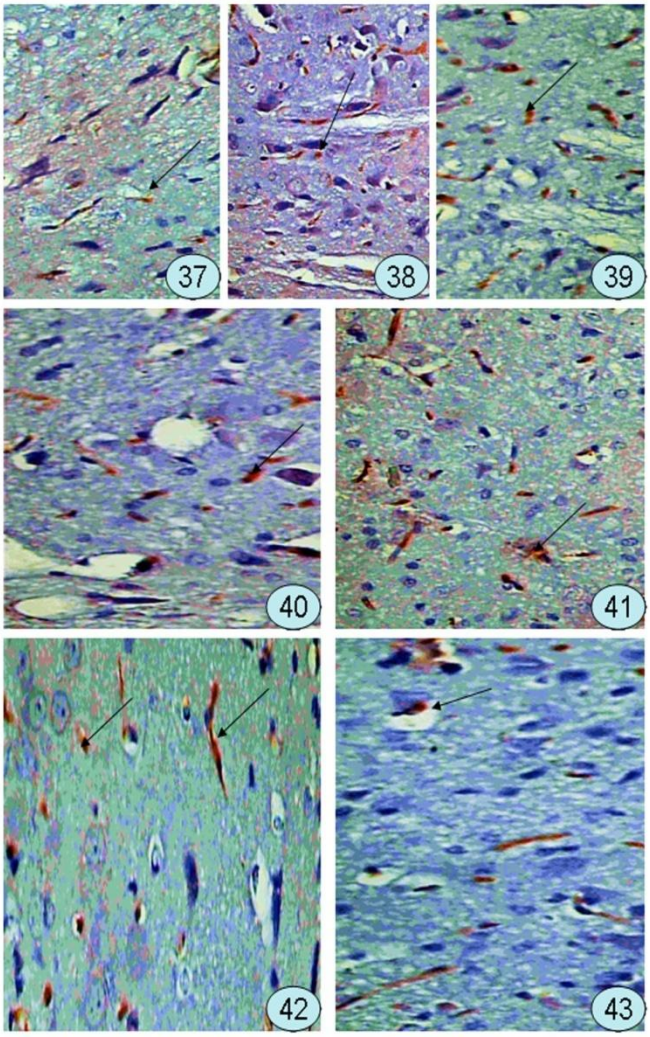
Figs. 16-22: Photomicrographs of transverse sections of the granular layer of the dentate gyrus showing the reactivity (↓) towards p53 antibody in different groups; the control (Fig.16), the 24-hours epileptic (Fig.17), the 21-days epileptic (Fig. 18); the topirmate-treated for 21days (Fig. 19), the pumpkin oil-treated for 21days (Fig. 20), the epileptic treated with topirmate for 21 days (Fig. 21) and the epileptic-treated with pumpkin oil for 21 days (Fig. 22). (X 160)



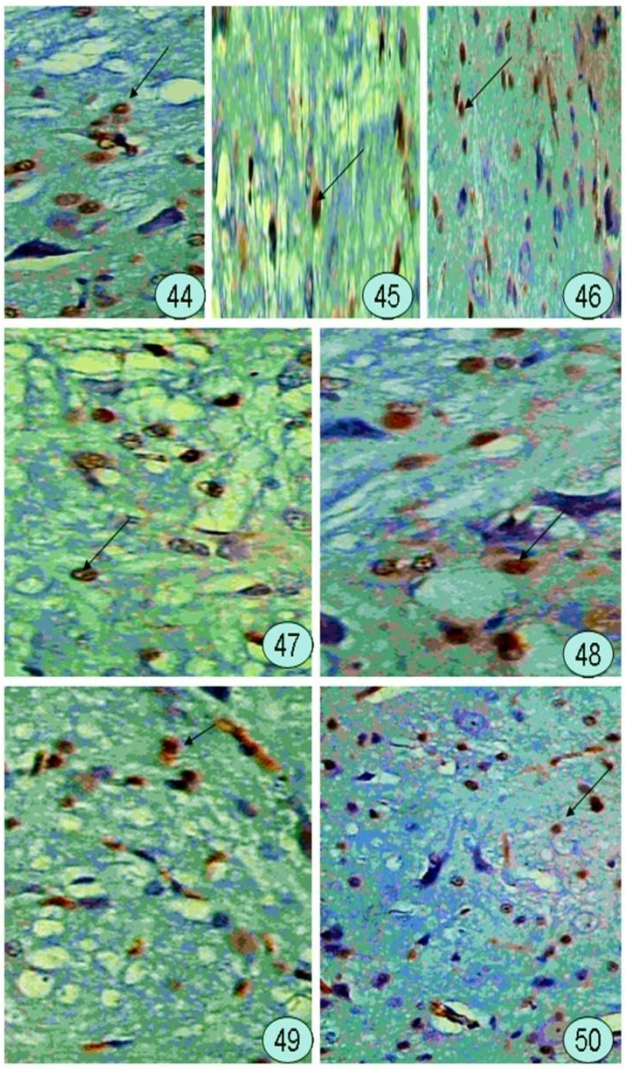
Figs. 23-29: Photomicrographs of transverse sections of the granular layer of the dentate gyrus showing the reactivity (↓) towards PC-10 antibody (proliferative index) in different groups; the control (Fig.23), the 24-hours epileptic (Fig.24), the 21-days epileptic (Fig. 25); the topirmate-treated for 21days (Fig. 26), the pumpkin oil-treated for 21days (Fig. 27), the epileptic treated with topirmate for 21 days (Fig. 28) and the epileptic-treated with pumpkin oil for 21 days (Fig. 29). (X 160)



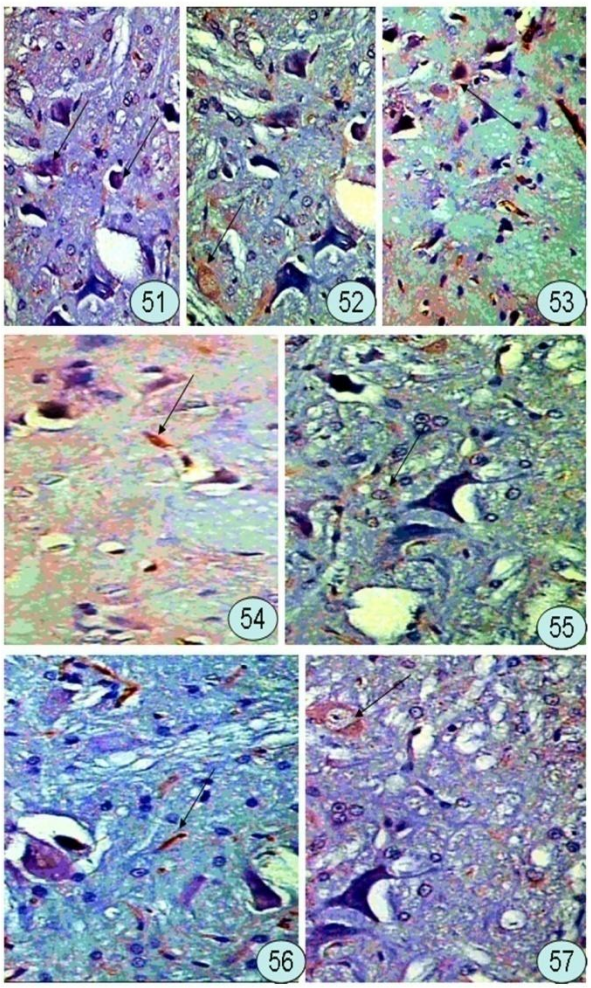
Figs. 30-36: Photomicrographs of transverse sections of the molecular layer of the dentate gyrus showing the reactivity (↓) towards Bcl-2 antibody in different groups; the control (Fig.30), the 24-hours epileptic (Fig.31), the 21-days epileptic (Fig. 32); the topirmate-treated for 21days (Fig. 33), the pumpkin oil-treated for 21days (Fig. 34), the epileptic treated with topirmate for 21 days (Fig. 35) and the epileptic-treated with pumpkin oil for 21 days (Fig. 36). (X 160)



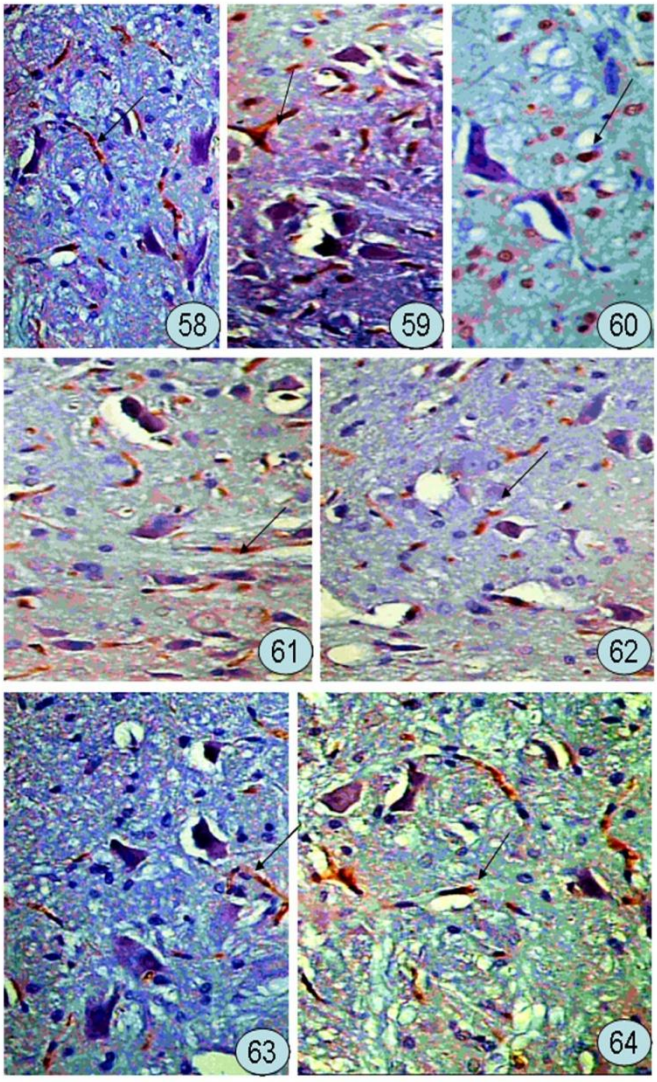
Figs. 37-43: Photomicrographs of transverse sections of the molecular layer of the dentate gyrus showing the reactivity (↓) towards p53 antibody in different groups; the control (Fig.37), the 24-hours epileptic (Fig.38), the 21-days epileptic (Fig. 39); the topirmate-treated for 21days (Fig. 40), the pumpkin oil-treated for 21days (Fig. 41), the epileptic treated with topirmate for 21 days (Fig. 42) and the epileptic-treated with pumpkin oil for 21 days (Fig. 43). (X 160)



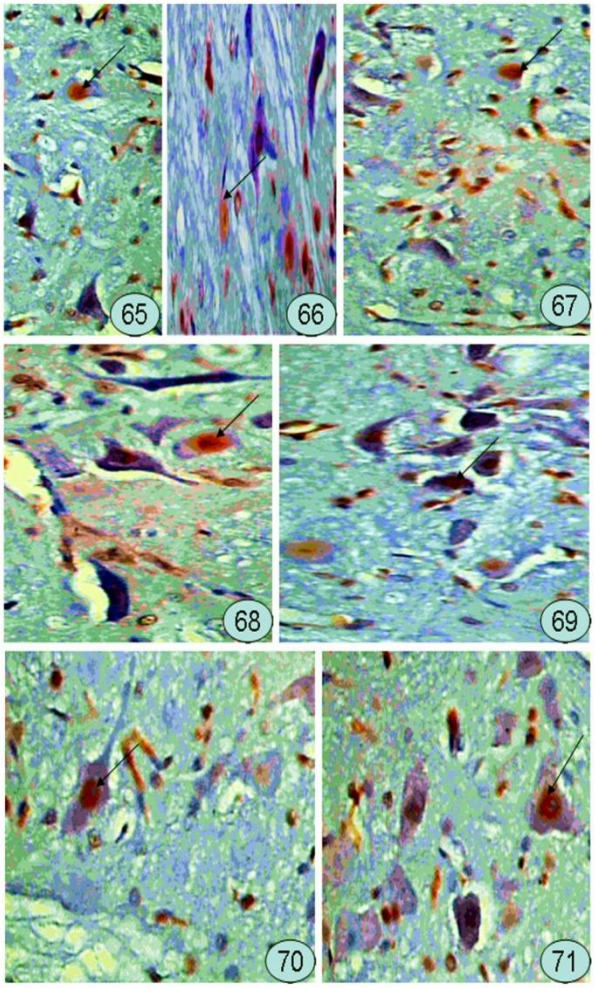
Figs. 44-50: Photomicrographs of transverse sections of the molecular layer of the dentate gyrus showing the reactivity (↓) towards PC-10 antibody (proliferative index) in different groups; the control (Fig.44), the 24-hours epileptic (Fig.45), the 21-days epileptic (Fig. 46); the topirmate-treated for 21days (Fig. 47), the pumpkin oil-treated for 21days (Fig. 48), the epileptic treated with topirmate for 21 days (Fig. 49) and the epileptic-treated with pumpkin oil for 21 days (Fig. 50). (X 160)



Figs. 51-57: Photomicrographs of transverse sections of the polymorphonuclear layer of the dentate gyrus showing the reactivity (↓) towards Bcl-2 antibody in different groups; the control (Fig.51), the 24-hours epileptic (Fig.52), the 21-days epileptic (Fig. 53); the topirmate-treated for 21days (Fig. 54), the pumpkin oil-treated for 21days (Fig. 55), the epileptic treated with topirmate for 21 days (Fig. 56) and the epileptic-treated with pumpkin oil for 21 days (Fig. 57). (X 160)



Figs. 58-64: Photomicrographs of transverse sections of the polymorphonuclear layer of the dentate gyrus showing the reactivity (↓) towards p53 antibody in different groups; the control (Fig.58), the 24-hours epileptic (Fig.59), the 21-days epileptic (Fig. 60); the topirmate-treated for 21days (Fig. 61), the pumpkin oil-treated for 21days (Fig. 62), the epileptic treated with topirmate for 21 days (Fig. 63) and the epileptic-treated with pumpkin oil for 21 days (Fig. 64). (X 160)



Figs. 65-71: Photomicrographs of transverse sections of the polymorphonuclear layer of the dentate gyrus showing the reactivity (↓) towards PC-10 antibody (proliferative index) in different groups; the control (Fig.65), the 24-hours epileptic (Fig. 66), the 21-days epileptic (Fig. 67); the topirmate-treated for 21days (Fig. 68), the pumpkin oil-treated for 21days (Fig. 69), the epileptic treated with topirmate for 21 days (Fig. 70) and the epileptic-treated with pumpkin oil for 21 days (Fig. 71). (X 160)

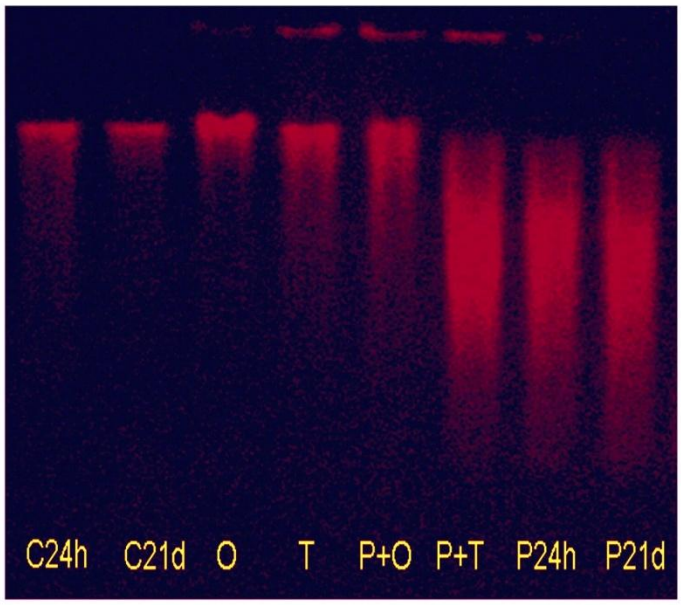
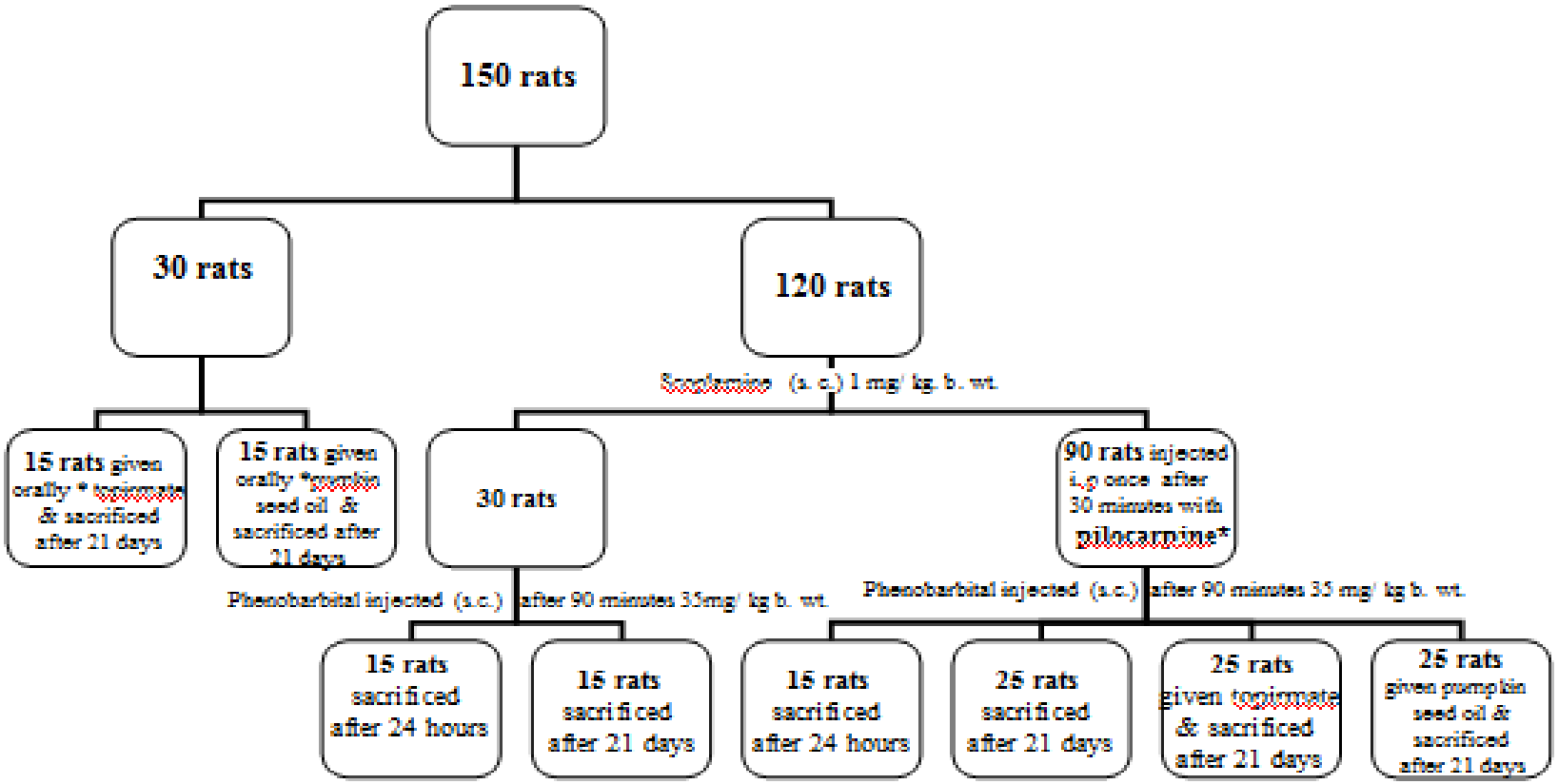


Fig. 72: Evaluation of topirmate and pumpkin oil effects on epilepsy-induced DNA fragmentation by DNA electrophoresis C24: control group 24 hours; C21: control group 21 days; O: pumpkin seed oil; T: topirmate; P+O: pilocarpine+ oil; P+T pilocarpine + topirmate; P24h: pilocarpine 24 hours; P21d: pilocarpine 21 days.

Scheme 1: Animal grouping and schedule of treatment



*Pilocarpine dose is 300 mg/kg b. wt. (Turski *et al.*, 1983). *Topirmate dose is 20mg/ kg b. wt. (Borowicz *et al.*, 2003)
*Pumpkin seed oil dose is 40mg/ kg b. wt. (Al-Zuhair *et al.*, 2000).
i.p: intraperitoneal, s.c: sub coetaneous.

Table (1): Amino acid concentrations (µmol/g tissue) in the hippocampi of different groups.

Groups	Amino acids (µmol/g tissue)						
	Aspartate	**Glutamate**	**Serine**	**Aspargin**	**Glycine**	**Histidine**	**GABA**
24 hrs-control group	2.945 ^{cd} ± 0.235	4.821^{d} ± 0.262	0.181^{e} ± 0.044	0.315 ^{a} ± 0.048	0.275 ^{e} ± 0.023	0.201^{bc} ± 0.037	1.603 ^{b} ± 0.143
24 hrs-epileptic- group	5.687 ^{b} ± 0.262	9.799 ^{a} ± 0.383	0.636 ^{a} ± 0.032	0.123 ^{c} ± 0.012	0.461^{b} ± 0.023	0.137 ^{d} ± 0.018	0.558 ^{c} ± 0.062
21 day-control group	2.607 ^{cd} ± 0.203	6.411 ^{c} ± 0.285	0.304^{d} ± 0.030	0.314 ^{a} ± 0.022	0.360 ^{cd} ± 0.012	0.294 ^{a} ± 0.031	2.121 ^{a} ± 0.202
21 day-epileptic-control group	6.940 ^{a} ± 0.384	7.504 ^{b} ± 0.365	0.600 ^{ab} ± 0.043	0.072 ^{c} ± 0.007	0.827 ^{a} ± 0.02	0.100^{e} ± 0.009	0.467 ^{c} ± 0.101
Topiramate -treated group	2.306 ^{d} ± 0.17	3.166 ^{ef} ± 0.257	0.474 ^{c} ± 0.038	0.085 ^{c} ± 0.006	0.404b^{c} ± 0.039	0.273 ^{ab} ± 0.011	1.552 ^{b} ± 0.236
Topiramate-treated epileptic group	3.247 ^{c} ± 0.286	4.561 ^{e} ± 0.493	0.520 ^{bc} ± 0.042	0.257 ^{ab} ± 0.021	0.292d ^{e} ± 0.037	0.285^{a} ± 0.007	1.216 ^{b} ± 0.133
Pumpkin seed oil-treated group	2.426 ^{d} ± 0.186	2.731 ^{f} ± 0.174	0.29 ^{d} ± 0.035	0.117 ^{c} ± 0.006	0.190 ^{f} ± 0.020	0.226 ^{b} ± 0.024	1.322 ^{b} ± 0.221
Pumpkin seed oil-treated epileptic group	2.718 ^{cd} ± 0.296	3.908 ^{de} ± 0.217	0.487 ^{c} ± 0.020	0.229 ^{b} ± 0.020	0.295d ^{e} ± 0.026	0.196 ^{c} ± 0.011	0.713 ^{c} ± 0.099
F-propability	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD at 5 %	0.854	0.923	0.104	0.062	0.076	0.052	0.465
LSD at 1 %	1.015	1.243	0.140	0.083	0.103	0.069	0.626

- Data are expressed as Means ± SE.
- Number of preparations in each group is six.
- Values with a common letters are not significantly different.

Table (2): Neurotransmitters concentrations (µg/g tissue) in hippocampi of different groups.

Groups	Neurotransmitters (µg/g tissue)		
	Norepinephrine	**Dopamine**	**Sertonine**
24 hrs-control group	2.047 ± 0.146 [bc]	0.742 ± 0.024 [c]	0.064 ± 0.003 [bc]
24 hrs-epileptic- group	1.248 ± 0.016 [d]	0.509 ± 0.052 [d]	0.022 ± 0.0004 [e]
21 day-control group	2.420 ± 0.97 [a]	0.713 ± 0.023 [c]	0.075 ± 0.004 [a]
21 day-epileptic-control group	2.002 ± 0.018 [c]	0.921 ± 0.008 [a]	0.042 ± 0.002 [d]
Topiramate -treated group	2.126 ± 0.144 [bc]	0.729 ± 0.045 [c]	0. 076 ± 0.002 [a]
Topiramate-treated epileptic group	1.981 ± 0.083 [c]	0.883 ± 0.046 [ab]	0.072 ± 0.04 [ab]
Pumpkin seed oil-treated group	2.332 ± 0.98 [ab]	0.750 ± 0.054 [c]	0.068 ± 0.005 [ab]
Pumpkin seed oil-treated epileptic group	2.466 ± 0.105 [a]	0.788 ± 0.057 [bc]	0.058 ± 0.004 [c]
F-propability	P < 0.001	P < 0.001	P < 0.001
LSD at 5 %	0.288	0.122	0.010
LSD at 1 %	0.388	0.64	0.013

- Data are expressed as Means ± SE.
- Number of preparations in each group is six.
- Values with a common letters are not significantly different.

Table (3): Electrolytes concentrations (mM/ml) in the serum and the hippocampi of different groups.

Groups	Electrolytes (mM/ml)					
	Serum			Hippocampus		
	Na^+	K^+	Ca^{2+}	Na^+	K^+	Ca^{2+}
24 hrs-control group	142.333 ± 1.890 [a]	5.100 ± 0.118 [de]	3.169 ± 0.096 [a]	24.67 ± 0.56 [c]	93.58 ± 0.73[a]	0.935 ± 0.620 [d]
24 hrs-epileptic-control group	116.667 ± 0.670 [d]	6.983 ± 0.830 [a]	1.572 ± 0.051 [d]	30.13 ± 0.15 [a]	72.33 ± 2.01[d]	1.965 ± 0.089 [a]
21 day-control group	139.500 ± 1.669 [a]	4.750 ± 0.139 [ef]	3.149 ± 0.143 [a]	24.33 ± 0.92 [cd]	92.00 ± 1.65 [a]	0.842 ± 0.061 [d]
21 day-epileptic-control group	119.517 ± 641 [d]	6.367 ± 0.184 [b]	1.801 ± 0.069 [d]	29.63 ± 0.06 [a]	75.00 ± 1.10 [d]	1.797 ± 0.082 [a]
Topiramate -treated group	140.00 ± 0.966 [a]	4.650 ± 0.085 [f]	2.786 ± 0.102 [b]	23.00 ± 0.97 [d]	93.00 ± 1.83[a]	0.900 ± 0.055 [d]
Topiramate-treated epileptic group	134.333 ± 0.760 [b]	5.450 ± 0.243 [cd]	2.408 ± 0.083 [c]	27.73 ± 0.23 [b]	79.67 ± 0.56 [c]	1.236 ± 0.041[c]
Pumpkin seed oil-treated group	138.830 ± 1.515 [a]	5.037 ± 0.083 [ef]	2.900 ± 0.167 [ab]	23.83 ± 0.38 [cd]	91.00 ± 1.67 [a]	1.001 ± 0.042 [d]
Pumpkin seed oil-treated epileptic group	129.833 ± 1.922 [c]	5.570 ± 0.082 [c]	2.133 ± 0.143 [c]	27.4 ± 0.48 [b]	85.66 ±0.56 [b]	1.460 ± 0.042 [b]
F-propability	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
LSD at 5 %	3.934	0.409	0.327	1.63	3.99	0.178
LSD at 1 %	5.299	0.551	0.440	2.19	5.37	0.280

- Data are expressed as Means ± SE.
- Number of preparations in each group is six.
- Values with a common letters are not significantly different

Table (4): MDA (n mol/g tissue), Nitric oxide (u.mol/100g tissue), GSH (u.mol/g tissue) and GPx (mU/g tissue) concentrations hyppocampi of different groups

Groups	MDA(n.mol/g tissue)	Nitric oxide (u.mol/100g)	GSH (u.mol/g tissue)	GPx (mU/g tissue)
24 hrs-control group	55.53±0.98 [b]	16.07±0.26 [c]	2.79±0.34 [a]	34.76±4.79 [bc]
24 hrs-epileptic-control group	69.84±2.54 [a]	40.08±4.59 [b]	1.43±0.14 [b]	21.88±1.99 [d]
21 day-control group	56.83±0.78 [b]	19.97±1.88 [c]	2.76±0.22 [a]	33.73±2.11 [bc]
21 day-epileptic-control group	69.37±4.14 [a]	51.24±3.77 [a]	1.22±0.16 [b]	58.29±4.23 [a]
Topiramate -treated group	56.57±0.81 [b]	20.98±1.49 [c]	2.76±0.03 [a]	28.26±1.50 [cd]
Topiramate-treated epileptic group	61.11±1.09 [b]	43.59±3.26 [ab]	2.67±0.18 [a]	33.07±4.91 [bc]
Pumpkin seed oil-treated group	55.53±2.85 [b]	15.72±0.42 [c]	2.76±0.17 [a]	29.09±2.99 [bcd]
Pumpkin seed oil-treated epileptic group	62.35±1.83 [b]	39.04±4.29 [b]	2.69±0.18 [a]	39.38±4.63 [b]
F-propability	0.001	0.001	0.001	0.001
LSD at 5 %	6.82	8.56	0.56	10.51
LSD at 1 %	9.19	11.53	0.76	14.16

- Data are expressed as Means ± SE.
- Number of preparations in each group is five.
- Values with the same letters are not significantly different.

DISCUSSION

Epilepsy, as a common neurological disorder, is accompanied by behavioural changes (Tejada *et al*., 2006), and may be partly mediated by neuronal firing, oxidative stress and/or neuronal cell death (Heinemann *et al*., 2002; Liang *et al*., 2007; Xu *et al*., 2009).

In our study, after 21 days of pilocarpine injection, some behavioural changes accompanied with mossy fibres sprouting and activation of progenitor cells formation with granular cell loss in the dentate gyrus were observed. These changes were associated with significant decrease in GABA, aspragin, histidine, NE, 5-HT, serum Ca^{2+}, Na^{+} and hippocampal K^{+} levels in addition to decreased reduced glutathione level and abated reactivity towards Bcl-2 antibody. Though, the concentration of glutamate, aspartate, DA, Ca^{2+} and Na^{+} in the hippocampus, serum K^{+}, lipid peroxidation, nitric oxide, glutathione peroxidase activity and reactivity against p53 and PCNA antibodies showed significant increase.

The results followed the Racine's scale, which is the common way for researchers to describe seizure behaviour in rodent models of epilepsy (Lopez-Picon, 2008) and support the hypothesis that aspartic acid, glutamic acid, glycine, and possibly serine, play an important role in the mechanism of seizure activity and seizure-related brain damage in the human epileptic focus, as the induction of convulsive seizures seemed mainly related to a marked increase of these amino acids while, the maintenance and frequency of seizures seemed related to a marked increase of serine and glycine (Ronne-Engström *et al*., 1992). Furthermore, glycine antagonist protect against ischemia-induced neurodegeneration and NMDA-mediated neurotoxicity (von Essen *et al*., 1996). In the last years the ideas on the role of brain serotonin in epilepsy have been turned upside down: increasing the available brain serotonin is thought now to have an antiepileptic effect and the antidepressant drugs like selective serotonin re-uptake inhibitors have proved to be useful in seizure control (Mainardi *et al.*, 2008). Also it was found that elevation of NE and 5-HT increase the seizure threshold (Ziai *et al*., 2005). On the other hand, elevated DA may be related to the decreased GABA, which modulates or reduces its levels and abolishes the cue-induced increase (Gerasimov, *et al*., 2001) also it may referred to the hypothesis that, it may accumulate as a result of decreased NE formation *i.e*. dopamine did not changed to NE (Murray *et al.*, 1993).

The results also agreed with many studies on the electrolyte imbalance in epilepsy (Konrad and Vigier , 2005; Rail *et al*., 2006; Broberg *et al*., 2008) and gave confirmed evidence on unbalanced redox status in patients and animal models of epilepsy where elevated levels of MDA (López *et al*., 2007; Santos *et al*., 2008), decreased GSH levels (Kim *et al*., 2000 and Ong *et al*., 2000) and increased or unchanged GPx activity (Bellissimo *et al*.,2001 and Sudha *et al.*, 2001) and lowered GPx activity (Erakovic *et al*., 2000) were reported in the hippocampus of rats treated with pilocarpine. Moreover, it proved the pathological role for NO (Guix *et al*., 2005), mossy fibers sprouting (Kang *et al*., 2006) and neuronal death (Scharfman *et al*., 2002; Araujo *et al*., 2008) in epileptic seizure in adult humans and experimental animals. This death was reported to be apoptotic in certain studies (Bengzon *et al*., 2002; Ebert *et al*., 2002) resulting from abnormal expression of apoptotic genes, including Bcl-2, p53 and Fas (Araki *et al*., 2004, Engel *et al*., 2007; Xu *et al*., 2009). In contrast, increased number of dividing cells was also reported in adult patients of TLE and experimental models of seizures (Cha *et al*., 2004; Zhu *et al*., 2005). The mechanisms initiating seizure-induced increase of neurogenesis remain largely obscure, but several, however, been proposed including increased cell death and alterations in levels of neurotransmitters or growth factors (Parent, 2002; Hagihara *et al*., 2005). Originally, the birth of new neurons was considered to be a potential mechanism of neuronal repair and thereby beneficial for the brain. Currently, altered neurogenesis was proved to be harmful and lead to hyperexcitability and promotion of seizure state (Shapiro and Ribak, 2005).

Recent studies have reported that altering nor adrenaline levels can change the rate of progenitor proliferation in the hippocampus where noradrenergic depletion with the selective noradrenergic neurotoxin; N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4) decreased the number of proliferating cells in the DG of the adult rat (Kulkarni *et al*, 2002). Conversely, increasing synaptic levels of nor adrenaline with antidepressants, including selective nor adrenaline reuptake inhibitors, enhanced neurogenesis (Malberg *et al*, 2000). Furthermore, activation of the noradrenergic system by blockade of pre synaptic inhibitory auto receptors with an alpha2-adrenoceptor antagonist has been reported to increase adult neurogenesis in the olfactory bulb, by preventing the loss of newly formed neurons (Bauer *et al*, 2003).

Though, it was found that if intracellular calcium $[Ca^{2+}]_I$ concentration is elevated, it results in mitochondrial depolarization (Duchen, 1999) which may exploit O2 incompletely, resulting in an increased production of radical oxygen species (ROS). The redox balance is particularly important in epileptic patients, not only because of the cell damage that ROS cause, but also because free radicals can be a source of seizure activity, as evidenced in the following facts: free radicals can modulate $GABA_A$ gated chloride channel (Sah *et al.*, 2001) inactivate glutamine synthase and inhibit glutamate decarboxylase, which can increase glutamate concentration and diminish GABA (Sudha *et al.*, 2001); in turn, glutamate concentration can rise too as a consequence of glutamate transporter

inactivation by ROS (Trotti *et al*., 1996). As a result, mitochondrial function may be compromised, leading to reduced generation of NADH and subsequently reduced production of adenosine triphosphate (ATP) (Heinemann *et al*., 2002).

During seizures, extracellular potassium concentration $[K^+]_O$ increases whereas extracellular sodium $[Na^+]_O$, extracellular calcium $[Ca^{2+}]_O$, and the size of the extracellular space decrease (Lux *et al., 1*986). After seizures, the transmembrane ion gradients have to normalize, a process that is ultimately dependent on the supply of ATP. About 60% of cerebral ATP consumption is used for operation of the electrogenic Na-K pump, which transports 3 Na ions out of the cell in exchange for 2 K ions (Ames, 2000). The ATP content within a nerve cell is rather limited, and other stores for energy production are also scarce. Neurons in the central nervous system (CNS) can use γ-aminobutyric acid (GABA) for ATP production through the GABA shunt and also metabolize lactate (Schousboe *et al.*, 1997). Consumption of GABA for ATP synthesis may, of course, be a dangerous event, as this might lead to depletion of the GABA pool during recurring seizures (Heinemann *et al*., 2002).

It is generally accepted that the over activation of excitatory amino acid receptors, with the subsequent marked $[Ca^{2+}]_I$ rises, has been implicated in the mediation of injury caused by neurotoxins and ischaemia-related insults. Intracellular free Ca^{2+} overload may damage the neurons in various ways. Activation of phospholipase A2, phospholipase C, protein kinase C, endonucleases, nitric oxide synthase as well as Ca^{2+}-dependent proteases may contribute to the Ca^{2+}-mediated cell damage (Tymianski and Tator, 1996) as many of these enzymatic reactions are free radicals generative.

The role of NO in epileptic seizures cell death has been investigated in several experimental models, but the results are contradictory (Noyan and Gülec, 2000). Although, NO is a multifunctional biomolecule involved in a variety of physiological and pathological processes, but excess NO is toxic and may cause certain pathological conditions such as apoptosis, septic shock, and diabetes mellitus (Kröncke *et al*.,1997, Chen *et al*., 2008). To date, the cascade underlying NO-mediated cell death has not been fully understood. NO-induced apoptosis may be mediated by a DNA damage pathway that involves the accumulation of p53 and the activation of poly (ADP-ribose) polymerase (PARP) (Pieper *et al*., 1999)**.**

Seizures in experimental models induce and activate p53 (Morrison *et al*., 1996; Araki *et al*., 2004) and recent immunohistochemical findings suggest that p53 expression may be elevated in human epilepsy brain (Xu *et al*., 2007). The p53 protein plays a central role in the cellular response to DNA damage (Toledo and Wahl, 2006). p53 expression leads to arrest of the cell cycle so that DNA repair can occur or can activate apoptotic pathways when repair seems impossible (Toledo and Wahl, 2007). Excitotoxic stimulation caused by over activation of postsynaptic glutamate receptors and the release of endogenous glutamate, with the subsequent marked $[Ca^{2+}]_I$ rises, has been shown to stimulate p53 production by neurons, suggesting that p53 may also be responsible for triggering apoptosis under these circumstances (Sakhi *et al*., 1996) leading to p53 stabilization and transcription of p53's target genes, which include death receptors and pro-apoptotic Bcl-2 family proteins (Hofseth *et al*., 2004). In mammalian cells, anti-apoptotic Bcl-2 family protects the mitochondrial integrity by inhibiting pro-apoptotic Bcl-2 family members. In dying cells, several pro-apoptotic members of Bcl-2 family may antagonize the anti-apoptotic Bcl-2 family proteins to induce mitochondrial damage. The subsequent release of cytochrome c from damaged mitochondria induces the formation of apoptosome by recruiting caspase-9 and Apaf-1. Active caspase-9 cleaves and activates caspase-3, which in turn cleaves a variety of cellular substrates such as spectrin, lamins, poly-(ADP-ribose) polymerase (PARP), and inhibitor of caspase-activated DNase (ICAD), and allows caspase-activated DNase (CAD) to induce DNA laddering (Blomgren *et al*., 2007).

From the above mentioned data, the significant increase in glutamate and significant decrease in GABA with the neuronal cell loss after 21-days from pilocarpine injection, in the present study, may be explained by the increased ROS production manifested by significant increase in lipid peroxidation, NO formation and GPx activity and significant decrease in GSH concentration. Elevated hippocampal peroxidase activity may be a compensatory mechanism against oxidative damage (Župan *et al*., 2008) or may be due to the depletion of its substrate, GSH, as noted in our data. However the increased level of lipid peroxidation and the depleted glutathione concentration showed that the rise in peroxidase activity was not enough to avoid oxidative damage. Also, the increased glutamate, aspartate and serine values with the over stimulation of the excitatory receptors may explain the significant increase in hippocampal Ca^{2+} level which increased ROS formation and Ca^{2+}-dependent cell death. The elevated Ca^{2+} in the hippocampus may cause the decrease in serum Ca^{2+} that may resulted in the significant increase in NO formation observed herein and thereafter NO-dependent cell death which was confirmed by the shearing effect noticed by gel electrophoresis. Apart from this circulated pathway, GABA decrease can be explained by the consumption of increased ATP production during seizures. In addition, the imbalance observed in significant decrease in serum Na^+ and hippocampal K^+ with the reversed significant increase in hippocampal Na^+ and serum K^+ could be resulted also from the over stimulation of the excitatory receptors with the observed increase in excitatory amino acids.

Moreover, the increased neurogenesis in dentate gyrus (proliferative index and progenitor cell formation) may be, in part, an adaptive response to the degree of granule cell death as a result of increased p53 reactivity, NO-, Ca^{2+}-dependent cell death or decreased Bcl-2 reactivity and could be the cause of significant decrease in this loss after 21 days compared to that recorded after 24 hours. This process may not reflect a repairing process but an increased hyper excitatory where the newly formed neurons were present in different locations than they should be as proved by the presence of granular cells in both the molecular and polymorphonuclear layers.

All the changes reported after 21 days of pilocarpine injection showed similar pattern after 24 hrs, except for decreased DA level, GPx activity and proliferative index associated with severe mossy fibres sprouting and granular cell loss. Hara *et al*. (1993) reported a decrease in DA level and a significant decrease in monoamines was previously reported in epileptics (Macêdo *et al.*, 2004) and was confirmed by the use of monoamine oxidase inhibitors to reduce seizures (Mago *et al*., 2008).

A decrease in the GPx activity in the hippocampus of rats treated with pilocarpine (Erakovic *et al*., 2000) has also been reported. The low GPx activity reported in 24 hrs-epileptic group can be the consequence of the oxidative unbalance itself. The excess of ROS, originated as consequence of the epileptic activity, can result in the loss of the catalytic activity of GPx due to post-translational oxidative damage (Erakovic *et al.*, 2000). Moreover, it has been shown with a silver staining method that seizures lead to injury of hippocampal neurons with granular cell loss in 24 hrs, but within 4 weeks after seizures, no significant neuronal loss was detected (Toth *et al*., 1998).The severe granular cell loss after 24 hours compared to that reported after 21 days of pilocarpine injection could be related to the significant decrease in the poliferative index reported herein. Generally, neuronal loss in hippocampus is frequently accompanied by robust reorganization of the granule cell axons (mossy fibres sprouting) leading to changes in neurotransmitters and their receptors and ion channels (Pirttila, 2006).

Numerous studies were directed towards treating epilepsy and decreasing its implications (Ryvlin *et al*., 2008; Gómez-Alonso *et al*., 2008; Bauer *et al*., 2009; Skarpaas and Morrell, 2009). Most of these studies failed to identify any drug which is capable of preventing or attenuating epileptogenesis (Lo¨scher, 2002a; Pitka¨nen, 2004). However, AEDs attenuating the severity of the initial insult by being administered during an SE, improved the outcome by reducing epileptogenesis, resulting in a milder disease (Pitka¨nen and Kubova, 2004). TPM is one of the most recently used antiepileptic drugs (Sendrowski and Sobaniec, 2005; Gomer *et al.*, 2007; Faught, 2007).

In our study, TPM administration to epileptic rats for 21 days revealed significant ameliorations in most of the tested parameters. All the serum and hippocampal measured electrolytes revealed balanced measurements. Also the measured amino acids were significantly ameliorated except serine while 5-HT only increased significantly after TPM treatment. This treatment significantly reduced the tissue concentrations of MDA and restored the values of GSH and GPx to the control value. Also TPM treatment greatly reduced the mossy fibres sprouting, significantly diminished the proliferative index, increased the antiapoptotic marker (Bcl-2) activity but with no impact on p53.

TPM was found to treat various seizure types, this broad spectrum of activity may reflect multiple mechanisms of action contributing to its anticonvulsive activity. These mechanisms are: 1) voltage-gated sodium channel blockade (McLean *et al.*, 2000); 2) kainate-type glutamate receptor inhibition (Gryder and Rogawski, 2003); 3) reduction of L-type voltage-sensitive calcium currents (Zhang *et al*., 2000); 4) increased frequency of GABA-mediated chloride channel opening (White *et al*., 2000); 5) carbonic anhydrase inhibition (Dodgson *et al.*, 2000); 6) increased potassium conductance (Herrero *et al*., 2002).

The first three actions would serve to reduce neuronal excitation, the last three would enhance inhibition. It is not known which of these proposed mechanisms is most important for the anti seizure effects. Neuroprotective effects against damage from status epilepticus (Fischer *et al*., 2004; Wang *et al*., 2008) have also been described. Also, it has the ability to attenuate seizure-induced neuronal cell death (Rigoulot *et al*., 2004; Park *et al*., 2008).

It is therefore possible that modulation of voltage-gated sodium and calcium channels are the main cause of the observed electrolyte balance by topiramate. Topiramate seems to have protective effects on the Pentylentetrazol-induced seizures, blood toxicity and nephrotoxicity by regulating calcium-dependent processes, inhibiting free radical, and supporting the antioxidant redox system. (Naziroğlu *et al*., 2008; Armagan *et al.*, 2008). Also, the modulation of voltage-gated calcium channels is contributing to the inhibition of NO formation, as a result of increased $[Ca^{2+}]_O$. It has been proved that inhibition of voltage-gated calcium channels prevents the release of transmitters by preventing calcium influx into cells and thus preventing exocytosis and the mechanism of transmitter release (Williamson and Hargreaves, 2001). In addition, its effect on inhibiting glutamate receptor, and increasing the frequency of GABA channel opening resulted in the decrease in glutamate and the increase in hippocampal GABA levels, which leads to the inhibition of both *N*-methyl-D-aspartate and ά-amino-3-hydroxy-5-methyl-4-isoaxoleproprionate (AMPA)/kainate receptors and finally leads to the inhibition of neuronal firing (Classey *et al.*, 2001). The mechanisms leading to the diminished apoptotic death in epileptic rats treated with topirmate in the present study seems to be p53-independent, Ca^{+2}- and/or NO-dependent or finally a result of the activation of antiapoptotic members of Bcl-2 family. This decreased apoptotic death may be the result of decreased proliferative

index as an adaptive mechanism. Though, the diminished apoptosis appeared non significant as shearing effect was still noticed by gel electrophoresis.

Pumpkin (*Cucurbita pepo L*.) seed oil is a common salad oil of a high nutritional value (Idouraine *et al*. 1996). Seeds and oil can be used in official and alternative medicine, pharmacology, and cosmetics, especially when organically produced (Bavec *et al*., 2007).

In the present study, pumpkin seed oil administration for 21 days to epileptic rats showed similar effects as TPM in abating the electrolyte imbalance and oxidative stress beside ameliorating amino acids and neurotransmitters levels except for NE. Its administration, also, highly reorganizes the sprouting of mossy fibres and significantly abated the p53 value, while it showed no effect on either Bcl-2 activity or proliferative index. The modulatory effect of pumpkin seed oil on epileptic cell death, contrary to topirmate, seems to be p53-dependent and this inhibitory effect appears significant where shearing was highly reduced applying gel electrophoresis.

Pumpkin seed oil was found to decrease potassium values in urine (Suphiphat *et al*., 1993). Moreover, it has the ability to modify the potency of the calcium antagonist felodipine (FEL) in spontaneously hypertensive rats (SHR) (Al-Zuhair *et al*., 2000). These 2 effects could explain its K^+-decreasing and Ca-increasing effects in epileptic rats in the present study. Also, it has shown to possess strong antioxidant compounds, such as vitamin E, especially gamma-tocopherol (Fahim *et al*., 1995; Murkovića *et al*. 2004) which could explained the improvement in the current tissue concentrations of MDA, nitric oxide, GSH and glutathione peroxidase activity. These data were in accordance with Al Zuhair *et al*., (2000) who found an improvement in the measured MDA, glutathione peroxidase activity and GSH in the heart and kidney in spontaneously hypertensive rats, and Nkosi *et al*., (2006) who reported significant increased levels of serum GPx activity and decreased liver lipid peroxidation levels in carbon tetrachloride-induced acute liver injury.

Pumpkin seed oil is also rich in unsaturated fatty acids as high omega-3 (6 and 9)-fatty acids (Murkovića *et al*. 1996). Recent studies have revealed that polyunsaturated fatty acids (PUFAs) have anticonvulsive properties (Borges, 2008; Porta *et al.*, 2009; Taha *et al.*, 2009) depending on its ability to modify the membrane fluidity (Jones *et al*. 1992) and cell membrane ionic channels and/or receptors, as PUFA-induced openings of voltage-gated K channels (Xu *et al*., 2008). Also, arachidonic acid is released upon stimulation of NMDA-type glutamate receptors and inhibits the rate of glutamate uptake in neuronal synaptic (Volterra *et al.*, 1992). The beneficial outcome of docosahexaenoic acid (DHA) on the brain could derive from specific modulation of AMPA-mediated toxicity, so, it might be useful in preventing neurodegenerative diseases (Ménard *et al.*, 2009). Also, DHA has an important role as structural constituents in brain development, and dietary supply of n-3 and n-6 PUFA could modify aspects of the dopaminergic and serotonergic system and, consequently, cognitive performance and behavior (Bosch *et al.*, 2007) and several studies found that eicosapentaenoic acid DHA deficiency is associated with dysfunctions of neuronal membrane stability and transmission of serotonin, norepinephrine and dopamine (Su, 2008; 2009; Fedorova *et al.*, 2009). Tryptophan, as one constituent of pumpkin oil (Eagles, 1990), which is also the precursor of serotonin, may affect the incidence of aggression, self-mutilation and stress resistance (Paredes et al., 2009).

Recently, pumpkin seed oil has become more popular as a medical treatment for urinary complaints associated with benign prostatic hyperplasia (BPH) (Dreikorn *et al*., 2002; Gossell-Williams *et al*., 2006) where it appears to interrupt the triggering of prostate cell multiplication (Abdel-Rahman *et al*., 2006). This inhibitory effect on cell proliferation oppose the current results concerning the non-significant change of the increased proliferative index in epeliptic rats as a consequence of pumpkin seed oil administration. Abdel-Rahman *et al*. (2006) related the changes in cell multiplication in prostatic hyperplasia to moderating hormones in the body that are vital to prostate function which may be unchanged in our results.

All these effects of various constituents of pumpkin seed oil may explain its ameliorative impact on epileptic changes in our study.

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