# Effect of Different Intensities of Static Electric Fields on the Hepatic Functions and Cerebral Cortex of Male Mice

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## Abstract

This study determined the oxidative modification of total proteins and activities of antioxidant enzyme response in the liver, such as superoxide dismutase (SOD), malonaldehyde (MDA), glutathione (GSH), lactate dehydrogenase (LDH) exposed to the effect of external static electric fields (SEFs) of 67 and 133 kV/m for 0.5, 1, and 1.5 h daily for 21 days. The results revealed that the exposure of albino mice to SEFs with intensities of 67 and 133 kV/m caused a significant increase for the short-term exposure, compared with that for the long-term exposure. However, a significant increase was observed for the MDA content, and the SOD, GSH, and LDH activities decreased during the SEF exposure. Interestingly, the liver enzyme activities of total bilirubin, alanine transaminase, aspartate aminotransferase, and alkaline phosphatase in the serum and liver tissue significantly increased. The brain homogenate of Gamma-aminobutyric acid decreased, and glutamate increased. Numerous histopathological changes were detected in the liver tissue and cerebral cortex of the albino mice of the exposed groups.

**Keywords**: static electric field, hepatic function, oxidative stress, antioxidant enzyme, neurotransmitter and cerebral cortex.

## Statements and Declarations

**Author Contributions:** M. E., A. G., and N. K. conceived and designed the research. M. E. and H. A. conducted experiments and biochemical analysis. H. A. contributed new analytical tools and histopathological studies. M. E., N. K. and A. G. analyzed the data. M. E., N. K., A. G. and H. A. wrote the manuscript. All authors read and approved the manuscript.

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## Availability of data materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

**Declarations**

**Consent for publication**

Not applicable.

**Conflicts of interest**

The author declares that there are no conflicts of interest.

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## 1. Introduction

The biological effects of static electric fields (SEFs) have attracted interest and generated debates because the worldwide distribution of electricity, which has markedly increased during the last half-century, has resulted in increased human exposure to electromagnetic fields (EMFs) . Reports explaining the mechanism(s) of the response to exposure to an EF have suggested that either the EF-induced electric current or the perception of the EF through the skin surface acts as a trigger on cellular, humoral, or behavioral responses . Certain investigators have reported that reactive oxygen species (ROS) might be active in the mechanisms of extremely low frequency (ELF)-EMF effects. Plasma levels of melatonin secreted from the pineal gland and regulated by circadian rhythms may be suppressed by exposure to an EMF, but these results are considered controversial . Additionally, the balance between the generation of ROS and the reuptake of melatonin at the cellular level may have modulated the mechanisms of the EMF effects . The increase of he phagocytosis activity in mouse bone marrow cells exposed to 50-Hz EMF concomitantly upregulated the intracellular level of ROS . However, these reports of the possible harmful effects caused by ROS generated by exposure to EMF do not consider the effects of an EF. If the biological effects of various electrical treatments or environments including MFs or EMFs involved free radical metabolism as a common target, it would be extremely important to confirm these results for EF exposure alone. Long-term exposure to various external physical factors (among them, different forms of EMFs) causes so-called oxidative stress in living organisms, resulting in the generation of an increased amount of ROS, changes in the activity of the antioxidant defense system, stimulation of lipid peroxidation in biological membranes, and subsequent apoptotic cell death .

The divergence of reported experimental results regarding disturbances in the prooxidant-antioxidant balance in animal tissues under the influence of an EMF is mainly related to different physical parameters of EFs and experimental models used in particular studies . Recently, the transport of electric power using air high-voltage direct current (HVDC) transmission lines has become extremely common. To date, there are only a few experimental studies on the influence of strong SEFs on the prooxidant–antioxidant balance, although, in our opinion, investigations

regarding this problem are of great importance .

Hashish et al. reported that an ELF-EMF caused physiological disturbances in mice by affecting the redox balance through the measurement of the activities of lactate dehydrogenase (LDH), g-glutamyl transferase, glutathione-S-transferase, and lipid peroxidation levels in the liver. The disturbance of free radical homeostasis in organisms is a common hypothesis for explaining the effects of ELF-EMFs . However, studies on the relationship between environmental SEF exposure and oxidative stress are limited, and the results are controversial . Thus, additional studies on the oxidative effects of environmental SEFs are required. The official reports of the World Health Organization (WHO) and other responsible organizations contain minimal biological evidence suggesting any adverse effect of SEFs on human health. A few animal studies also appear to have yielded no data supporting the existence of adverse effects of SEFs . In another study, there was a fragmentary and coherent approach to investigating the biological consequences of SEF exposure. In many areas, the data was insufficient to draw conclusions regarding possible health effects, particularly following chronic exposure .

Changes in the serum protein fractions were reported by Marino et al. where rats were exposed to vertical fields (0.6–19.7 kV/m) for 30 days and by Harutyunyan and Artsruni where rats were exposed to a vertical field (200 kV/m) for an hour in a week. The results indicated a decrease in fast α1 and α2 globular proteins in plasma, coinciding with a clotting acceleration after the short-term ESF and the attenuation of clotting-dependent proteome modifications reflected with incomplete coagulation after the long-term ESF exposure. Ohta described the effect of an ESF of 180 kV/m and higher on action potentials from afferent fibers innervating various sensory receptors in the hind limbs of cats. His results indicated that a high DC EF-induced the movement of hairs, which eventually evoked action potentials that innervated the hair receptors. There are several reports on SEF effects on the pro/antioxidant system. Cieślar et al. described the transient inhibition of antioxidant enzyme activity in erythrocytes with subsequent adaptive stimulation. Seyhan and Güler reported an increase in the level of thiobarbituric acid reactive substances (TBARS) in the plasma, liver, lung, and kidney tissues of white guinea pigs after exposure to SEFs of 0.8–1.8 kV/m. Artsruni et al. provided evidence of the effect of a SEF of 200 kV/m on the physical parameters of the red blood cell membranes of rats. The superoxide dismutase (SOD)/catalase (CAT) ratio increase in the blood plasma and hemolysates of rats exposed to a SEF (200 kV/m) was observed in our earlier investigation.

## 2. Material and Methods

### 2.1. Experimental animals

#### 2.1.1. Ethics statement

All animal protocols were approved by the Ethical Committee of Zagazig University **(ZU-IACUC committee)**, approval number **(ZU-IACUC/1/F/257/2022)**

**2.1.2. Experimental animals**

Male albino mice, from a same batch (n=35, 4-week-old, weighing 27 ± 30 g) were used in experiments. The mice obtained from the Experimental Animal Care Center and were housed in metabolic cages under controlled environmental conditions (25 ± 1 °C and a 12 h light/dark cycle) one week before starting the experiment as acclimatization period. The mice fed a standard diet provided ad libitum. After an acclimatization period of one week with standard basal diet, thirty-five mice divided into seven groups each group have five mice and exposed to dose 4 and 8 KV where their equivalent are 67 and 133 KV/m after measuring the distance that applied by the device, so the doses that applied for the mice where calculated by the following equation.

Dose calculation:

**E = V/d = 4 (KV)/ 0.06 (m) = 66.666 ∼ 67 KV/m**

**E = V/d = 8 (KV)/ 0.06 (m) = 133.333 ∼ 133 KV/m**

**Where:**

**V = Applied voltage**

**d = Distance between two plates of used system as fig. 1.**

The doses and the period of exposure to SEF for each group are explained in Table 1.

**Table 1. Treatment group, applied stat**ic electric field (SEF), daily exposured time and total experimental time

| Treatment Groups | E (kV/m) | Exposure time (h) | Total time (day) |
|---|---|---|---|
| T1 | 0 | 0 | 21 days |
| T2 | 67 (4) | 0.5 | |
| T3 | | 1 | |
| T4 | | 1.5 | |
| T5 | 133 (8) | 0.5 | |
| T6 | | 1 | |
| T7 | | 1.5 | |

## 2.2. SEF exposure system

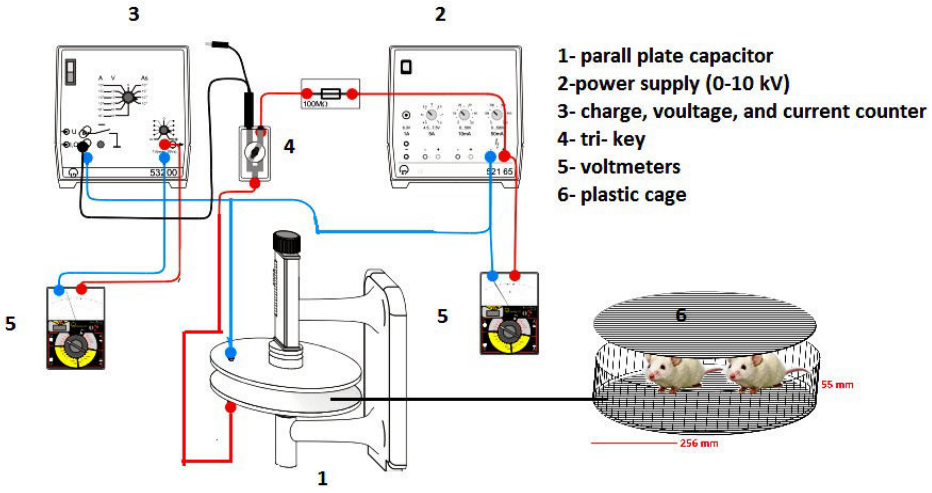


Fig. 1 shows the device for producing SEF. The input AC voltage was 220 V, and the output DC voltage was 0–10 kV where the SEF strength produced DC voltage was up to 160 kV generated between two parallel-electrode capacitors. The device comprised two plates separated by a distance within the range of 0–7 cm that was continuously adjustable, fine adjustment of the plate spacing of 1/10 mm intervals over a length of 2 mm, plate diameter of 255 mm, and plate thickness of 7 mm.

**2.3. Determination of liver enzymes and antioxidant enzyme activity**

After a 21-day SEF exposure cycle, 5 mice per group were sacrificed under ether anesthesia. Their liver tissue was obtained and immediately weighed. Next, 0–2 g of the liver samples were homogenized in cold saline at a ratio of 1:9 (mass/volume) using an automatic sample grinder. After centrifugation (2000 r/min), the supernatants of the liver homogenate were obtained. For the assessment of liver enzymes, alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured in the serum and liver tissue using a kinetic method according to the International Federation of Clinical Chemistry (IFCC) , while the total protein (TP) was determined using the Biuret method . The activities of antioxidant enzymes such as total SOD , glutathione (GSH) and LDH , and malonaldehyde (MDA) were detected using the enzyme-linked immunosorbent assay (ELISA) method.

**.24. Determination of neurotransmitters**

After the extraction and derivatization, glutamate (GLU) was quantitatively determined using the ELISA kit . Gamma-aminobutyric acid (GABA) was also determined using the ELISA kit .

**2.5. Histological and histochemical techniques**

All steps were conducted in the Histology and Cell Biology Department of the Faculty of Medicine at Zagazig University. Specimens from the liver and cerebral cortex of each animal were fixed in 10% neutral formol saline, dehydrated, embedded in paraffin wax, and processed into 5-µm thick sections. The sections were stained with hematoxylin and eosin, and the stained slides were analyzed by light microscopy in the Image Analysis Unit .

## 2.6. Statistical analysis

The statistical analysis of the data was conducted using one-way analysis of variance (ANOVA), followed by posthoc tests, including multiple comparison tests between the groups. $p < 0.001$ was considered a statistically significant difference. Data are presented as mean ± standard error of mean or as median and percentiles depending on the data distribution (n = 5).

## 3. Results and Discussion

### Influence of SEFs on MDA and antioxidant enzyme activity

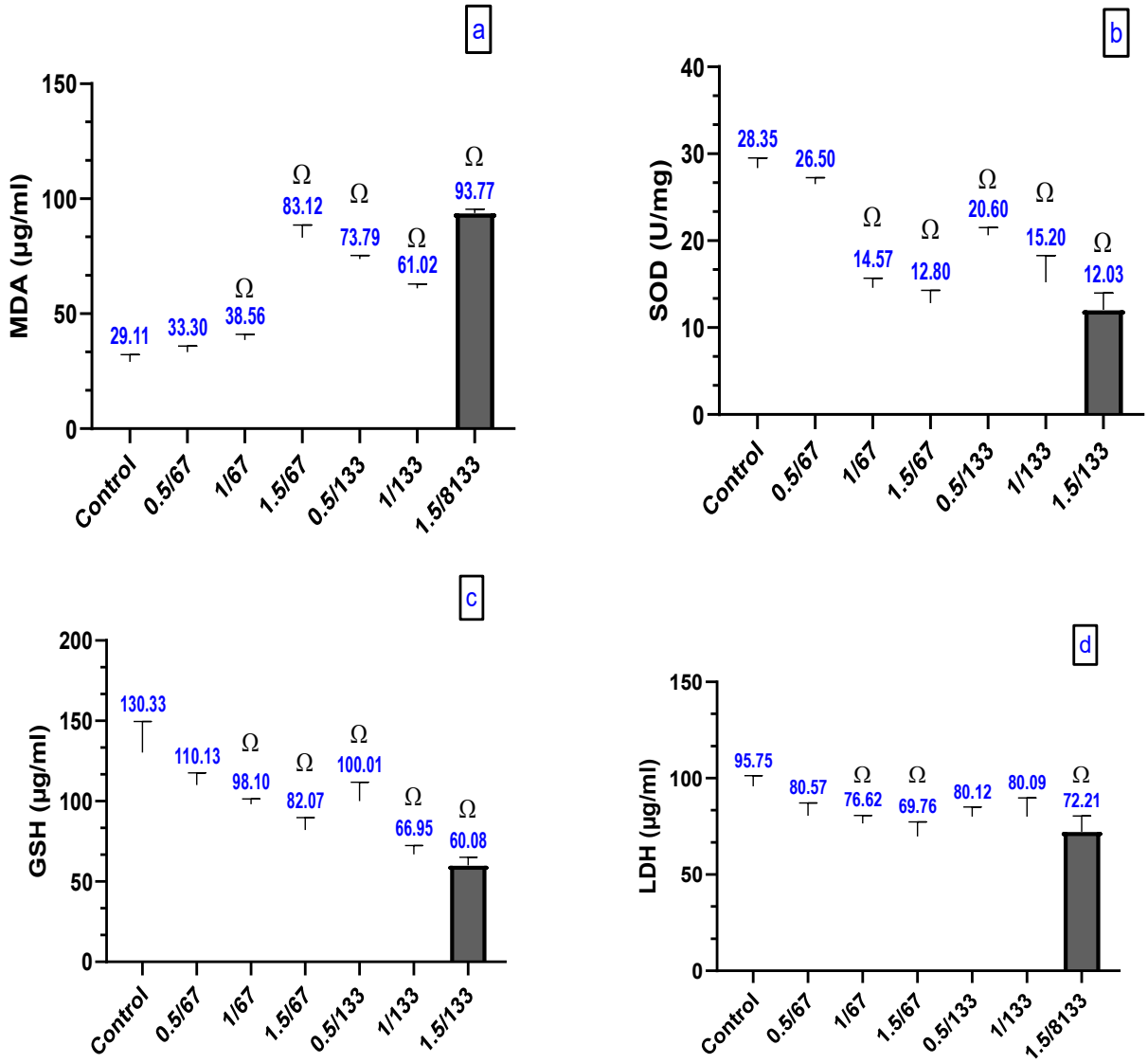


**Fig 2:** Bar charts representing MDA (a), SOD (b), GSH (c) and LDH (d) in the studied groups. Analysis was performed using one-way ANOVA, followed by post hoc Tukey test. Values are presented as mean, SD. Ω significant difference compared with control group, *P <0.001*.

**a- MDA content in liver**

The MDA contents in the liver of the albino mice in control and exposed groups (n = 5 in

each group) on different days of the exposure cycle are shown in Fig. 2a . As the time of exposure increased, the MDA content increased. However, there was a significant difference ($p < 0.001$) in the MDA content between the groups exposed to SEF with intensities of 67 and 133 kV/m and the control groups.

**b- SOD activity in liver**

The SOD activity in the liver of mice exposed to SEF with intensities of 67 and 133 kV/m was lower in 1/67, 1.5/67, 0.5/133, 1/133, and 1.5/133 groups than that of the mice in the control groups (Fig. 2b). Thus, there was a significant difference ($p < 0.001$) between them.

**c- GSH activity in liver**

The GSH activity in the liver of the control and the groups (n = 5 in each group) exposed to SEFs (67 and 133 kV/m) are shown in Fig. 2c. There was a significant decrease ($p < 0.001$) in the GSH activity of mice exposed to SEF in 1/67, 1.5/67, 0.5/133, 1/133, and 1.5/133 groups compared with that of mice in the control groups.

**d- LDH activity in liver**

The LDH content in the liver of mice exposed to SEF with intensities of 67 and 133 kV/m and mice from control groups are shown in Fig. 2d. There was a significant decrease ($p < 0.001$) in the LDH activity of mice exposed to SEF in 1/67, 1.5/67, and 1.5/133 groups compared with that of mice in the control groups.

A source of oxygen free radicals in organisms is ionizing radiation and other physical factors generating great amounts of active ions. The electric field produced permanently in nearby transmission lines generates an ionization current, which charges all the ungrounded elements in this field. The continuous generation of ions creates a cloud of space charge, which increases the ion density in the air even a hundred meters away from the transmission line. The inhaled air with an increased amount of ions may indirectly stimulate free oxygen radical synthesis, thereby resulting in many unfavorable effects on human body.

The potential of the SEF adversely affecting the health of the human population is an issue that continues to receive increased attention in public and scientific forums. There is increasing evidence that the effects of SEF can be mediated by the formation of ROS and highly reactive free radicals, thereby removing hydrogen atoms from fatty acids, causing lipid peroxidation, and consequently, cell death . Toxic oxygen free radicals are extremely reactive and can cause considerable damage to biomolecules, such as RNA, enzymes, membranes, proteins, and lipids, which may lead to various pathological consequences. Generally, oxygen free radicals are neutralized by highly efficient systems in the body. These include antioxidant enzymes, such as SOD and LDH.

Free radical scavengers, which can be divided into two major categories of antioxidant enzymes and antioxidants, are a group of substances that can prevent and repair damage by free radicals. GSH is a

powerful antioxidant that plays an important role in the antioxidant defense system in humans. It is mainly produced by the liver and transported to other parts of the organism. GSH eliminates free radicals by directly reacting with a variety of ROS and combines and removes certain organic peroxides catalyzed by GSH-peroxidase (PX) and GST. Thus, GSH-PX and GST are considered to be sensitive markers of free radical content and oxidative reaction intensity.

The present data showed a pronounced decrease in the hepatic GSH content of the exposed mice; a highly significant decrease was recorded at the highest strength (133 kV/m) of exposure. The obtained results appeared to be conceivable to those obtained by who reported that the exposure of mice to a 10-GHz microwave for 30 days resulted in a highly significant depletion of GSH levels in the intestine, liver, testis, and spleen, compared with that for the control group. Here the decrease in the GSH activities in the liver following the SEF exposure may have been due to the damaging effect of the free radicals produced, following the SEF exposure or by a direct effect of the formaldehyde formed from the oxidation of free radicals on these enzymes. GSH acts as a free radical scavenger and regenerator of alpha-tocopherol and plays a significant role in sustaining protein sulfhydryl groups .

Decreased hepatic GSH contents result in increased susceptibility to hepatic injury via the induction of lipid peroxidation . GSH is the main antioxidant found in liver cells. It plays a protective role in the metabolism of many toxic agents, including oxidative stress. Enhanced SEF toxicity has been associated with decreased hepatic GSH, which may reflect the depletion of GSH by the overproduction of ROS and subsequent oxidative stress caused by the SEF. This may account for increased levels of oxidized lipids in the serum lipoproteins of irradiated mice, following the consumption of a diet rich in oxidized lipids since the hepatic GSH detoxifies dietary lipids before they enter circulation . reported that exposure to 2450-MHz 0.25-$mW/cm^2$ continuous waves MW significantly decreased the antioxidant enzyme activities of GSH-Px, SOD, and CAT, compared with that of the control group. Additionally, the decreased LPO and GSH contents in the testes and epididymis of rats exposed to 0.9/1.8 GHz . Additionally, the decreased GSH contents in the liver, heart, kidney, and plasma of rats exposed to ELF-EMF (60 Hz). An enhanced ROS production after combined exposure to RF radiation (930 MHz, SAR 1.5 $Wkg^{-1}$) and iron ions was reported in an experimental model of rat lymphocytes and induced lipid peroxidation, accompanied by a decrease in the SOD, myeloperoxidase (MPO), and GSH-Px activities by RF exposure in various organs, such as a rat kidney and guinea pig liver. Moreover, in the latter animal model, treatment with epigallocatechin-gallate, the main active component of green tea, and N-acetyl cysteine, a GSH precursor, protected against oxidative stress-induced liver injury caused by RF-EMFs .

SOD is another antioxidant enzyme that functions with GSH-PX and GST to protect organisms from toxic free radicals, mainly superoxide anion free radicals . The liver is the most important detoxification organ in the body and is abundant in mitochondria, the main organelles generating superoxide anion free radicals . Thus, estimating the hepatic activity of SOD reflects the oxidative stress state to an extent.In our study, a statistically significant decrease in the hepatic activity of SOD was observed in the mice exposed to the high intensity of 133 kV/m after an exposure time of 1.5 h/day for 21 days, compared with that in the control group. The decrease in the SOD activity may be regarded as an indicator of increased superoxide anion free radical production after exposure time. However, the SOD activity significantly decreased in both exposed groups (133 kV/m). This may have been because the adverse effect induced by the SEFs was cumulative after the exposure, and the organism was unable to regulate itself when the adverse effect exceeded a certain threshold level. The difference became significant ($p < 0.001$) in the serum when the exposure level was increased to 133 kV/m.

The MDA concentrations in the hepatocytes were also determined in this study. MDA is a specific and chemically stable product of lipid peroxidation, which is the process during which free radicals attack polyunsaturated fatty acids in cell membranes, resulting in cell damage. Thus, MDA is regarded as a reliable biological marker of oxidative stress **.** Furthermore, observed a significant increase in the MDA levels and a significant decrease in the antioxidant enzyme activities in guinea pigs exposed to an ELF-EF. They also discovered that N-acetyl-L-cysteine application has protective effects on ELF-EF-induced oxidative stress. observed a significant increase of the MDA concentration in mice exposed to 9.2 kV/m for 14 and 21 days, compared with those in the control groups. Additionally, detected an increase in the MDA levels due to oxidative stress in the cornea, similar to previous studies. In corneas exposed to PC monitor radiation, the MDA level, as an indicator of lipid peroxidation, significantly increased. The results of this study correlated with those of previous studies showing a significant increase in MDA concentrations after different exposures (133 kV/m) to SEF. These data indicated that SEFs of certain intensities increased the lipid peroxidation in the biological membranes in liver tissues. LDH is antioxidant enzyme and has been studied as a general marker of cellular health. These findings suggested that LDH increased in the exposed groups (67 and 133 kV/m).

**Influence of SEFs on the activities of Total protein (TP), total bilirubin, ALT, AST, and ALP in serum**

**TP content in liver**

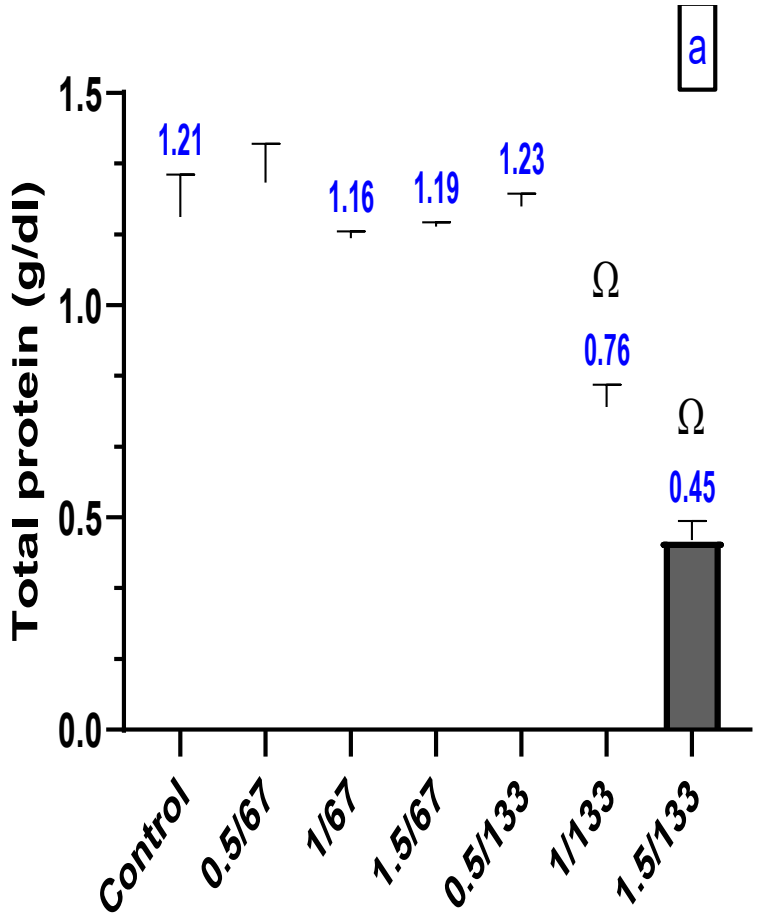


**Fig 3:** Bar charts representing Total protein in the studied groups. Analysis was performed using one-way ANOVA, followed by post hoc Tukey test. Values are presented as mean, SD. Ω significant difference compared with control group, *P* <0.001.

The TP contents in the liver of the mice in the exposed and control groups are shown in Fig. 3. After exposure to a SEF with an intensity of 67 kV/m for 21 days, the TP contents decreased in the exposed group had a higher significant value ($p < 0.001$) than that of the control group. The results revealed that the groups (n = 5 in each group) exposed to SEFs with intensities of 67 and 133 kV/m significantly increased the TP content for short-term exposure, compared with that for long-term exposure.

**Total bilirubin, ALT, AST, and ALP in serum**

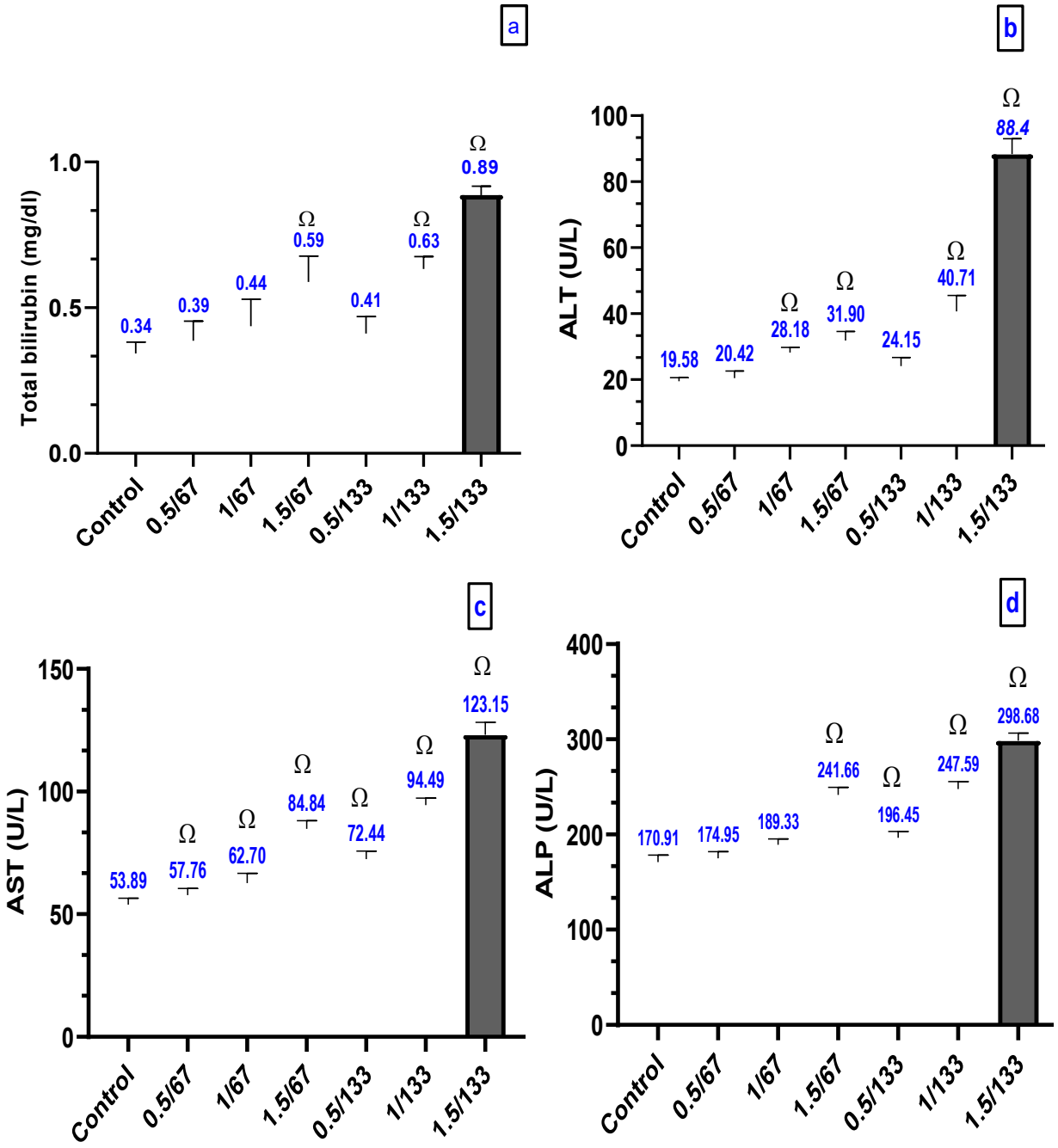


**Fig. 4.** T. bilirubin (**a**), ALT (**b**), AST (**c**) and ALP (**d**) levels in various studied groups. Statistical analysis was done using one-way ANOVA, followed by Tukey's Post-test. Values are represented as mean ± SE. (n = 5). Ω significant difference from control group P < *0.001*.

The results of T. bilirubin, ALT, AST, and ALP in serum after exposure to SEFs with the intensities of 67 and 133 kV/m are shown in Fig. 4. After a 21-day exposure cycle to SEF for 0.5, 1, and 1.5 h, daily. The T. bilirubin, ALT, AST, and ALP contents had significantly higher means between the mice from the groups exposed to SEFs with intensities of 67 and 133 kV/m and those from the control groups (p < *0.001*).

## Histopathological changes

### Liver Histopathology: Fig. 5

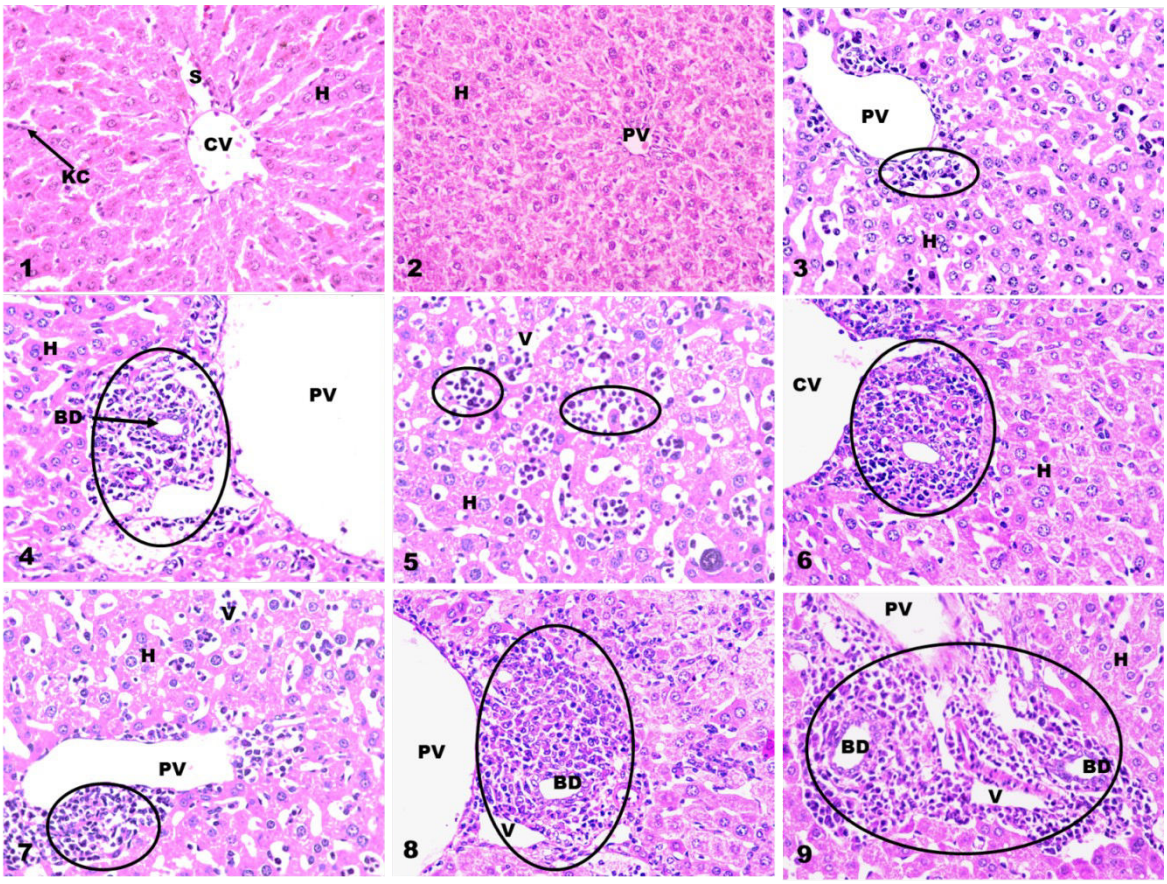


**Fig. 7** Photomicrographs of liver sections of adult male mice of different groups: **(1) control group** showing the hepatic central vein (CV), hepatocytes (H) forming normal organized hepatic strands, sinusoids (S), and Kupffer cell (KC). **(2) 0.5/67 group** showing a constricted hepatic portal vein (PV) and disorganized hepatocytes (H). **(3) 1/67 group** showing a ruptured hepatic PV and surrounded with mononuclear inflammatory leucocytic infiltration (circle). **(4) 1.5/67 group** showing hepatic cells (H) undergoing different degrees of degeneration with an increased incidence of inflammatory leucocytic infiltration (circle). The hepatic strands are in disorganized patterns, and the bile duct (BD) can be observed. **(5) 0.5/133 group** showing a normal appearance of most hepatocytes (H). To an extent, most hepatocytes with prominent nuclei (N) and nucleoli, small distributed inflammatory cells (circles), and vacuolation (V) were observed. **(6&7) 1/133 group** showing the hepatic CV and PV in shattered shapes, and vacuolation (V) inflammatory infiltration increased and became scattered (circles). **(8&9) 1.5/133 group** showing destroyed PVs surrounded with huge and massive mononuclear inflammatory leucocytic infiltration (circles); BDs and vacuoles (V) became dilated, and disorganized hepatocytes (H) can be observed. **(H & E, x400)**

As observed in fig. 5, **0.5/67 group** showed constricted hepatic portal vein and disorganized hepatocytes. **1/67 group** illustrated a ruptured hepatic portal vein and surrounded with mononuclear inflammatory leucocytic infiltration. **1.5/67 group** clarified the hepatic cells undergoing different degrees of degeneration with increased incidence of inflammatory leucocytic infiltration, the hepatic strands are in disorganized pattern and bile duct is noticed. **0.5/133 group** displayed normal appearance of most hepatocytes, to some extent, most of hepatocytes with prominent nuclei and nucleoli, and small distributed inflammatory cells and some vaculation appeared. **1/133 group** exhibited the hepatic central vein and portal vein in shattered shape while avculation (V) inflammatory infiltration are increased and become scattered. **1.5/133 group** showed a destroyed portal veins surrounded with huge and massive mononuclear inflammatory leucocytic infiltration while bile ducts and vacuoles become dilated and disorganized hepatocytes are noticed.

The release of enzymes in liver is an important indicator of the hepatocytes damage, in which ALT and AST are the two most sensitive indices . Thus, the activities of ALT and AST in serum can directly reflect the damage of hepatocytes. Our results in this study explained that After a 21-day exposure cycle to SEF for 0.5, 1, and 1.5 h, daily with the intensities of 67 and 133 kV/m, T. bilirubin, ALT, AST, and ALP contents had significantly higher means between the mice from the groups exposed to SEFs with intensities of 67 and 133 kV/m and those from the control group. While the TP contents decreased in the exposed group had a higher significant value than that of the control group. When hepatocytes are damaged, the permeability of cell membrane will increase. Accordingly, the ALT and AST inside the cell will pour into the blood, causing a substantial increase of their activities in serum . This illustrated where exposing to EF-EMFs in humans or animals resulted in increasing glucorticoids (cortisol), stress oxidative compounds, and produced hipoxy. This is an important reason for increasing the amount of transaminases in experimental groups. Hipoxy production could increase the AST and ALT value in serum, up to thousands of units in liter .

**Influence of a SEF on the brain homogenate of GABA and Glutamate (GLU)**

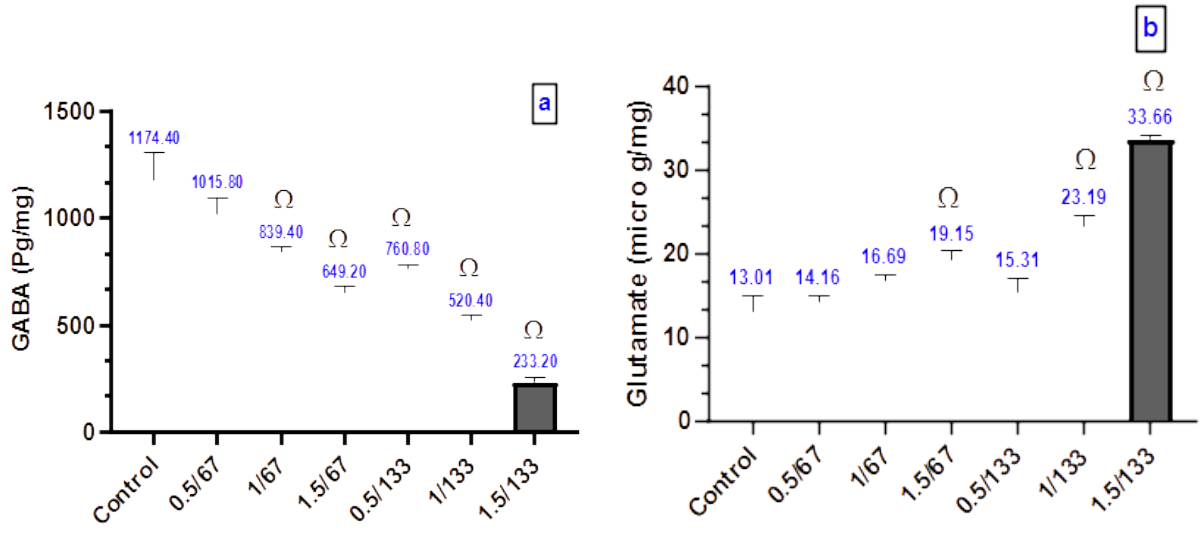


**Fig. 5.** GABA (a) and Glu (b) levels in various studied groups. Statistical analysis was done using one-way ANOVA, followed by Tukey's Post-test. Values are represented as mean ± SE. (n = 5). Ω significant difference from control group P < 0.001.

The results of neurotransmitters such as GABA and GLU are shown in Fig. 5. After a 21-day exposure cycle of SEF for 0.5, 1, and 1.5 h, daily, there was a significantly higher mean of the GLU content between the mice from the groups exposed to SEF with intensities of 67 and 133 kV/m and those from the control groups ($p < 0.001$). Although there was a significantly lower mean of the GABA content of the exposed groups, compared with that of the control group ($p < 0.001$).

**Brain Histopathology: Fig. 6**

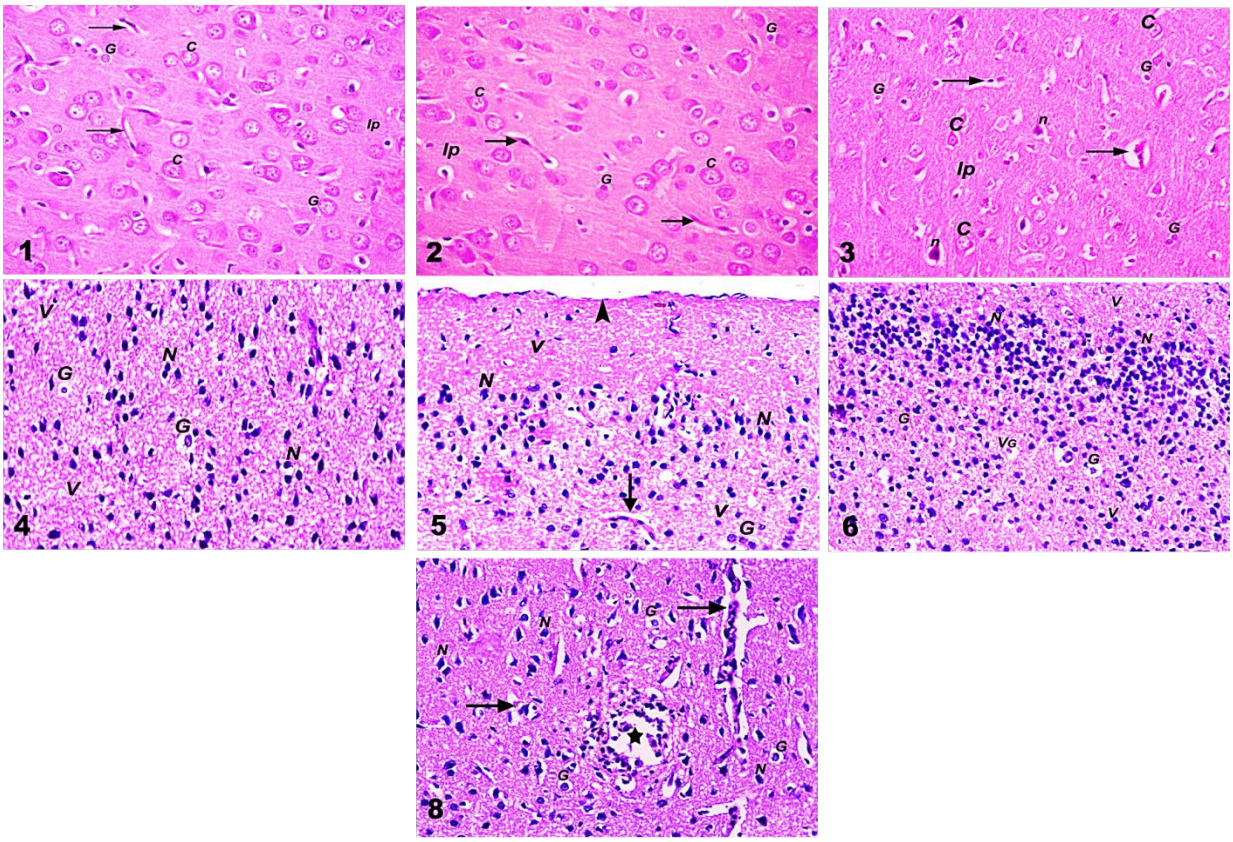


**Fig. 6** Photomicrographs of cerebral cortex sections of adult male mice in different groups: **(1) control group** cerebral cortex showing internal pyramidal (Ip). Pyramidal cortical neurons with rounded pale nuclei and basophilic cytoplasm (C). The neuropils exhibited different types of neuroglia (G) and blood vessels with narrow perivascular spaces (arrow). **(2) 0.5/67 group** showing Ip. Large pyramidal cortical neurons with rounded pale nuclei and basophilic cytoplasm (C). The neuropils exhibited different types of neuroglia (G) and blood vessels with normal perivascular spaces (arrow), compared with the controls. **(3) 1/67 group** showing Ip. Pyramidal cortical neurons with rounded pale nuclei and basophilic cytoplasm (C). The neuropils exhibited different types of neuroglia (G) and blood vessels with narrow perivascular spaces (arrow). A few neurons with darkly stained nuclei and wide pale spaces (n) were observed. **(4) 1.5/67 group** showing the inner portion of the cerebral cortex (internal granular and pyramidal layers). Neurons with darkly stained nuclei (N) surrounded by white spaces and vacuolated neuropil (V) can be observed. Glial cells or astrocytes with rounded nuclei and pale wide cytoplasm (G) can be observed. **(5) 0.5/133 group** showing the outer portion of the cerebral cortex (molecular, external granular, and external pyramidal layers). The dura mater appeared normal (arrowhead). A few blood vessels with narrow perivascular spaces (arrow) can be observed. The neurons with darkly stained nuclei (N) were surrounded by white spaces. Vacuolated neuropil (V) containing glial cells with pale nuclei (G) can be observed. **(6) 1/133 group** showing the inner portion of the cerebral cortex (internal granular and pyramidal layers). Most of the

neurons with darkly stained nuclei (N) were surrounded by white spaces and extensive vacuolated neuropil (V). Glial cells or astrocytes with pale rounded nuclei and pale wide cytoplasm (G) can be observed. **(7) 1.5/133 group** showing the inner portion of the cerebral cortex (internal granular and pyramidal layers). Extensive dilated blood vessels (arrow) with wide perivascular spaces can be observed. Nearly all the neurons with irregular darkly stained nuclei (N) were surrounded by white spaces. Glial cells were most probably astrocytes with pale nuclei, and cytoplasm (G) was easily identified. A degenerated area was observed with irregularly arranged nuclei (star). **H&E, ×400**

As observed in fig. 6, **1/67 group** presented the internal pyramidal, pyramidal cortical neurons with rounded pale nuclei and basophilic cytoplasm. The neuropils show different types of neuroglia and blood vessels with narrow perivascular spaces. Notice, few neurons with darkly stained nuclei and wide pale spaces. **1.5/67 group** illustrated the inner portion of cerebral cortex (internal granular and pyramidal layers). The neurons with darkly stained nuclei surrounded by white spaces and vacuolated neuropil are observed. Glial cells or astrocytes with rounded nuclei and pale wide cytoplasm are noticed. **0.5/133 group** showed the outer portion of cerebral cortex (molecular, external granular and external pyramidal layers). The dura matter appears normal. Few blood vessel with narrow perivascular space can be seen. The neurons with darkly stained nuclei surrounded by white spaces. Vacuolated neuropil with glial cells having pale nuclei (G) can be observed. **1/133 group** appeared the inner portion of cerebral cortex (internal granular and pyramidal layers). Most of the neurons with darkly stained nuclei surrounded by white spaces and extensive vacuolated neuropil are observed. Glial cells or astrocytes with pale rounded nuclei and also pale wide cytoplasm are noticed. **1.5 /133 group** showed the inner portion of cerebral cortex (internal granular and pyramidal layers). Extensive dilated blood vessels with wide perivascular spaces can be seen. Nearly all of the neurons with irregular, darkly stained nuclei surrounded by white spaces. Glial cells most probably astrocytes with pale nuclei and also cytoplasm are easily identified. A degenerated area with irregular arranged nuclei are noticed .

Signal transmission among neurons is the basis of learning and memory formation. Considering that an electric signal is a neuronal signal transduction pathway, an EF can drive a current in the conducting body and may impact cognitive function . This study investigated the effects of SEF exposure with different intensities of 1, 5, and 10 kV/m for 5 days (10 min/day) on the GABA level in the mice brain. It was observed that the GABA level insignificantly ($p > 0.001$) decreased during the low-intensity (1 kV/m) exposure to SEF. However, the mice exposed to the higher intensity (133 kV/m) experienced a significant increase ($p < 0.001$) in GABA

levels. Similarly, reported that a full-body exposure of mice to SEF at a certain intensity (2.30 kV/m) for 7, 21, and 35 days (24 h/day) did not cause significant changes in the glutamic acid and GABA levels in the hippocampus. However, exposure to SEF at a strong intensity (9.20 kV/m) for 21 and 35 days increased the GABA level.

GABA is the primary inhibitory neurotransmitter in the central nervous system . It can act on its receptor and regulate appropriate levels of inhibitory signals essential for the plasticity of synapses, which is the basis for learning and memory. Over the last several decades, GABA has attracted considerable attention because of its diverse physiological implications for plants, animals, and microorganisms .

**Conclusion and outlook**

Different histopathological changes were observed in the liver tissue and cerebral cortex of albino mice of the groups exposed to a SEF of 133 kV/m at 1.5 h/day for 21 days. The exposure of albino mice to SEFs with intensities of 67 and 133 kV/m significantly increased the long-term exposure, compared with that of short-term exposure. However, a significant increase was observed in the MDA content and the SOD, GSH, and LDH activities decreased with an increase in the SEF exposure time. In addition, there was a significant increase in the liver enzyme activities of total bilirubin, ALT, AST, and ALP in the serum and liver tissue; the brain homogenate of GABA decreased, and GLU increased.

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**Abbreviations:**

**SEF : Static Electric Field**
**EMF: Electromagnetic Field**
**HVDC : high-voltage, direct current**
**kV/m: kilovolt per meter**
**AC: Alternating Current**
**MDA: malondialdehyde**
**SOD: superoxide dismutase**
**GSH: glutathione**
**ROS: reactive oxygen species.**
**AST: Aspartate amino transferase**
**ALT: Alanine amino transferase**
**LDH: lactate dehydrogenase**
**ALP : Alkaline phosphatase**
**ALT : Alanine aminotransferase**

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