Effect of gamma radiation on combination therapy of certain antiepileptic drugs in rats

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Abstract: The current study was designed to estimate the effect of γ-radiation on male rats pretreated with Levetiracetam (LEV) and/ or Oxcarbazepine (OXC). Poly-treatment of rats with LEV, OXC and γ-radiation showed a significant elevation in the activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and isoenzyme creatinine kinase-MB (CK-MB) along with, an increase in the level of creatinine, urea, cardiac troponin (cTnI) and glutamate. These increases were associated with a decrease in acetylcholine (Ach) and γ-aminobutyric acid (GABA) levels. The data further revealed a significant increase of the apoptotic mediator's tumor necrosis factor alpha (TNF-α) and brain caspase3 as well as, alterations in the oxidative stress parameters. The Results of the histopathological examination of liver, kidney, heart and brain tissues indicated coincidence with those recorded by the biochemical analysis. It seems promising to conclude that the exposure to γ- radiation intensified the deleterious and detrimental effect of dual treatment of LEV and OXC in rats.

Keywords: γ-irradiation, levetiracetam, oxcarbazepine, troponin, caspase3 and tnf-alpha.

INTRODUCTION

Drug interaction is the modification of patient`s medical response to the prescribed drug by co-therapy of another drug. Drug interactions may cause serious side effects and can result in early termination or withdrawal of drugs (Palatini and De Martin, 2016). Epilepsy is a chronic neurological condition wherein groups of nerve cells in the brain sometimes signal abnormally resulted in epilepsy (Riviello, 2003). Nearly 60% of all epilepsies are idiopathic. Epilepsy is common in patients exposed to brain radiotherapy (Werner-Wasik *et al*., 1999). An imbalance among glutamate and GABA neurotransmitter can lead to hyper excitability (Engelborghs and De, 2000). Augmentation of excitatory transmission and the concurrent decline of inhibitory mechanisms results in repetitive neuronal liberations (Duncan, 2006).

Levetiracetam (LEV) is an anti-epileptic drug (AED) frequently used for the treatment of partial onset and generalized seizures. LEV has excellent bioavailability, slight plasma protein binding, and quick attainment of stable state concentrations (Surges 2008 and Zou, 2013). LEV has several mechanisms of action: mainly via the inhibition of the synaptic vesicle protein 2A leading to decrease in the rate of vesicles release (Surges, 2008), by blocking N-type calcium channels and indirectly reversing the inhibition of GABA and glycine pathways therefore diminish the excessive neuronal activity. Metabolism takes place through hydrolysis; and elimination arises via the kidneys (Lyseng, 2011). Several investigators have reported that LEV has considerable neuroprotective effect in epileptic and non-epileptic conditions (Zou, 2013). Oxcarbazepine (OXC) is an equivalent of carbamazepine, with anequivalent anticonvulsant efficiency, low incidence of allergic reactions. Elger and Bauer (1998) reported that OXC is usually better than carbamazepine in case of combination therapy with other antiepileptic drugs. However, the involvement of OXC in neuronal toxicity has not been investigated. The mechanism of action of OXC and its active metabolite, the monohydroxy derivative, involve blockade of voltage dependent sodium and calcium channels. Clinically, the drug is effective against generalized and partial seizures (Wellington and Goa, 2001). Even though the improvement in the treatment of epilepsy, 30–40% of patients still uncontrolled on a single anti-epileptic drug. For these patients, polytherapy is conventional (Kwan and Brodie, 2006). Polytherapy aims to attain better seizure control and to minimize the side effects, as well as control of multiple seizure types that respond to different drugs (Bourgeois *et al*., 2005).

More than 200,000 patients treated with partial or whole brain irradiation per year (Greene-Schloesser *et al*., 2013). Portions of healthy adjacent brain tissue exposed to radiation, causing neurotoxicity and radiation encephalopathy (Lofdahl *et al.*, 2012). Radiation induced vascular brain injuries, including endothelial cell damage in arterioles and venules, and inflammation round the

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vessels (Lengyel *et al*., 2003) leading to swelling and seizure (Tang *et al*., 2011).

Patients with brain tumor related epilepsy required an exceptional and multidisciplinary approach. There are many factors to be taken into concern: First, there is the management of pharmacological therapies, the concurrent use of antiepileptic drugs, chemotherapy, radiation and AEDs. Secondly, a great concern has to be given to patients receiving these therapies to assure maintenance of a healthy lifestyle (Fonkem *et al*., 2013). Consequently; this study was aimed to explore the effect of γ irradiation of rats on different organs pretreated with LEV and / or OXC.

MATERIALS AND METHODS

Animals

Male albino rats weighing 150 - 200g were obtained from the animal house of Animal Health Research Institute (AHRI) Giza; Egypt. Animals were kept under standard conditions and were provided free access to a standard diet and water adLibitum. The animal's treatment protocol has been accepted by the Animal Care Committee of the Scientific Ethics at Faculty of pharmacy, Al-Azhar University, Cairo, Egypt, following the guidelines of the National Institutes of Health (NIH).

Chemicals and drugs

Levetiracetam syrup was purchased from Galaxo Company, Cairo, Egypt. Oxcarbazepine suspension was manufactured by Novartis Company, Cairo, Egypt.

Irradiation

Whole body γ-irradiation of animals was achieved using γ cell 40 which a cesium- 137 irradiated units is belonging to the National Center of Radiation and Technology (NCRRT) Cairo, Egypt. The dose rate was 0.758rad / Sec.

Experimental design

The rats were divided into eight groups, each of 6 rats. Group one: was considered as the control group. The second group was administered LEV (150mg/kg/p.o.) for 3weeks (Jia *et al*., 2011). Group three was received OXC (100mg /kg/p.o.) for 3 weeks (Gonzalez *et al*., 2000). The fourth group was exposed to a single dose of γ-radiation (6 Gy). Animals in fifth group were treated with LEV and OXC orally for 3weeks. Group six was administered LEV (150mg/kg/p.o.) for 3weeks, 24hrs later rats were irradiated (6Gy). Group seven was received OXC (100mg/kg/p.o.) for 3 weeks, after 24hrs rats were exposed to 6 Gy of γ-radiation. The last group was orally treated for 3 weeks with the two the drugs of LEV and OXC followed by exposure to the single dose of γ-radiation. Twenty-four, hours, post γ-irradiation the rats were sacrificed. Blood was immediately collected, centrifuged at 3000 RPM for 20 min, to attain serum for biochemical assays. Brain, liver, kidney and hearts were quickly excised, washed with saline, and homogenized to make 20% homogenate. Histopathological examinations were done on liver, kidney, heart and brain samples accordingly to the method of Drury and Wallington (Drury and Wallington, 1976).

Evaluation of biochemical parameters

Assessment of oxidative stress biomarkers

Hepatic, renal, cardiac and brain Malondialdehyde (MDA) level and Glutathione-Stransferase (GST) activity were determined by the method of Ohkawa and Habig respectively (Ohkawa *et al*., 1979; Habig *et al*., 1974) using Kit belongs to Biodiagnostic Company, Dokki, Giza, Egypt. Serum antioxidant capacity was measured according to the method of Koracevic (Koracevic *et al*., 2001) using a TAC Kit (Biodiagonostic).

Assessment of anti-apoptotic mediators

Caspase-3 was estimated in brain tissue homogenate using caspase-3 Elisa kit for research only provided by Uscn life science Inc. Tumor necrosis factor alpha was assayed in serum using TNF-alpha rat Elisa kit delivered by KOMA Biotch Inc.

Assessment of serum hepatic marker enzymes

The activity of AST and ALT was measured using Kit (Biodiagonostic) according to the method of Reitman and Frankel (1957). ALK activity was quantitatively estimated as stated by Tietz *et al*., (1983).

Assessment of renal functional tests

Urea was estimated by kits according to the procedure described by Halled and Cook (1971). Creatinine level was measured following the method of Henery (1964).

Assessment of cardiac biomarkers

Rat Cardiac Troponin-I level was detected in serum samples using Elisa kit supplied by (Kamiya, biomedical company, USA) according to manufacturer`s instruction. Serum CK-MB was detected using colorimetric assay Kit of Behring Diagnostics Company.

Assessment of neurotransmitter

Glutamate was assayed in serum using a glutamate assay kit (Abnova). GABA was estimated in serum sample using Rat aminobutyric acid, GABA ELISA Kit supplied by (Eiaab) according to manufacturer`s instruction. Serum acetyl cholinesterase level was measured by rat Elisa kit supplied by (Cloud- Clone-Crop) according to manufacturer's instruction.

STATISTICAL ANALYSIS

The data were expressed as mean ±SEM of six animals and analyzed statistically at $p<0.05$ by means of one way analysis of variance (ANOVA) followed by Tukeys-Kramer test for multiple comparison using Graph pad software prism (version 5).

RESULTS

Biochemical evaluations

Antioxidant status of AEDs and /or γ irradiated treated rats was studied. LEV and /or OXC administration as well as exposure to γ-radiation showed a significant decrease serum TAC level. The calculated percentage for the group treated with dual therapy AEDs was 56%, while for group treated with both AEDs followed by γ-radiation was 80% as compared to normal values (fig 1). Rats treated with AEDs either individually or in combination with γ-radiation indicated a decrease in liver, kidney, heart and brain tissue GST activities which recorded 65%, 74.9%, 74.9% and 59.4% respectively for the group treated with both AEDs compared to normal control and 80.4%, 75%, 76.7% and 80% for group treated with both AEDs then exposed to radiation, as compared to of AEDs control values (table 1). However; the level of MDA in all tested tissues increased in all treated groups table (2). The percentage increase was 294, 46% (liver), 422, 103% (kidney), 465,115% (heart) and 586.1, 92.3% (brain) for group five and eight respectively as compared to their corresponding control values table (2).

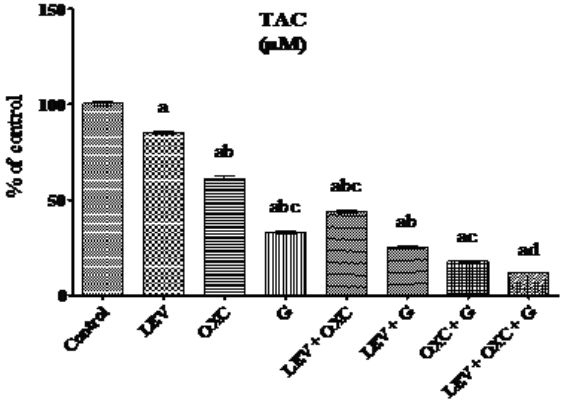

Fig. 1: Effect of γ-irradiation on the activity of TAC in male albino rats pretreated with LEV and/ or OXC. Results are expressed as mean± SE (n=6). [a]p<0.05 Significantly different from control group. [b]p<0.05 significantly different from LEV treated group. [c]p<0.05 Significantly different from OXC treated group. [d]p<0.05 Significantly different from (LEV+OXC) treated group.

Serum hepatic marker levels

Liver toxicity was detected by measuring activity of liver biomarkers in serum. Serum AST, ALT and ALK activity showed a significant increase due to the administration of LEV, OXC or exposure to radiation. Dual therapy of rats with LEV and OXC exerted a marked increase in serum AST (227.6%), ALT (202%) and ALK (336%) as compared to that of control. The most pronounced increase in liver enzyme activity was obvious in group eight which administered both AEDs followed by γ-irradiation as it recorded 117.6%, 148.2% and 212.2% for AST, ALT and ALK respectively as compared to LEV+OXC group table (3).

Serum renal function markers levels

Data in table (4) shows insignificant change in serum urea and creatinine level of rats treated with LEV and /or OXC as compared to the normal control level. Exposure of animals to single dose of γ- radiation showed a significant increase in both serum urea and creatinine in comparison with those of control values. This increase was more pronounced in groups treated with LEV and OXC followed by γ- irradiation and the percentage increase were 180% and 70% for creatinine and urea respectively comparing to LEV+OXC group table (4).

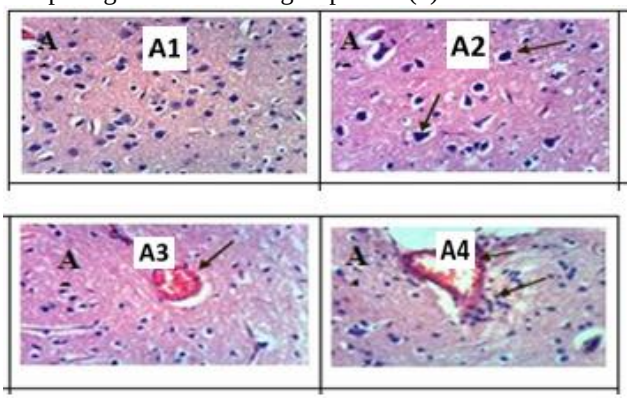

Fig. A1: Brain of rats from control group showed no histopathological changes (H & E X 400). Fig. (A2): Brain of rat from animals group exposed to γ radiation (6Gy) showed necrosis and pyknosis of neurons). Fig. (A3): Brain of rats treated with LEV + OXC showed cellular oedema (25a) and congestion of cerebral blood vessel (25b). Fig. (A4): Brain of rat from animals group treated with f LEV (150mg/kg) + OXC (100mg/kg) once daily for three weeks then exposed to a single dose (6Gy) of gamma radiation showed congestion of cerebral blood vessel associated with perivascular cuffing with mononuclear cells and focal gliosis.

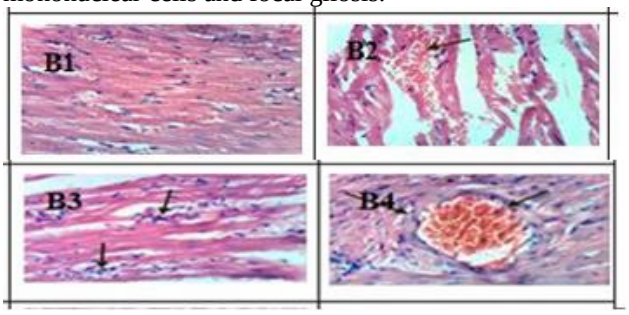

Fig. B1: Heart of rat from control group showed normal cardiac myocytes. Fig. (B2): Heart of rat from animals group exposed to γ radiation (6Gy) showed congestion of myocardial blood vessels and intramuscular haemorrhage. Fig. (B3): Heart of rats treated with LEV+ OXC showed focal myocarditis, inflammatory cell infiltration between cardiac myocytes and intermuscular oedema. Fig. (B4): Heart of rat from animals group treated with LEV + OXC then exposed to (6Gy) of γ radiation showed mononuclear cell infiltration between cardiac myocytes and congestion of myocardial blood vessel and hyalinosis of its wall.

Table 1: Effect of γ-irradiation on the level of GST in rats pretreated with LEV and/ or OXC .

Parameters Group	GST(nmol/g.tissue)			
	Liver	Kidney	Heart	Brain
Control	70.05±6.8	7.18±0.63	14.58±1.3	9.0±0.63
LEV	50±4.8[a]	4.46±0.44[a]	8.35±0.6[a]	7.05±0.48[a]
OXC	45.35±3.72[a]	4.45±0.39[a]	6.41±0.45[a]	4.45±0.42[a]
IRR	23.43±2[abc]	1.68±0.15[abc]	1.81±0.15[abc]	1.76±0.16[abc]
LEV+OXC	24.1±1.84[abc]	1.8±0.13[abc]	3.66±0.28[abc]	3.65±0.27[abc]
LEV+IRR	12.68±1.3[ac]	1.46±o.08[ab]	1.65±0.1[ab]	1.63±0.13[ab]
OXC+IRR	12.12±1[ac]	0.93±0.08[ac]	1.7±0.15[ac]	1.6±0.14[ac]
LEV+OXC+IRR	4.7±0.46[ad]	0.45±0.04[ad]	0.85±0.07[ad]	0.68±0.06[ad]

Table 2: Effect of γ-irradiation on the level of MDA in rats pretreated with LEV and/ or OXC

Parameters Group	MDA(nmol/g.tissue)			
	Liver	Kidney	Heart	Brain
Control	30.25±3.01	21.43±1.73	19.8±1.59	17.27±0.85
LEV	58.83±4.4[a]	56.3±5.56[a]	55.13±4.68[a]	54.32±4.38[a]
OXC	87.73±4.04[ab]	80.58±4.59[a]	69.52±6.21[a]	67.2±4.8[a]
IRR	132.6±5.4[abc]	143.7±6.3[abc]	134.1±6.74[abc]	128.3±7.85[abc]
LEV+OXC	119.2±6.3[abc]	112±7.7[abc]	112±8[abc]	118.5±9.5[abc]
LEV+IRR	160.6±6.3[ab]	174.2±6.41[ab]	167.4±9.63[ab]	167.9±6.32[ab]
OXC+IRR	189.6±3.9[ac]	194.4±5.44[ac]	178.9±6.2[ac]	188.9±9.6[ac]
LEV+OXC+IRR	223±10.05[ad]	227.7±9.7[ad]	214.8±9.9[ad]	227±14.11[ad]

Table 3: Effect of γ-irradiation on liver enzymes (AST, ALT and ALK) in rats pretreated with LEV and/or OXC.

Parameters Groups	AST(U/I)	ALT(U/I)	ALK(U/I)
Control	15.67±0.88	18.83±1.07	41.33±1.58
LEV	29.5±0.76[a]	29.83±1.24[a]	110.2±2.85[a]
OXC	29.0±0.96[a]	32.17±1.07[a]	108.5±2.61[a]
IRR	65.5±1.33[abc]	80±2.25[abc]	214.7±2.33[abc]
LEV+OXC	59.5±1.56[abc]	57±1.23[abc]	180.2±1.57[abc]
LEV+IRR	93.67±1.62[ab]	88.33±1.33[ab]	310.8±2.8[ab]
OXC+IRR	92.33±1.21[ac]	89.33±1.22[ab]	308.8±2.91[ac]
LEV+OXC+IRR	129.5±1.33[ad]	141.5±1.47[ad]	562.7±3.92[ad]

Results are expressed as mean± SEM (n=6). A,b,c or d Significantly different from control, LEV, OXC and LEV and OXC treated groups, respectively at $p<0.05$ using one way ANOVA followed by using Tukey Kramer test for multiple comparison.

Cardiac biomarkers levels

The effect of AEDs administration to rats beside exposure to γ- rays on cardiac biomarkers is in shown table (5). Administration of LEV showed non-significant change in serum cTnI and CK-MB concentrations. In contrast, animals treated with OXC as mono therapy revealed a significant increase in both cTnI and CK-MB levels. Co therapy of rats with LEV and OXC showed a marked increase in the activity of cTnI (56.7%) and CK-MB (121%) as compared to normal control values. Moreover, animal groups exposed to γ-radiation (6Gy) either individually or pretreated with either mono or co-therapy of AEDs exhibited a significant increase in serum cTnI and CPK concentrations, which was more pronounced in animal group subjected to co therapy with both AEDs followed by γ- irradiation as it recorded 55.3% (cTnI) and 89.7% (CK-MB) compared to (LEV+OXC) control group values.

Neurotransmitter levels

Table (6) displays the effect of AEDs administration to rats beside γ- irradiation on serum neurotransmitters. LEV and/ or OXC administration caused non-significant changes in the level of serum glutamate, GABA, as well as ACH. On the other hand, exposing animals to γ-radiation showed a significant increase in serum glutamate. This increase was accompanied by a fall in the level of GABA and ACH as compared to the normal level. Adequate repair of neurotransmitter was noticed in the group treated with LEV and/or OXC prior to γ-irradiation; such repair was more pronounced in the group subjected to the co therapy with AEDs before exposure to γ- radiation.

Table 4: Effect of γ-irradiation on kidney function (creatinine and urea) in rats pretreated with LEV and/ or OXC.

Parameters Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control	0.38±0.3	22.17±1.07
LEV	0.35±0.022	23.67±1.11
OXC	0.38±0.03	23.17±1.35
IRR	1.00±0.036^{abc}	38.83±1.86^{abc}
LEV+OXC	0.38±0.03	24.5±1.25
LEV+IRR	1.033±0.049^{ab}	40.17±1.77^{ab}
OXC+IRR	1.00±0.36^{ac}	40.00±1.86^{ac}
LEV+OXC+IRR	1.067±0.033^{ad}	41.67±2.06^{ad}

Table 5: Effect γ-irradiation on Cardiac biomarkers (cTnI and CPK) in rats pretreated with LEV and / or OXC.

Parameters Group	cTnI (ng/ml)	CK-MB (ng/ml)
Control	0.6±0.011	25.5±2.14
LEV	0.6±0.012	30.67±0.88
OXC	0.93±0.011^{ab}	56.33±0.88^{ab}
IRR	1.26±0.01^{abc}	77.33±0.66^{abc}
LEV+OXC	0.94±0.01^{ab}	56.5±0.76^{ab}
LEV+IRR	1.30±0.01^{ab}	78.83±1.7^{ab}
OXC+IRR	1.44±0.012^{ac}	108.8±1.99^{ac}
LEV+OXC+IRR	1.46±0.02^{ad}	107.2±2.52^{ad}

Table 6: Effect of γ-irradiation on the activity of neurotransmitter (GABA, Glutamate and ACH) in male albino rats pretreated with LEV and /or OXC.

Parameters Groups	Glutamate (mg/ml)	GABA (ng/ml)	ACH (ng/ml)
Control	9.43±0.79	36.72±0.47	26.0±1.9
LEV	8.88±0.8	36.93±0.44	25.92±0.21
OXC	9.13±0.0.79	36.83±0.44	25.83±0.25
IRR	46.08±0.96^{abc}	11.53±0.24^{abc}	8.21±0.15^{abc}
LEV+OXC	7.73±0.47	36.57±0.37	25.67±0.24
LEV+IRR	28.35±1.68^{abe}	20.03±0.2^{abe}	13.75±0.16^{abe}
OXC+IRR	30.17±1.67^{ace}	20.28±0.24^{ace}	13.93±0.15^{ace}
LEV+OXC+IRR	20.9±0.21^{ade}	28.77±0.55^{ade}	21.2±0.26^{ade}

Table 7: Effect of γ-irradiation on apoptotic biomarkers (Caspase3 and TNF-α) in rats pretreated with LEV and/ or OXC.

Parameters Group	Caspase3(Brain) nmol/g tissue	TNF alpha(Serum) Pg/ml
Control	8.5±0.28	38.48±2.02
LEV	15.72±0.23^{a}	59.65±2.54^{a}
OXC	20.8±0.15^{ab}	68.22±1.53^{a}
IRR	70.35±0.87^{abc}	146.2±2.87^{abc}
LEV+OXC	45.55±0.5^{abc}	82.12±1.74^{abc}
LEV+IRR	91.12±0.67^{ab}	162.6±1.33^{ab}
OXC+IRR	109.8±0.57^{ac}	167.8±1.42^{ac}
LEV+OXC+IRR	136.5±0.26^{ad}	203.0±1.82^{ad}

Results are expressed as mean± SEM (n=6). A,b,c or d Significantly different from control, LEV, OXC and LEV + OXC treated groups, respectively at $p<0.05$ using one way ANOVA followed by using Tukey Kramer test for multiple comparison.

Anti-apoptotic mediator's levels

As shown in table (7) administration of LEV and/ or OXC to rats either alone or followed by exposure to γ-radiation caused a significant increase in altitudes of serum TNF-alpha and Caspase3 in brain tissue. The percentage increase was 113.4% (TNF-alpha) and 435.8% (caspase-3) for group five (LEV+OXC) as compared to normal control values and 199.6% (TNF-alpha) and 147.2% (caspase-3) for group treated with LEV and OXC followed by γ-irradiation as compared to their respective control. The Results of the histopathological examination of liver, kidney, heart and brain tissues indicated coincidence with those recorded by the biochemical analysis.

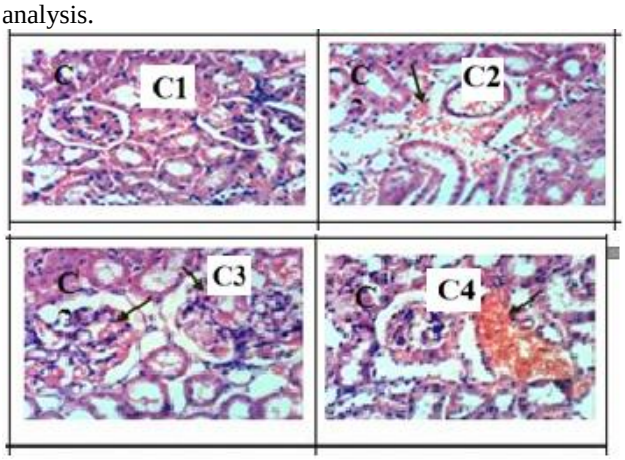

Fig. (C1): Kidney of rat from control group showed the normal histological structure of renal parenchyma. Fig. (C2): Kidney of rat from animals group exposed to γ radiation (6Gy) showed vacuolation of epithelial lining renal tubules, congestion of glomerular tuft and focal renal hemorrhage. Fig. (C3): Kidney of rats treated with oral doses of LEV (150mg/kg) and OXC (100mg/kg) once daily for three weeks showed hypertrophy, congestion of glomerular tufts and protein cast in the lumen of renal tubules. Fig. (C4): Kidney of rat from animals group treated with oral doses of LEV and OXC three weeks then exposed to a single dose (6Gy) of γ radiation showed congestion of renal blood vessel and congestion of glomerular tuft (H & E X 400).

DISCUSSION

Patients undergoing radiotherapy are often treated with glucocorticosteroids, anti-epileptic drugs (AED) as symptomatic treatment for coexisting conditions (Arif *et al*., 2007). A great concern has to be given to patients receiving these therapies to assure maintenance of healthy lifestyle consequently, the present study has investigated the effects of LEV and/ or OXC administration to rats followed by γ-irradiation on different body organs.

Administration of LEV and/ or OXC together with γ-radiation caused oxidative stress in the rats tested tissues, which recorded by the decrease in serum TAC and tissue GST along with an increase in the level of MDA. This alteration in oxidative stress could be owed to the generation of metabolites that increased body burden of free radicals (Thanoon, 2007), as well as ROS generation from water radiolysis inducing damage to DNA and cell membranes (Bellon *et al*., 2004). Combination treatment of LEV, OXC and gamma irradiation induced severe oxidative damage rather than the combination treatment of LEV and OXC.

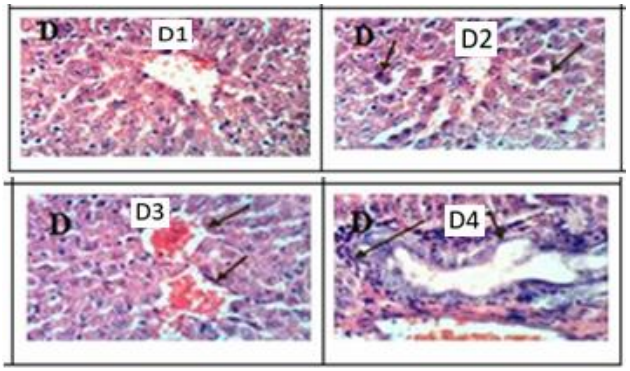

Fig. D1: Liver of rat from control group showed the normal histological structure of hepatic lobule (H & E X 400). Fig. (D2): Liver of rat from animals group exposed to γ radiation (6Gy) showed necrosis of sporadic hepatocytes. Fig. (D3): Liver of rats treated with oral doses of LEV and OXC showed congestion of central veins and slight dilatation of hepatic sinusoids (H & E X 400). Fig. (D4): Liver of rat from animals group treated with oral doses of LEV + OXC daily for three weeks then exposed to a single dose (6Gy) of γ radiation showed vacuolization of Centro lobular hepatocytes , necrosis of sporadic hepatocytes and hyperplasia of epithelial lining bile duct and scanty fibroblasts proliferation (H & E X 400).

Besides the oxidative stress effect of radiation, apoptosis can be induced by ROS formed from ionizing radiation (Cardenas-Rodriguez *et al*., 2013). Apoptosis, a gene-regulated phenomenon, undergoes two major apoptotic pathways, ''extrinsic'' and ''intrinsic'' pathways; in which Caspases are the key players in both pathways (Adams and Cory, 1998). During these pathways, the production of pro-inflammatory cytokines such as interleukin IL-6 and TNF-a increases (Sochocka *et al*., 2013), which in turn activate caspases leading to cell apoptosis (Chang *et al*., 2003) that induced by oxidative stress. Since caspase 3, existing as a proenzyme, can become activated during the cascade of events associated with apoptosis (Said *et al*., 2012) therefore, its level is a good indicator of apoptosis. According to the previous findings the observed increase in the activity of serum TNF alpha and brain caspase3 after treatment of rats with LEV and OXC followed by-radiation can be explained. In the current study, rats treated with LEV and/or OXC together with γ-radiation exposure showed an elevation in serum liver enzyme activity as well as severe histopathological

changes of liver; indicating hepatocytes injuries. Injury to the hepatocytes may be due to increase in free radical production and/ or decrease free radical utilization (Planjar-Prvan, 2013). The oxidative stress and lipid peroxidation causes damage of the membrane bilayer and cell integrity that leads to escape of these cytoplasmic enzymes in blood (Mansour *et al*., 2014). Concerning radiation effect on urea and creatinine serum levels, the present study revealed that irradiation of rats pretreated with LEV and OXC exerted a significant rise in serum creatinine and urea levels as compared to LEV+OXC group , in addition to severe histopathological changes of the kidney including; congestion of renal blood vessel and focal renal hemorrhage. The increase in creatinine level after irradiation indicates development of nephritis and renal dysfunction (Borg *et al*., 2002). Which may be attributed to impairment of glomerular selective properties (Berry *et al*.,2001).The increase in serum urea may be due to the augmentation in glutamate dehydrogenase enzyme due to irradiation that cause increase in carbamoyl phosphate synthetase activity leading to rise in urea concentration (Nduka, 1999). Elevations of serum urea and creatinine are reflected nephrotoxicity induced by drug in animals and humans (Adelman *et al*., 1981). Cardiac biomarkers are proteins that escape out of injured myocardial cells, resulting in elevated levels in blood; elevated creatinine kinase (CK-MB) as well as troponin I was used to assess cardiac injury (Hamada *et al*., 2015). Combined therapy of AEDs (LEV+OXC) followed by γ-irradiation showed significant increase in the level of cardiac biomarkers as compared to rats subjected to (LEV+OXC) alone indicating that γ-irradiation intensified the toxic effect of AEDs on the heart. Free radicals generated after administration of AEDs and γ-irradiation of rats altered the permeability of the membrane allowing leakage of cardiac biomarkers and excessive calcium influx causing death from calcium overload (Nellessen *et al*., 2010). Histopathological abnormalities, including infiltration and congestion of myocardial blood vessels confirm the biochemical results. Consequently, patients who treated with LEV and /or OXC alone or in combination with radiotherapy must periodically check their liver, kidney functions and cardiac biomarkers.

Glutamte is the excitatory amino acid neurotransmitter responsible for many neurological functions, including cognition, memory, behavior (Sundaram *et al*., 2012). However, excessive activation of glutamate receptors facilitated neurological disorder (Choi, 1988). GABA is the main inhibitory neurotransmitter in the mammalian central nervous system. It has a role in declining neuronal excitability (Watanabe *et al*., 2002).

Rats exposed to γ-radiation exhibited a significant increase in the glutamate level with concomitant decrease in the levels of GABA and ACH. These alterations in the neurotransmitters may be attributed to the increased rate of formation of O_2- and H_2O_2 (Hassan *et al*., 2012) as well as the damaging effect of γ-radiation on GABAergic neuron (Burke *et al*., 2004) and glutamate receptor composition (Roth *et al*., 2001). Moreover, Kaminski *et al*., (2008) demonstrated that γ-radiation showed a significant increase in acetylcholine-esterase activity leading to decrease ACH concentration. The present study revealed that administration of rats with AEDs either as mono or poly therapy prior to γ irradiation modulate the harmful risk of γ-radiation. This modulatory effect of LEV may be due to the suppression of calcium release from endoplasmic reticulum through binding to neuronal synaptic vesicle protein 2A receptor (SV2A) (Said 2004). Since calcium accumulation in response to repetitive stimulation causes glutamate over flow. Therefore, the binding of the SV2A receptor by LEV restored the ability of neurons to reduce the excessive glutamate overflow (Stahl, 2004). The present results further indicate that rats treated with combination therapy of LEV and OXC exhibited a lower glutamate level and higher GABA level as compared to irradiated rats treated with one of the two drugs alone, this may be due to a synergy of LEV and OXC associated with beneficial anticonvulsant pharmacodynamics action (Czuczwar *et al*., 2009).

CONCLUSION

It is clear from the assessment of toxicity parameters that the combination of LEV and OXC followed by γ-irradiation increase the level of toxicity. Therefore, the combination of these antiepileptic drugs followed by γ-irradiation should be avoided. Whether this phenomenon occurs with other antiepileptic drugs wants further investigations.

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