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STUDY ON DNA DAMAGE AND OXIDATIVE STRESS AND SOME BIOCHEMICAL ALTERATIONS OF LONG TERM ADMINISTRATION OF ALPHA-2 DELTA (*α2-δ)* LIGAND PREGABALIN AND THE POSSIBILITY OF *ZINGIBER OFFICINALE* IN AMELIORATING THESE EFFECTS IN RATS

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ABSTRACT

The present study was carried out to examine the protective effect of *Zingiber officinale* against the possible harmful effects induced by the α2-*δ* ligand, pregabalin, a novel antiepileptic drug with demonstrated efficacy. The results of this study put on show that the highest DNA damage (tail length, tail DNA % and tail moment) was in the group which received pregabalin only. The serum activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gama-glutamyl transferase (γ-GT) were significantly increased in pregabalin treated group whereas, a significant decrease in serum total proteins was observed. The obtained results showed a significant decrease in both superoxide dismutase (SOD) and catalase (CAT) activities in pregabalin administered group. *Zingiber officinale* (Ginger) administration before pregabalin for two weeks then its co-administration with pregabalin concurrently till the end of the experiment (90 days), showed lower DNA damage (tail length, tail DNA % and tail moment), with a significant improvement in serum ALP, ALT and γ-GT besides clinical improvements in serum total proteins levels. Antioxidant enzymes SOD and CAT values showed significant increase. From the obtained results, it could be concluded that pregabalin in a dose of 20mg/kg body weight does has an oxidative DNA damage, elevating some serum biochemical parameters related to liver and produce an oxidative stress. The pretreatment with ginger two weeks before the co-administration of both drugs significantly minimize those harmful effects and considered to be a valuable aid in minimizing the hazards of pregabalin.

KEYWORDS: Pregabalin, Ginger, Comet assay, Oxidative stress, Liver enzymes.

INTRODUCTION

In our continuous effort to investigate the possible harmful effects of pregabalin (Kamel and Khalifa, (2015), we demonstrate a study to discuss the adverse effects of the α2-δ ligand, pregabalin on the level of DNA damage and oxidative stress and the possibility of ginger to protect against those potential harmful effects. Pregabalin (δ-[+]-3-Isobutylgaba) (PGA) was designed as a lipophilic GABA analog substituted at the 3 position to facilitate diffusion across blood brain barrier (Lauria-Horner and Pohl, 2003). It is a novel antiepileptic drug that had been used as an adjunctive therapy for uncontrolled partial seizures; it possesses analgesic, anxiolytic and anticonvulsant properties (Noor, 2007). The (α2-δ) is an auxiliary protein associated with voltage-gated calcium channels, the antiepileptic agent binds to this protein and the end result is inhibition of calcium influx at the nerve terminals together with other neurotransmitters including glutamate, noradrenalin, serotonin, dopamine and substance P.

Pregabalin is well known to have adverse effects as cognitive impairments, somnolence and dizziness among other common side effects such as dry mouth, asthenia, amblyopia, nausea and peripheral edema (French *et al.,* 2003). Also, there have been an increasing number of reports of heart failure in patients using the drug (De Smedt *et al.,* 2008).

Focus on plant research has become worldwide and the beneficial outcome of using medicinal plants in various systems has already been existed. Ginger (Zingiber officinale), family Zingiberaceae has been used in traditional medicine to aid in digestion and to treat stomach upset, diarrhea, nausea and arthritis for centuries. Beside these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help in common cold, flu-like syndrome, headache and even painful menstrual periods. Some researchers showed that ginger oil has dominative protective effect on DNA damage induced by H2O2 and might act as a scavenger of oxygen radical and it could be used as an antioxidant (Lu *et al.,* 2003). The dried extract of ginger contains monoterpenes and sesquiterpenes beside the main antioxidants in ginger, gingerols and shogoals as well as some phenolic ketone derivatives (Sekiwa *et al.,* 2000 and Pal et al., 2001). The high concentration of potassium found in ginger protects the body against a variety of disorders as bone fragility, paralysis, sterility, muscle weakness, mental apathy and confusion, kidney damage and damage to the heart (Qian and Liu, 1992).

The present study was undertaken to discuss the possible harmful effects of pregabalin administration and the possibility of ginger to counteract these effects through studying DNA damage by comet assay in both liver and brain tissues, studying the oxidative stress by estimating the antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), measuring some liver function parameters, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and gama-glutamyl transferase (γ-GT), and the estimation of serum total proteins.

MATERIALS AND METHODS

1- Drugs

Pregabalin 150mg (Lyrica® 150mg, Pfizer Pharmaceutical Industries): the dose was 20mg/kg body weight (kamel and Kalifa, 2015). Pregabalin was given orally via stomach tube for 90 consecutive days.

Ginger oil: Method of extraction**:** The fresh ginger plant (rhizomes) were obtained from local market (Cairo, Egypt), washed, homogenized. The oil of ginger was prepared in the Faculty of Science, Zoology Department, Zagazig University by distillation of 400 gm of the plant after grinding by using an electrical mixer and then the plant powder was soaked in methanol for 48 h and then we take the filtrate and obtain the oil by using distillation instrument with heat supply (Heater) for several hours (10 h) and then the oil extract was sent to Department of Pharmacognsy, Faculty of Pharmacy, Zagazig University to assure its identity and purity and it was daily freshly prepared at it was administered at dose 3.3mg/ 100 gm rat orally by stomach tube for 90 consecutive days plus two weeks prior to pregabalin administration in the 4th group (Kamel *et al.*, 2014).

2- Experimental Animals

Forty mature male albino rats were obtained from Laboratory Animal Breeding Unit, Faculty of Veterinary Medicine, Zagazig University. Body weights were 150 to 180 gm and they were classified into 5 equal groups each of 8 rats. Animals were kept under hygienic conditions, housed in metal cages; feed and water were given *ad libitum*. The light system was 12/12 hours dark/light cycle. Rats were accommodated to the laboratory conditions for 2 weeks before applying the experiment.

3- Experimental Design

Animals were randomly allocated into five equal groups each of 8 rats as follows:

G1: control animals that received only saline

G2: ginger treated animals (3.3mg/ 100 gm rat orally by stomach tube for 90 consecutive days).

G3: pregabalin treated animals (20mg/kg body weight orally by stomach tube for 90 consecutive days).

G4: ginger was orally given for 2 consecutive weeks prior to pregabalin administration and then the both drugs continued till the end of the experiment.

G5: ginger and pregabalin were concurrently administered orally daily allover the experimental period (90 consecutive days).

4- Collection of samples

1- Liver and brain tissues were been collected after slaughtering of the animals at the end of the trial (90 days). They were kept in deep freezer for measuring the antioxidant enzymes (SOD and CAT) and DNA damage by the comet assay.

2- Blood samples were been collected from tail vein at the end of the trial in test tubes without anticoagulant to obtain serum. Blood samples were going under centrifugation at 3000rpm for 15 minutes for the collection of serum for estimation of some liver enzymes ALP, ALT and GGT plus measuring of total proteins.

Comet Assay (Single Cell Gel Electrophoresis: SCGE)

The procedure for slide preparation performed using the standard technique was described by Singh *et al.,* (1988) with some modifications. Comet slides were prepared by pre-coating clean regular microscope slides with 0.75% (w/v) normal melting point (NMP) agarose. Slides were allowed to dry for 1-2 h at room temperature. The cell containing layer was generally prepared from mixing 25µL of treated cells with 75µL of 0.5% (w/v) low melting point (LMP) agarose at 37ºC and the cell suspension was rapidly spread onto a pre-coated slide. The slides were gently covered with the cover slips and placed on a cold flat surface to allow the agarose to solidify for about 5min. the cover slips were gently removed by sliding them sideways from the slides and 80 µL of 0.5% LMP agarose was spread on glass slides, recovered with the cover slips and left on cold surface for agarose to solidify. At least two slides were made for each treatment. The cover slips were gently removed and the slides were submerged into freshly prepared lysis solution (2.5M NaCl, 100mM EDTA, 10mM Tris, 10%

dimethyl sulfoxide (DMSO), 1% Triton x-100, PH 10; 4ºC) for 2 hours. After lysis, the slides were equilibrated in freshly prepared electrophoresis buffer containing alkaline buffer (300mM NaOH, 1mM EDTA, PH more than 13 at 4ºC) to allow unwinding of double-strand DNA for approximately 20 min. the slides were then transferred into an electrophoresis unit with the same buffer and subjected to an electrophoretic field at 300mA and 25V at 4ºC for 20 min. The level of electrophoresis buffer was adjusted to achieve 300mA.

Following electrophoresis, the slides were neutralized in 0.4M Tris (PH 7.5) for 5 minutes for three times. After removing of the neutralization buffer, the slides were washed with cold water and left to dry at room temperature. The DNA was stained with 50µL of 0.2% ethidium bromide.

From each slide, fifty comet cells were randomly selected for the comet analysis. The comet images were scored using the fluorescence microscope (at 200x magnification) connected with charge coupled device (CCD) camera. The camera was linked to a personal computer containing automatic comet image analysis software. The two parameters selected as indicator of DNA damage were *tail length* (TL, the distance of DNA migration measured from the center of the nucleus towards the end of the tail, unit of measure is *µm*) and *tail moment* (TM, a measure of the distance between the center of both of the tail and head, multiplied by the percentage of DNA in the tail, unit of measure is *%*).

Estimation of antioxidant enzymes

Superoxide dismutase (SOD) and catalase (CAT) activities in the liver and brain tissue homogenates were determined according to the methods described by Nishikimi *et al.*, (1972) and Aebi, (1984) respectively.

Estimation of some liver function tests

The serum activities of serum alanine aminotransferase (ALT) was determined colorimetrically according to Tietz, (1987), alkaline phosphatase (ALP) according to Belfield and Goldberg, (1971) and gamma glutamyl transferase activity according to method described by Kaplan and Pesce, (1992).

Determination of serum total proteins

Serum total proteins were determined using the method described by Gassbaro *et al.*, (1972).

Statistical Analysis

Data were analyzed using computerized **SPSS** programs version 21 (2011). The results were expressed as Means ± S.E. The total variation was analyzed by performing one-way analysis of variance **(ANOVA)**. Duncan test was used for determining significance. Probability levels of less than 0.05 were considered significant (Snedecor and Cochran, 1982).

RESULTS

1- Effects of pregabalin and/or *Zingiber officinale* on the oxidative DNA damage in both liver and brain of mature male albino rat.

Concerning the effect on the oxidative DNA damage, the current study was carried out to evaluate the protective effects of *Zingiber officinale* in rats treated with pregabalin using the single cell gel electrophoresis (comet assay). The present study revealed that the highest ($P<0.05$) DNA damage was in the group which received PGA only (20 mg/Kg b.wt. orally for 90 days) compared to the control and ginger (3.3 mg/kg b.wt. orally for 90 days) received groups. This damage was in the form of damaged nuclei (tail length, tail DNA% and tail moment) in both liver and brain, **(Tables 1, 2 and Figures 1, 2).**

The prior exposure of rats with ginger for two weeks and its concurrent administration with pregabalin for the end of the experiment and the group that received both ginger and pregabalin along the entire experiment, showed lower ($P <0.05$) DNA damage compared to the pregabalin received group that showed the highest DNA damage.

Table (1): Effect of pregabalin (20 mg /Kg b.wt.) and/or *Zingiber* officinale (3.3 mg/ kg b.wt.) on tail length, tail DNA (%) and tail moment in liver of mature male albino rat.

Mean ± S.E (n=8)

Parameters Groups	Tail length (px)	Tail DNA (%)	Tail moment
G1 (control)	7.00 ± 0.86^{d}	1.12 ± 0.02^{c}	0.22 ± 0.01^{d}
G2 (Ginger orally)	7.07 ± 0.34^{d}	1.20 ± 0.04^{c}	0.21 ± 0.01^{d}
G3 (pregabalin orally)	21.97 ± 0.67^{a}	2.59 ± 0.08^{a}	1.16 ± 0.05^{a}
G4 (Ginger for 2 weeks then combined with PGA orally)	14.90 ± 0.51^{c}	1.75 ± 0.06^{b}	0.59 ± 0.02^{c}
G5 (Ginger and PGA orally)	17.60 ± 0.31^{b}	1.89 ± 0.05^{b}	0.69 ± 0.02^{b}

Means within the **same column** carrying different superscripts are significantly different at $P < 0.05$ based on **Duncan's Multiple Range Test (DMRT).**

Table (2): Effect of pregabalin (20 mg/kg b.wt.) and/or *Zingiber officinale* (3.3 mg/ kg b.wt.) on tail length, tail DNA (%) and tail moment in brain of mature male albino rat.

Mean ± S.E (n=8)

Parameters Groups	Tail length	Tail DNA (%)	Tail moment
G1(control)	7.63±0.84 [c]	1.22±0.02 [c]	0.24±0.01 [d]
G2 (Ginger orally)	8.03±0.81 [c]	1.15±0.02 [c]	0.20±0.00 [d]
G3 (pregabalin orally)	18.33±0.22 [a]	2.07±0.01 [a]	1.20±0.02 [a]
G4 (Ginger for 2 weeks then combined with PGA orally)	15.43±0.41 [b]	1.90±0.06 [b]	0.58±0.05 [c]
G5 (Ginger and PGA orally)	16.38±0.35 [b]	1.96±0.09 [b]	0.69±0.04 [c]

Means within the **same column** carrying different superscripts are significantly different at P<0.05 based on **Duncan's Multiple Range Test (DMRT).**

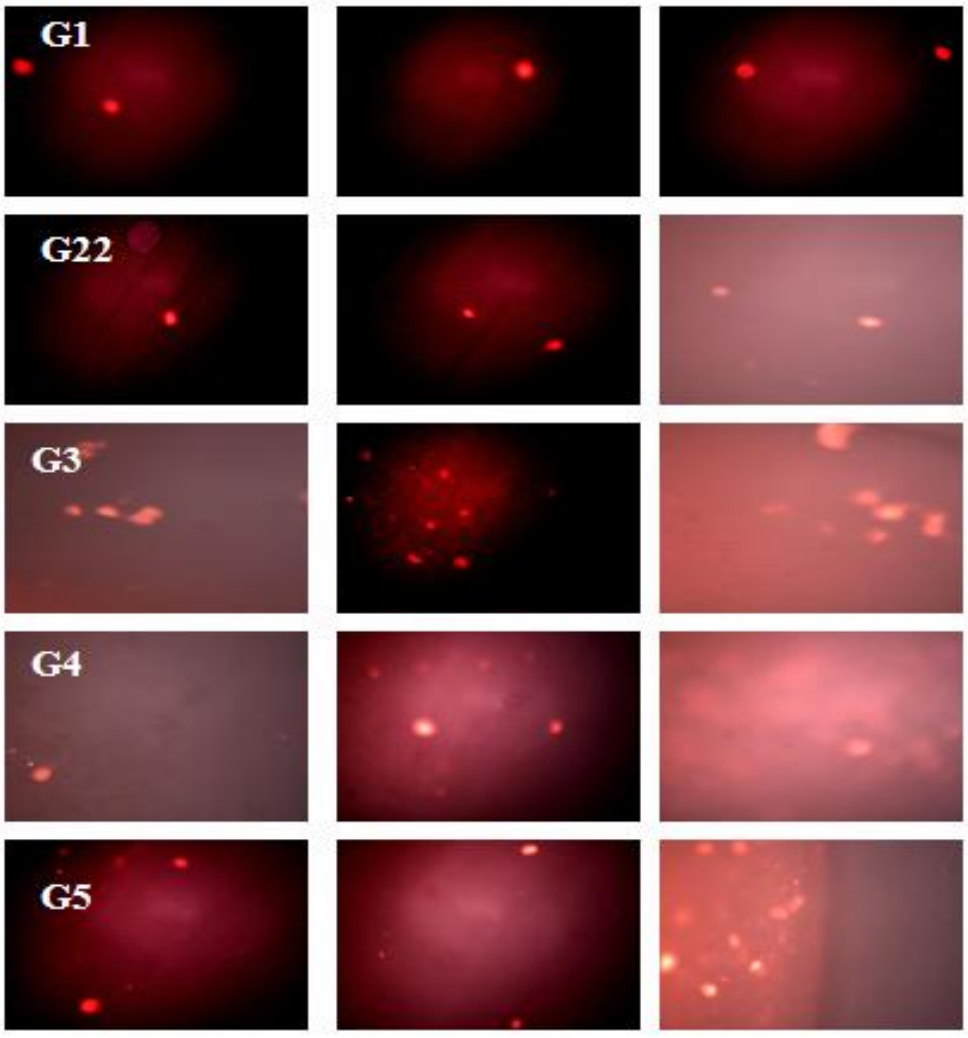

Fig (1): Photomicrograph with florescent microscope showing the effect of PGA and/or *Zingiber officinale* on the oxidative DNA damage in liver section of mature male albino rat.

G1) Control group: Normal condensed nucleus

G2) Group administered ginger (3.3 mg/Kg b.wt. orally for 90 days).

G3) Group administered PGA (20 mg/Kg b.wt. orally for 90 days)

G4) Group administered ginger for 2 weeks prior to PGA administration and continued with it till the end of the experiment.

G5) Group administered both PGA and ginger for the entire experiment.

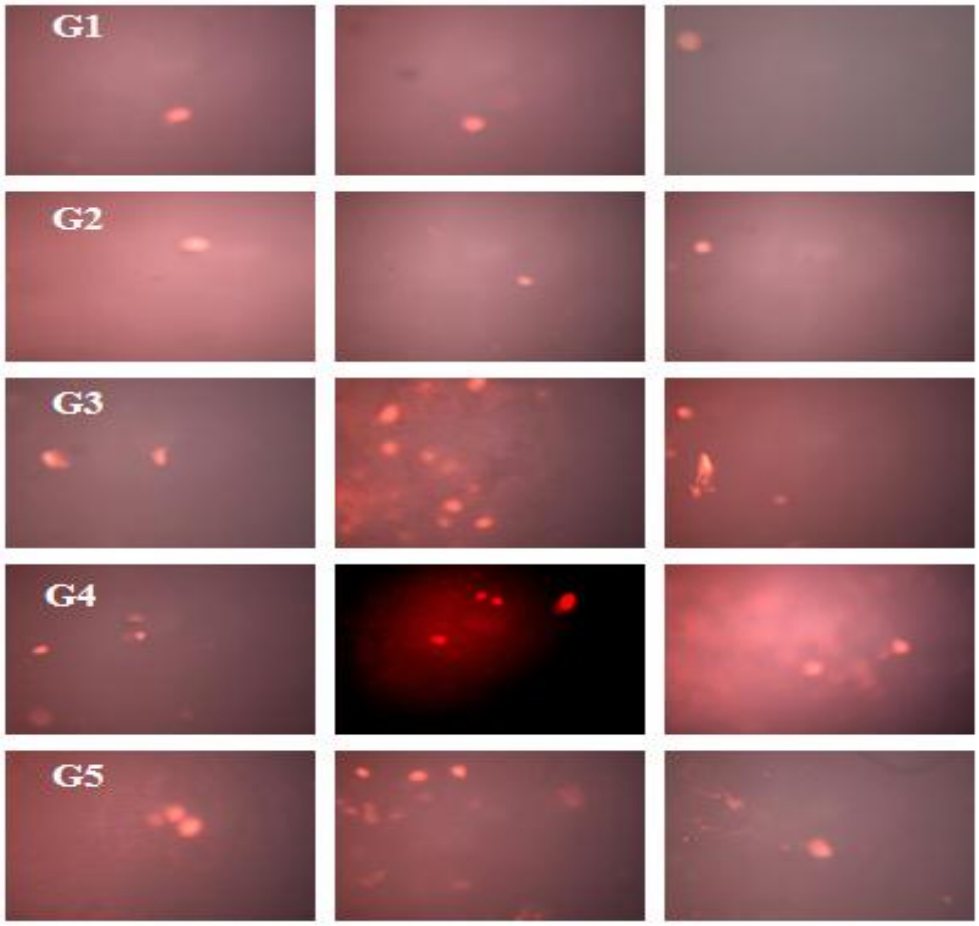

Fig.(2): Photomicrograph with florescent microscope showing the effect of PGA and/or *Zingiber officinale* on the oxidative DNA damage in brain section of mature male albino rat.

G1) Control group: Normal condensed nucleus

G2) Group administered ginger (3.3 mg/Kg b.wt. orally for 90 days)

G3) Group administered PGA (20 mg/Kg b.wt. orally for 90 days)

G4) Group administered ginger for 2 weeks prior to PGA administration and continued with it till the end of the experiment.

G5) Group administered both PGA and ginger for the entire experiment.

2- Biochemical parameters

Effects of Pregabalin and/or *Zingiber officinale* on some liver function parameters and total proteins of mature male albino rat.

The data illustrated in table (3) revealed the effect of PGA (20 mg/Kg b.wt.) and/or ginger (3.3 mg/Kg b.wt.) on the total protein (TP) and some liver enzymes activities. Total protein was the lowest (P<0.05) in PGA received group compared to the highest concentration in the control and ginger received groups.

On the other hand, there was a significant increase (P<0.05) in the total proteins in case of the prior exposure of rats to ginger for two weeks and its combined administration with PGA till the end of the experiment when compared with the group that given both PGA and ginger for the entire experimental period and when compared with the pregabalin received group.

Regarding the serum activities of alkaline phosphatase (ALP), Alanine aminotransferase (ALT) & Gama-glutamyl transferase (γ-GT), there were a significant increase (P<0.05) in PGA received group compared to the control and ginger administered groups.

Whereas the prior exposure of rats to ginger for two weeks and its combination with PGA for the end of the experiment, results showed a significant decrease (P<0.05) in the all enzymes compared to the group administered both pregabalin and ginger for the entire experiment. On the other hand, both groups had a lower enzyme activities compared to the pregabalin received group, but still higher than the control and ginger administered groups.

Table (3): Effect of pregabalin (20 mg/Kg b.wt.) and/or *Zingiber officinale* (3.3 mg/ kg b.wt.) on some liver enzymes and total proteins of mature male rat.

Mean ± S.E (n=8)

Parameters Groups	ALP U/L	ALT U/L	γ- GT U/L	TP gm/dl
G1(control)	24.38±0.52^{d}	81.75±0.65^{d}	29.61±0.57^{d}	7.98±0.12^{a}
G2 (Ginger orally)	24.30±0.31^{d}	80.41±0.47^{d}	26.50±0.43^{d}	7.93±0.25^{a}
G3 (pregabalin orally)	41.97±1.79^{a}	103.53±1.86^{a}	51.99±1.52^{a}	6.24±0.33^{c}
G4 (Ginger for 2 weeks then combined with PGA orally)	35.34±0.21^{c}	88.94±1.74^{c}	37.63±1.25^{c}	7.08±0.06^{b}
G5 (Ginger and PGA orally)	38.40±0.80^{ab}	93.50±1.05^{b}	44.83±0.94^{b}	7.08±0.11^{b}

Means within the **same column** carrying different superscripts are significantly different at P<0.05 based on **Duncan's Multiple Range Test (DMRT).**

3- Effects of Pregabalin and/or *Zingiber officinale* on the antioxidant enzymes in both liver and brain of mature male albino rat.

Regarding the effect of PGA (20 mg/Kg b.wt.) and/or ginger (3.3 mg/Kg b.wt.) on the antioxidant activities of mature albino rat in liver and brain, the obtained results showed a significant decrease (P <0.05) in both superoxide dismutase (SOD) and Catalase (CAT) activities in PGA administered group compared to the control and ginger administered groups which had the highest antioxidant activities, **tables (4, 5).**

Meanwhile the prior exposure of rats to ginger for two weeks and its combined administration with pregabalin till the end of the experiment and the group that administered both pregabalin and ginger for the entire experimental period, both showed significant increase (P <0.05) in the SOD and CAT activities compared to the PGA administered group, but still lower than that of the control and ginger received groups.

Table (4): Effect of pregabalin (20 mg/Kg b.wt.) and/or *Zingiber officinale* (3.3 mg/ kg b.wt.) on the antioxidant enzymes (SOD & CAT conc.) in liver of mature male albino rat.

Mean ± S.E (n=8)

Parameters Groups	SOD u/gm	CAT mmol/gm
G1(control)	91.63±1.74^{a}	77.12±4.70^{a}
G2 (Ginger orally)	84.91±4.48^{a}	76.97±4.06^{a}
G3 (pregabalin orally)	55.84±1.62^{c}	52.11±0.70^{c}
G4 (Ginger for 2 weeks then combined with pregabalin orally)	76.09±2.02^{b}	61.52±4.82^{b}
G5 (Ginger and pregabalin orally)	68.20±1.81^{b}	60.20±0.95^{b}

Means within the **same column** carrying different superscripts are significantly different at P < 0.05 based on **Duncan's Multiple Range Test (DMRT).**

Table (5): Effect of pregabalin (20 mg/Kg b.wt.) and/or *Zingiber officinale* (3.3 mg/ kg b.wt.) on the antioxidant enzymes (SOD and CAT conc.) in brain of mature male albino rat.

Mean ± S.E (n=8)

Parameters Groups	SOD u/gm	CAT mmol/gm
G1(Control)	95.16±0.64^{a}	77.90±4.79^{ab}
G2 (Ginger orally)	91.07±2.13^{a}	82.69±2.59^{a}
G3 (pregabalin orally)	54.11±1.10^{c}	50.77±1.34^{c}
G4 (Ginger for 2 weeks then combined with pregabalin orally)	72.92±2.73^{b}	66.30±7.16^{b}
G5 (Ginger and pregabalin orally)	78.52±1.50^{b}	65.40±3.27^{b}

Means within the **same column** carrying different superscripts are significantly different at P<0.05 based on **Duncan's Multiple Range Test (DMRT).**

DISCUSSION

Pregabalin is a medicine that relieves pain. Toth, (2014) had reviewed the clinical implications of pregabalin for the management of neuropathic pain. Clinical studies suggested that the administration of PGA is effective against diabetic peripheral neuropathy. But the doses up to 150mg/day are inefficacious (Satoh *et al.*, 2011). Several adverse central nervous system effects such as dizziness and somnolence and adverse systemic effects such as peripheral edema were observed besides, there had been reported that pregabalin possessing a potential for misuse. The incidence of aforementioned adverse effects increases with larger pregabalin doses (Semel *et al.*, 2010 and Toth, 2014).

The present study, focused on the possible side effects of pregabalin (20 mg/kg b.wt. orally) over the genotoxic effect, the biochemical impacts including the antioxidant enzymes activities (SOD & CAT) and some of the liver function tests as well as serum total proteins and the effect of ginger (3.3 mg/kg b.wt. orally) in ameliorating these potential side effects.

Ginger or *Zingiber officinale* (family: Zingiberaceae) is a perennial reed-like plant with annual leafy stems. The plant is about meter tall. The fragrant perisperm of zingiberaceae is used as sweetmeats by the Bantu tribe (Watt and Breyer-Brandwijk, 1962). Ginger is traditionally used as a common condiment for various foods and beverages such as soup, ginger ale, ginger bread, ginger snaps, parkin, ginger biscuits, and speculoos. Ginger rhizomes contain a number of pungent constituents and active ingredients (Dedov *et al.*, 2009). The steam distillation of ginger powder is used to produce ginger oil and contain high amount of sesquiterpene hydrocarbons, predominantly zingiberene. Gingerol is of the major pungent compounds in ginger and can be altered to shogoals, zingerone and paradol (Govindarajan and Connell, 1983). This takes part in several activities such as hepatoprotective effect (Ezeonu *et al.*, 2011).

Regarding the genotoxic effect, alkaline single-cell gel electrophoresis (comet assay) was been used, which has been proposed as a sensitive indicator of DNA damage as well as a biomarker in DNA kinetic repair studies. Thus, this assay is a reliable bioassay for monitoring exposure to hundreds of chemicals in a wide variety of ***in vitro*** and ***in vivo*** short-term studies as a biomarker of exposure, or biological dosimeter. The sensitivity of this

biomarker has enabled genetic toxicologists to monitor low-level, short and long-term exposure to chemicals, thus predicting genetic damage at any early stage (Valverde and Rojas, 2009).

It is a first record to determine the genotoxic effect of pregabalin using alkaline single-cell gel electrophoresis (comet assay). To our knowledge, there were no data available concerning the genotoxic effect of pregabalin in rats. Only a report by Yuksel *et al.*, (2010) who examined the potential genotoxicity of three antiepileptic drugs using the wing somatic mutation and recombination test (SMRT) in Drosophila melanogaster. They found that phenytoin clearly increased the frequency of total spots at all concentrations above 1.25 microg/ml. Gabapentin also increased the frequency of total spots at concentrations of 40 and 80 microg/ml. However, pregabalin displays lower genotoxicity in the SMAR assay when compared with the other two antiepileptics. The results also show that all antiepileptic drug concentrations lower the survival rate of the flies as the authors mentioned.

Although DNA is a stable, well-protected molecule, ROS can interact with it and cause several types of damage: modification of DNA bases, single and double-DNA breaks, loss of purines, damage to the deoxyribose sugar, DNA-protein cross-linkage and damage to the DNA repair system (Kohen and Nyska, 2002). The DNA damage detected in this study could be explained by the oxidative stress that was demonstrated through reduction in the antioxidant enzymes and elevation of lipid peroxidation. The increased ROS will result in an even higher negative impact into DNA (Azqueta *et al.*, 2011). Hence, under these circumstances, incidence of DNA damage is increased in the liver and brain cells. Thus, it is possible that pregabalin could cause alterations in DNA of mature albino rats resulting in formation of comets.

Ginger oil gives promising results improving damaged nuclei produced by pregabalin administration including (tail length, tail DNA% and tail moment) in both liver and brain caused by pregabalin as evidenced in tables 1&2 and figures 1&2. These results were in harmony with those of Polasa *et al.,* (2006) who recorded the anti-mutagenic effect of ginger in an in-vivo rat model, they found that ginger as a natural antioxidant, can protect DNA and other cellular molecules from cell damage induced by oxidation. Our findings were also in agreement with those of Siddaraju and Dharmesh, (2007) and Khaki *et al.,* (2009) who found that there was a protective role of ginger against DNA damage induced by H2O2.

The obtained results regarding pregabalin administration (20mg/kg b.wt.) showed a significant decrease in both superoxide dismutase (SOD) and catalase (CAT) when compared to the control and ginger administered groups. These results were in disagreement with those of Yilmaz *et al.,* (2012), who stated that SOD and CAT levels in liver tissues were significantly increased following pregabalin administration in albino rats. There is a possibility that there results were due to the induction of experimental epileptic seizures by bentylenetetrazole then comparing the antioxidant enzymes in the group with epilepsy to the one treated with pregabalin but in ours, there were no induction of epileptic seizures. Tufekci *et al.,* (2013) measured the SOD and CAT levels in brain cortex tissues after induction of epileptic seizures using bentylenetetrazole. Superoxide dismutase activities in pregabalin treated group were significantly lower than control group while catalase levels were similar.

Activities of SOD, CAT, GSH and MDA levels in the liver reflects the oxidative status and the serum enzymes like AST, ALT and ALP which represent the functional status of the liver (Cremer and Seville, 1982). SOD and CAT constitutes together a supportive team of defense against the reactive oxygen species (Bandopadhyay *et al.,* 1999). SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense mechanism via lowering the steady state level of O_2^-. CAT is a hemoprotein that catalyses the decomposition of H_2O_2 to water and oxygen so, it protects the cell from oxidative damage by H_2O_2 and OH^-.

A possible explanation of our findings could be returned to the ginger´s high antioxidant activity via scavenging a number of free radicals and protection of lipid cell membrane from oxidation, lower lipid peroxidation and raising the levels of antioxidant enzymes (Ahmed *et al.,* 2000 and Amin and Hamza, 2006). It also could be explained by interference with the mutagenic mechanism leading to enhancement of DNA repair system (Salah *et al.,* 2012).

Serum activities of ALP, ALT and γ-GT had shown a significant increase in their values in pregabalin received group compared to normal and ginger treated groups. The total proteins values on the other hand, showed the lowest levels in PGA received group when compared to the highest concentrations in the control and ginger administered groups.

Limited data is available on the hepatotoxicity of pregabalin. In prelicensure clinical trials in diabetic neuropathy and epilepsy, therapy with pregabalin was not associated with an increased frequency of serum aminotransferase elevations or liver toxicity. Since its approval and more wide scale use, however, pregabalin has been linked to rare instances of clinically

apparent liver injury. Most cases were mild and frequently without jaundice. The latency to onset of injury was short, symptoms of liver injury arising within 3 to 14 days. Both cholestatic and hepatocellular patterns of injury have been reported. Signs of hypersensitivity (fever, rash and eosinophilia) and autoimmunity were not present. Some cases have been severe and associated with marked jaundice and prolongation of the prothrombin time, but all cases ultimately resolved after the medication was stopped without evidence of residual injury, NIH, (2015).

Sendra *et al.,* (2011) reported a case of acute elevation of hepatic enzyme levels as a probable adverse reaction associated with pregabalin (25mg daily) for 59 years old man with a history of mantle cell lymphoma that developed neuropathic pain. Fourteen days after beginning the pregabalin treatment, patient developed left ankle edema and elevation of liver enzyme levels. Pregabalin was discontinued and hepatic enzyme levels returned gradually to the baseline levels. Furthermore, Kowar *et al.,* (2015) reported a case of developed liver failure due to new administration of pregabalin, 10 to 15 days after beginning of the treatment, patient developed jaundice, blood examination revealed elevated liver enzymes (ALT, AST, GGT and serum bilirubin). So, pregabalin should be taken into account as a cause of acute liver failure.

The prior exposure of rats to ginger for 2 weeks and its combination with PGA till the end of the experiment elicited a significant decrease in the all enzymatic values compared to the group administrated both PGA and ginger concurrently. On the other hand, both groups had a lower enzyme activities compared to PGA received group. Total proteins values showed a significant increase in case of the prior exposure of rats to ginger for 2 weeks and its combined administration with PGA till the end of the experiment when compared with the group that given ginger and PGA at the same time till the end of the experiment and when compared to PGA received group.

Infinite data are available regarding the hepato-protective effect of ginger. By way of illustration, Yemitan and Izegbu, (2006) found a significant decrease in serum activities of AST, ALT, ALP and lactate dehydrogenase (LDH) following ginger pretreatment in rats exposed to hepatotoxicity induced by both carbon tetrachloride and acetaminophen.

In conclusion, the present study validates the harmful effects that pregabalin could deliver on DNA level, oxidative stress and elevated liver enzymes. Pretreatment with ginger exerted a protective effect by augmenting host antioxidant defense mechanism.

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