MJFAS
Malaysian Journal of Fundamental and Applied Sciences

Nurhidayat *et al.* | Malaysian Journal of Fundamental and Applied Sciences, Vol. 18 (2022) 703-713

RESEARCH ARTICLE

# Evaluation of Static Electric Field Exposure on Histopathological Structure and Function of Kidney and Liver in DMBA-Induced RAT (*Rattus norvegicus* Berkenhout, 1769)

**Luthfi Nurhidayat[a], Ibnu Fajar[b], Afif Yati[b], Helmi Hana Prinanda[b], M. Irfan[b], Dalila Afina[b], Ahmad G. Fadlurrahman[b], Nyoman Yudi Antara[c], Firman Alamsyah[d], Warsito P. Taruno[d], Rarastoeti Pratiwi[c,e,*]**

[a] Laboratorium of Animal Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281; [b] Undergraduate Program, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281; [c] Graduate School of Biotechnology, Universitas Gadjah Mada Yogyakarta, Indonesia, 55281; [d] Ctech Labs Edwar Technology, Tangerang, Banten, Indonesia, 15320; [e] Laboratorium of Biochemistry, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

Abstract Intermediate frequency and low intensity static electric field have been developed for non-invasive cancer therapy nowadays. The evaluation of the vital organs, one of which is kidney and liver, is necessary for pre-clinical safety assessment. This research aimed to evaluate the effect of the intermediate frequency (150 kHz) and low intensity (18 Vpp) static electric fields in kidney tissue of rats induced by DMBA (7,12-Dimethylbenz [α] anthracene). This study was carried out in breast tumor models using Sprague Dawley (SD) rats induced with 7,12-Dimethylbenz [α] anthracene (DMBA) by 20 mg/kg body weight of dose ten times over five weeks. Twenty-four rats were divided into four groups, namely: Non-induction Non-therapy (NINT), Non-Induction Therapy (NIT), Induction Non-Therapy (INT), and Induction Therapy (IT) groups. Static electric field therapy is carried out for 10 hours (resting 2 hours after 5 hours exposure) per day using the Electro-Capacitive Cancer Therapy (ECCT) individual enclosure for 21 days. The blood samples were collected before and after therapy for AST, ALT, and Creatinine measurement. The samples of liver and kidney were processed using Paraffin Method and Hematoxylin-Eosin Staining for histopathological observation. The histopathological score was determined using the ordinal method and post-examination masking. This study reveals that the 150 kHz and 18 Vpp static electric field therapy doesn't significantly induce histopathological injuries on the liver and the kidney. Furthermore, it also does not have a negative impact on the creatinine, AST, and ALT levels of blood plasma.

**Keywords**: Static electric field, liver, kidney, histopathology.

***For correspondence:**
rarastp@ugm.ac.id

**Received:** 6 Sept. 2022
**Accepted:** 24 Dec. 2022



## Introduction

Breast cancer becomes the most common cancer case along with lung cancer, and it dominates the cancer case in women in 2018 [1]. The alternative cancer therapies using electric field exposure have

been widely developed and show encouraging results on inhibiting the growth and spread of cancer cells [2]–[4] with the induction of cytotoxic T cells and has a potency in wound healing [5] Moreover, several studies showing that electric field exposure for cancer therapy may bring some negative effects on other organs, but it still a way less harmful compared to chemotherapy [6]–[8].

In Indonesia, a 100 kHz and 18 Vpp non-contact static electric field therapies have been developed and have a positive effect on inhibiting the growth of cancer cells [3], [9]. In this study, we increased the frequency of static electric field up to 150 kHz for the DMBA-induced breast tumors model of rats. The safe use of these higher frequencies needs to be studied on other organs to determine their side effects. Therefore, this study aimed to know the effect of the intermediate frequency (150 kHz) and low intensity (18 Vpp) static electric fields in kidney and liver tissue of rats induced by DMBA (7,12-Dimethylbenz [α] anthracene).

# Materials and Methods

## Ethics

This study was performed using an experimental procedure approved by the Ethical Clearance Committee of Integrated Laboratory for Research and Testing of Universitas Gadjah Mada with ethical clearance number: 00029/04/LPPT/2018

## Specimens

Twenty-four 5-weeks-old female Sprague Dawley rats (*Rattus norvegicus*, Berkenhout 1769) with 50-80 gram of weight were used in this study. The rats were acclimatized in polypropylene cages for one week with a controlled environment. The rats were divided into four groups based on a combination of DMBA induction treatment and static electric field therapy. They were NINT (Non-Induction Non-Therapy), NIT (Non-Induction Therapy), INT (Induction Non-Therapy), and IT (Induction Therapy).

## Tumor Induction

The INT and IT groups were orally administered with 20 mg/Kg BW of dose DMBA (7,12 dimethylbenz [a] anthracene) for breast tumor induction. The DMBA administration was done ten times for five weeks. Subsequently, tumor nodules development was observed by palpation until they reached 1 cm of diameter. The NINT and NIT groups were only given corn oil as a DMBA solvent.

## Static Electric Field Therapy

The NIT and IT groups were exposed to 150 kHz and 18 Vpp non-contact static electric fields for 10 hours per day for 21 days. The rats were left to rest for 2 hours after 5 hours of exposure. The static electric field therapy was started after the tumor nodules of IT groups reached 1 cm of diameter. The NINT and INT groups were only put into treatment cages without turning it on. The rats were fed with cucumber during the therapy period as a substitute for drinking water.

## Blood sample collection

The blood samples were collected before and after therapy. The rats were anesthetized by intramuscular injection of ketamine (70 mg/kg of body weight) to blood sample collection. The blood was drawn through the orbital sinus using microhematocrit and collected in a microtube that previously added with EDTA. The blood was then centrifuged at 5000 rpm for 10 minutes to separate plasma and blood cell components. The plasma was subsequently used for creatinine, Alanine Transaminase (AST) and Aspartate aminotransferase (ALT) analysis.

## Creatinine, AST, and ALT analysis

The measurement of creatinine, AST, and ALT were performed by spectrophotometry using kits provided by Diasys (Holzheim, Germany) complying with the manufacturer's protocols. The absorbance measurements were performed with a 492 nm of wavelength for creatinine measurement, and 340 nm of wavelength for both AST and ALT measurements. The data were analyzed in SPSS using ANOVA($\alpha$=0.05) and Tukey test ($p<0.05$).

## Histopathological studies of Kidney and Liver

The rats were euthanized using an overdosed intramuscular injection of ketamine (150 mg/kg of body weight). The left kidney and one of the liver lobes of all groups were collected by necropsy and transversally incised into 3-5 mm of thickness. Afterward, the samples were then fixed with *Neutral Buffered Formalin* (NBF) and processed using the paraffin method and Hematoxylin-Eosin staining

procedure adapted from Bancroft and Cook Protocols [10] with some adjustment.
Histopathological conditions of the kidney and liver were examined and captured under a Leica DM750 photomicrographic microscope. The histopathological scoring was done using post-examination masking combined with ordinal scoring method [11]. The scoring system of the kidney included glomerular injury, tubular injury, interstitial injury, and congestion (Table 1), while the scoring system of the liver comprised cellular injuries, hemorrhage, and congestion (Table 2). The results of histopathological scoring were statistically analyzed in SPSS using the Kruskal-Wallis test which then proceeds to the Mann-Whitney test (α=0.05).

**Table 1.** The histopathological scoring system of the kidney

| Category | Injury | Score |
|---|---|---|
| **Glomerular** | No injuries | 0 |
| | Thickening of Bowman capsule | 1 |
| | Retraction of the glomerular tuft | 2 |
| | Glomerular fibrosis or glomerular segmentation | 3 |
| **Interstitial** | No injuries | 0 |
| | Inflammation and/or hemorrhage | 1 |
| | Inflammation and/or hemorrhage with necrosis in tissue less than 25% | 2 |
| | Inflammation and/or hemorrhage with necrosis in the tissue between 25%- 60% | 3 |
| | Inflammation and/or hemorrhage with necrosis in tissue more than 60% | 4 |
| **Tubular** | No lesion | 0 |
| | reversible injuries | 1 |
| | reversible injuries with necrosis in tissue less than 25% | 2 |
| | reversible injuries with necrosis in the tissue between 25%- 60% | 3 |
| | reversible injuries with necrosis in tissue more than 60% | 4 |
| **Congestion** | No congestion | 0 |
| | Congestion occupy less than 12,5 % of the area | 1 |
| | Congestion occupy between 12,5% - 25% of area | 2 |
| | Congestion occupy between 26%-50% of the area | 3 |
| | Congestion occupy more than 50% of the area | 4 |

**Table 2.** The histopathological scoring system of the liver.

| Category | Injury | Score |
|---|---|---|
| **Cellular** | No cellular injuries | 0 |
| | reversible injuries with necrosis in tissue less than 15% | 1 |
| | reversible injuries with necrosis in the tissue between 15%-40% | 2 |
| | reversible injuries with necrosis in the tissue between 41%-70% | 3 |
| | reversible injuries with necrosis in tissue more than 70 | 4 |
| **Hemorrhage** | No hemorrhage | 0 |
| | Hemorrhage occupy less than 15% of the area | 1 |
| | Hemorrhage occupy between 15-40% of the area | 2 |
| | Hemorrhage occupy between 41%-70% of the area | 3 |
| | Hemorrhage occupy more than 70 of area | 4 |
| **Congestion** | Number of Congestion | |

# Results and Discussion

## Effect of 150 kHz And 18 Vpp Static Electric Field Exposure on Kidney

High-voltage electric field exposure may cause many cases of renal failure and myoglobinuria [12], [13]. However, the electric field exposure for cancer therapy, both static and dynamic, is adjusted to be low in intensity and medium in frequency [3], [14]. We specifically set the non-contact static electric field device to 150 kHz and 18 Vpp. We then examined the histopathological structure of the kidney to look for deleterious effects.

A histological section of the kidney (Figure 1) reveals that the thickening of Bowman's capsule is a common injury found in the glomerulus. This thickening can also occur naturally due to aging, or to ischemia [15]. In this study, the thickening of Bowman's capsule might have been induced by nephrotoxic activity of DMBA [16] and electric field exposure [7], [17]. The thickening may contribute to filtration

leakage so that the proteins can escape to the filtrate [18]. Moreover, the filtration leakage may lead to proteinuria if the excreted protein reaches ≥ 3.5 g/d [19]. Nevertheless, the mean values of the glomerular injury score (Figure 2) were not significantly different among the treatments. Accordingly, neither DMBA induction nor electric field exposure are responsible for the glomerular injuries reported in this study.

Interstitial injury, similar to glomerular injury, is not significantly induced by DMBA induction or static electric field exposure, based on the mean value of interstitial injury score (Figure 2). However, some injuries can be observed in the interstitial tissue. These include inflammation, hemorrhage, and necrosis (Figure 1). Inflammation is characterized by interstitial cell swelling caused by leukocyte infiltration of antibody aggregates [20]. By contrast, the existence of blood cells can identify hemorrhage in a tissue or outside blood vessels [21].

The static electric field exposure and DMBA induction seem to have different mechanisms of tubular injury. The proximal part of tubular ren is sensitive to toxin exposure and to hypoxemia/ischemia [19]. Ischemia is capable of causing changes in cell polarity, which result in vasoconstriction of afferent arterioles , decreased glomerular filtration rate, and epithelial cell damage [22]. Hence, tubular injury will always develop due to the high rate of reabsorption by renal tubules even in non-treated rats; exposure of the rat to toxic substances amplify the injury. The tubular injuries of the kidney are dominated by cloudy swelling in the case of reversible injury; and pyknosis in irreversible injuries (Figure 1). Cloudy swelling is a reversible injury caused by an imbalance of ions in the cell, dilation of the endoplasmic reticulum or Golgi body, or influx of excess sodium and water [23]. If the reversible injury persists and continues to irreversible injury, it will continue to necrosis or cell death [19].

Based on the histopathological score of tubular injury (Figure 2), the DMBA induction harms renal tubules wherein the INT group has the highest damage score and is significantly different to the IT group. This result demonstrates the nephrotoxic effect of DMBA to the renal tubules. DMBA interacts with genetic material, such as DNA, and damages it, thus triggering necrosis or pyknosis[24]–[26].

On the other hand, static electric field exposure gives rise to decreasing tubular damage, as shown by the significantly lowest tubular injury score of NIT treatment compared to the other treatments (Figure 2). The static electric field therapy possibly provides compensatory tissue repair of the damage caused by DMBA. It may even give a better outcome for a control rat. The static electric field therapy can improve kidney function by increasing circulation and suppressing sympathetic reactions if applied using high tone electrical muscle stimulation (HTEMS) in acute kidney damage or acute kidney injury (AKI) [27]. Moreover, the electric field exposure can improve perfusion to the tissues and accelerate wound healing [28], and this will decrease the ischemic condition that can induce cell swelling and necrosis [29], [30].

The histopathological score of congestion demonstrates an almost similar condition with tubular injury (Figure 2). The DMBA induction is significantly upsurged the congestion while the electric field exposure potentially reduces the congestion for the NIT group. Several studies using microcurrent electricity [31] and NMES (neuromuscular electrical stimulation) electricity [32] reveals that electric therapy is capable of increasing blood circulation in the limbs. However, when it comes to the combination of DMBA induction and static electric field therapy, the congestion becomes much more severe (synergic effect). The respective roles of DMBA induction and static electric field therapy in promoting congestion cannot be ascertained because the congestion score of the NIT group and INT group are not significantly different. However, the sure things are DMBA may damage the endothelium [33], and heating due to the electric field may cause congestion [34].

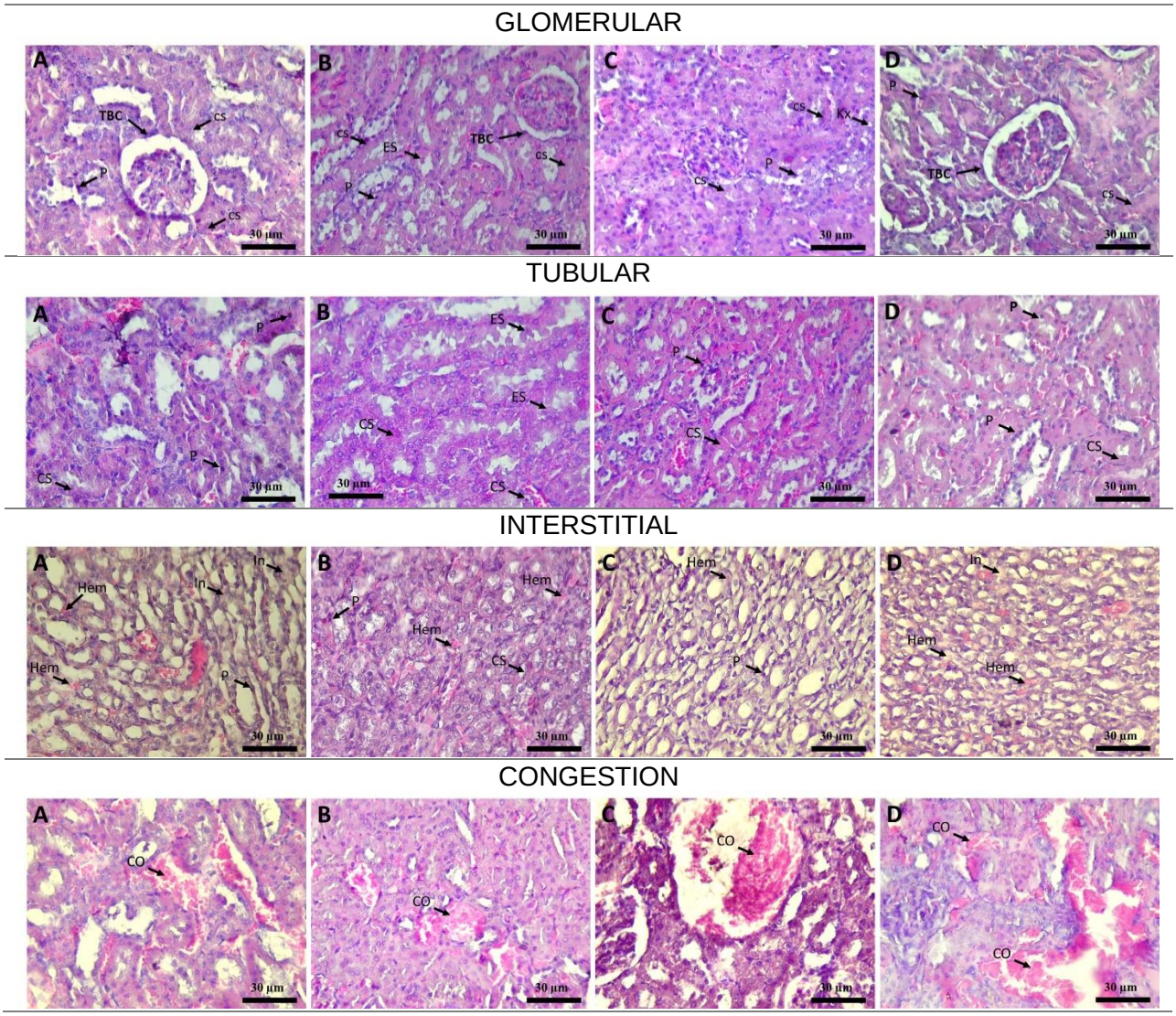


**Figure 1.** Histopathological images of rat kindey sections. A. NINT (Non-Induction Non-Therapy); B, NIT (Non-Induction Therapy); C, INT (Induction Non-Therapy); D, IT (Induction Therapy). *TBC, thickening of Bowman's capsule; CS, cloudy swelling; P, pyknosis; Hem, hemorrhage; Kx, karyorrhexis; ES, epithelial sloughing; P, pycnosis; In, inflammation; Co, congestion.*

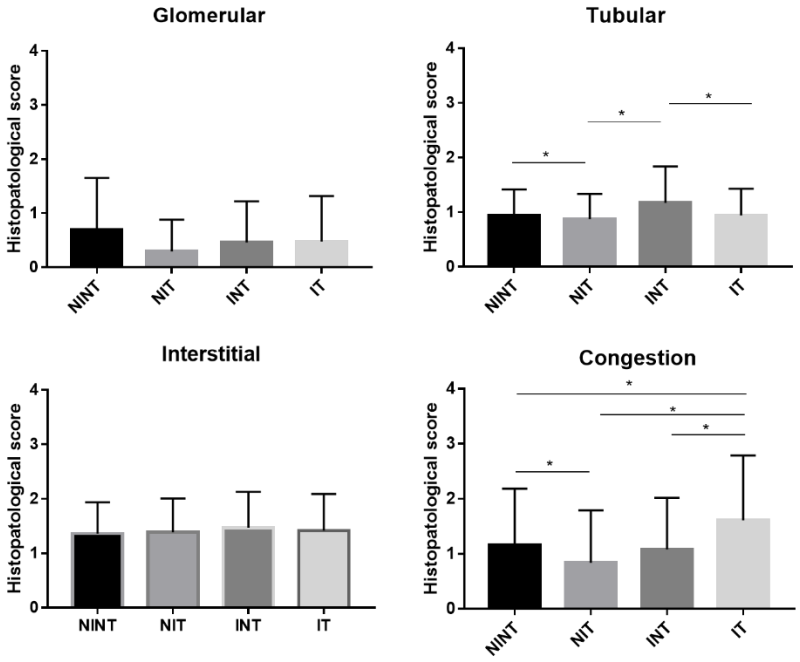


**Figure 2.** Histopathological score of rat kidney. NINT, Non-Induction Non Therapy; NIT, Non-Induction Therapy; INT, Induction Non-Therapy; and IT, Induction Therapy. *Statistically significant differences between treatment groups on each row/category (Mann-Whitney test; α=0.05).

We investigated creatinine levels for each treatment group at pre and post-treatment, as the results can be seen in Figure 3. We also measured creatinine levels in non-treatment rats, the baseline group, of which blood was collected with other groups. Creatinine is formed through the process of creatine and phosphocreatine breakdown derived from muscle tissue and brain [35]. Moreover, the creatinine levels can rise due to impaired kidney function [36]. Hence, the creatinine levels will be able to depict the kidney function after being treated with DMBA induction or/and static electric field exposure.

The mean value of the creatinine level in the baseline group did not increase significantly, of which pre-treatment and post-treatment results are 0.4 mg / dL and 0.45 mg / dL, respectively. All treatment groups, except the IT group, exhibit a significant increase in the creatinine level from pre-treatment to post-treatment. The mean value of creatinine levels of NINT, NIT, and INT creatinine levels ranged from 0.32 to 0.34 mg / dL at pretreatment and increased to the range of 0.51 to 0.59 mg/dl at post-treatment. The increase of creatinine levels can be caused by impaired function of the nephron in the kidneys, especially in the glomerulus. Consequently, it will lead to blood filtration interference resulting in the lessening of metabolic filtration in the blood, one of which is creatinine. The damage is allegedly due to ROS escalation in blood plasma. The ROS can harm cells in the glomerulus by altering the membrane structure, cytoplasm and nucleus biochemical compounds namely lipids, proteins, and nucleic acids [6]. For the NINT group, the increase of creatinine levels can be caused by muscle activity due to stress conditions that may appear on treatment since the variation of muscle mass and activity can affect the creatinine levels [37].

In contrary to the other groups, the IT group experienced a significant decrease in the mean value of creatinine level from 0.55 mg / dL at pre-treatment and 0.34 mg / dL at post-treatment. It is possibly caused by the continuous effects of DMBA induction with the different time intervals related to nodule growth as a starting point for therapy. This corresponds to the fact that DMBA metabolism increases ROS levels in the body through the enzymes CYP1A1, 1A2, and 1B1 [38].

However, based on comparisons between treatment groups, the mean value of creatinine levels in each group do not demonstrate significant differences. Moreover, when referring to the normal range of rats creatinine levels [37], the increase of creatinine levels in all treatment groups is still within the normal range. Therefore, it can be said that static electric field exposure does not have an adverse effect on kidney function.

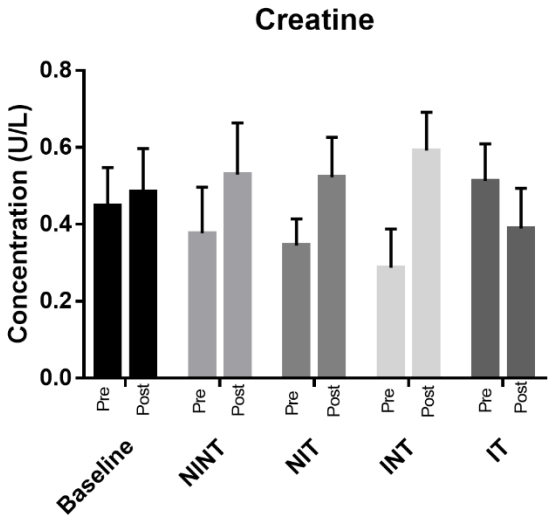


**Figure 3.** Level of creatinine in blood plasma before (pre) and after (post) exposure to non-contact electric fields. NINT: Non-Induction Non-Therapy); NIT: Non-Induction Therapy, INT: Induction Non-Therapy, and IT: Induction Therapy. Normal range of plasma creatinine concentration on rat is 0.3–1.0 mg/dL [37]. *Significant differences between treatment groups on each row/category (ANOVA; $\alpha$=0.05 and Tukey test; $p<0.05$).

## Effect of 150 kHz and 18 Vpp Static Electric Field Exposure on Liver

Histopathological image (Figure 4) and score (Figure 5) of the liver illustrate the insignificant difference of 150 kHz and 18 Vpp static electric field and DMBA induction effect on the cellular injury of rat liver. All groups share the close mean value of cellular injury score among them. The NIT group demonstrates the highest necrosis although the degree of necrosis is not significantly different compared to the other groups. This has possibly happened since a low-frequency electric field therapy (50 Hz) for ten weeks is able to cause abnormalities in rat liver function due to increased oxidative stress [39]. The same condition is also shown by a 900 MHz electromagnetic field exposure and even several diseases in humans such as fatigue, headaches, decreased learning ability, and cognitive impairment, can be triggered by this electromagnetic field exposure [40]. Nevertheless, the cellular injury caused by the static electric field is still in a reasonable range since it is not significant from the NINT group.

The DMBA induction appears to have a significant role in inducing hemorrhage on rat's liver. Endothelial cells of blood vessels are very sensitive to oxidative stress caused by DMBA induction [16]. This condition may lead to blood extravasation, or commonly called hemorrhage, due to blood vessels injury [19]. This condition also may contribute to the occurrence of congestion, although the DMBA induction had the lowest congestion score compared to the other groups in this study.

The congestion score of the liver exemplifies the synergic effect of static electric field exposure together with DMBA induction on escalating the congestion. The IT group shows the significantly highest congestion score among all groups. The congestion is induced by blood vessels vasoconstriction, reduced blood fluidity, increased blood concentrations and viscosity. As mentioned before, DMBA induction provides oxidative stress that possibly induces vascular injury. Moreover, Mendel *et al*. (2013) state that low-frequency (1-10 Hz) medium voltage (150V) electric field exposure for three hours can give rise to blood vasoconstriction which will lead to congestion and if it is getting worse, hemorrhage [41]. The DMBA induction, accompanied by static electric field exposure, will have a cumulative effect on making the congestion more severe.

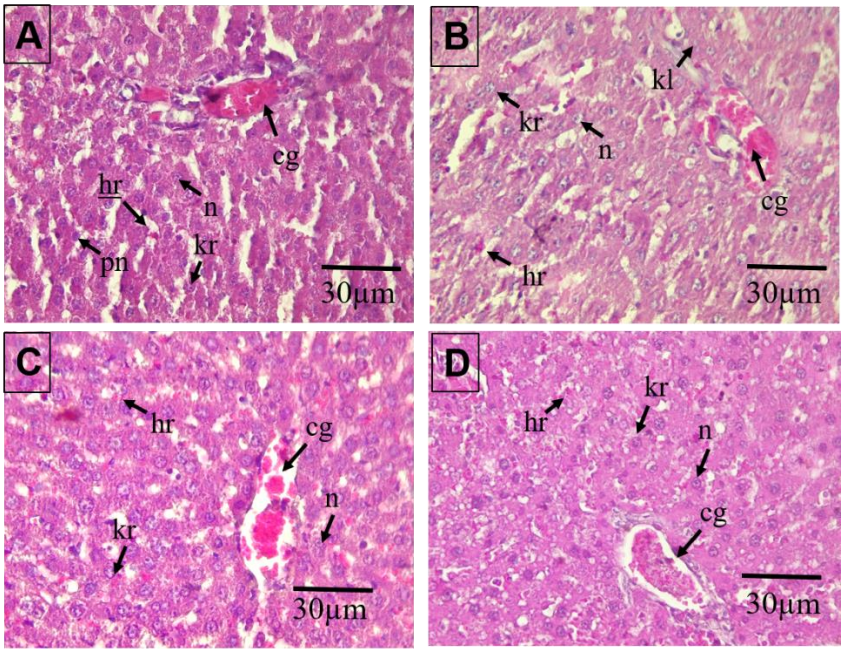


**Figure 4.** Rat liver histological sections. A, NINT (Non-Induction Non-Therapy); B, NIT (Non-Induction Therapy); C, INT (Induction Non-Therapy); D, IT (Induction Therapy). *N, normal*; c*s, congestion; kr, karyorrhexis; hr, hemorrhage; pn, pyknosis; kl, karyolysis.*

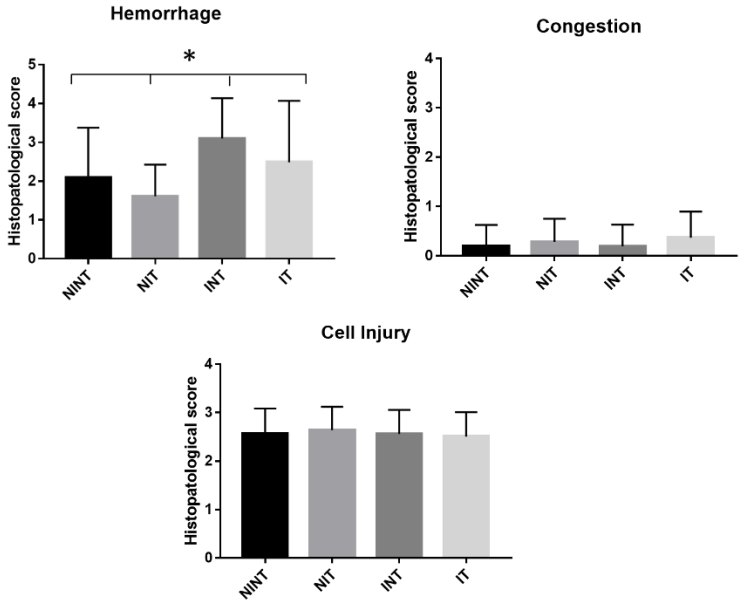


**Figure 5.** The histopathological score of rat liver. NINT: Non-Induction Non-Therapy, NIT: Non-Induction Therapy, INT: Induction Non-Therapy, and IT: Induction Therapy. *Significant differences between treatment groups on each row/category (Mann-Whitney test; α=0.05).

We also investigate the level of Aspartate aminotransferase (AST) and Alanine Transaminase (ALT) on blood (Figure 6) for confirming the histopathological result. AST is an enzyme that catalyzes aspartate transamination into alpha ketoglutarate to produce oxaloacetate and glutamate, while ALT is an enzyme that transfers amino groups from alanine to α-ketoacid, such as α-keto glutaric acid, then subsequently form pyruvate or other types of amino acids, usually glutamate [42], [43]. Furthermore, both enzymes can be used as an indicator for a clinical diagnosis of liver diseases such as hepatitis, jaundice, myocardial infarction, and even cancer when its activity increases sharply in the bloodstream [44]. AST is not only found in the liver, but also heart, brain, and skeletal muscle tissues, even the increase of AST

without ALT increasing indicate heart or muscle disease [45]. Hence, the AST analysis is usually carried out together with ALT analysis, which is more concentrated in the liver.

The results of measurement of AST levels showed that there was a significant change in AST levels between pre-treatment and post-treatment, both of which had increased or decreased. Nevertheless, The value still in the normal range or close to the range of normal values, which is 45-154 U / L [43]. ALT levels between pre-treatment and post-treatment also showed the same condition, but the post-treatment ALT levels of all treatment groups exceed the normal range. The exceeded level of ALT might be related to the acute stress condition that occurred due to the treatment. The acute stress can affect the liver function [46], and since the ALT is more concentrated in the liver so that it will show a greater fluctuation compared to AST.

AST levels in the baseline group were not significantly different from the NIT group and the IT group. The INT post group and the IT post group have significantly different AST levels indicating that there was a significant effect of treatment on rat blood plasma AST activity. It seems that there is a tendency in the IT group to decrease AST levels from a slightly higher value back into the normal range. This normalization also applies to ALT levels in the IT group, which demonstrate a decrease of mean value after static electric field exposure on DMBA induced rats. The lower water, sodium, and potassium content in cancer cells cause lower electrical resistance in cancer cells [47]. This lower electrical resistance allows the static electric field to inhibit the formation of the spindle in cell division. Since the spindle compiler protein subunits are charged compounds [48] so that the combination of static electric fields with low cell electrical resistance can minimalize cell division of cancer cells resulting in decreasing both AST and ALT levels in the blood. Thus, it can be said that exposure to static electricity in research plays a role in decreasing the levels of AST and ALT in the blood of DMBA induced rats.

Meanwhile, the DMBA induction significantly increases both AST and ALT levels in rat blood. These increases can be seen in the baseline group, which is significantly different from the INT group. The DMBA works by releasing ROS (Reactive Oxygen Species) compounds. The presence of ROS in cells may harm cell health and may lead to cell necrosis and release AST content into blood plasma [49].

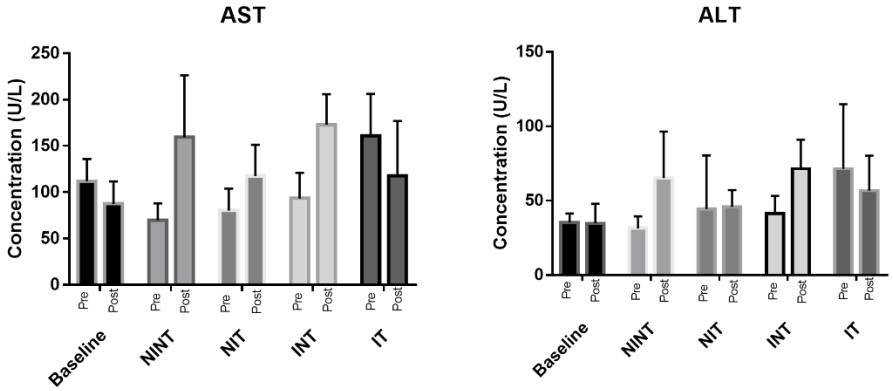


**Figure 6.** Level of AST and ALT in blood plasma before (pre) and after (post) exposure to static electric fields. NINT: Non-Induction Non-Therapy, NIT: Non-Induction Therapy, INT: Induction Non-Therapy, and IT: Induction Therapy. The normal range of plasma AST activity in rats is 45-154 U/L (according to [43]), while ALT activity is 5-40 U/L (according to [44]). *Significant differences between treatment groups on each row/category (ANOVA; α=0.05 and Tukey test; p<0.05).

## Conclusions

The150 kHz and 18 Vpp static electric field therapy do not significantly induce histopathological injuries on the liver and the kidney. Furthermore, it also does not harm the creatinine, AST, and ALT levels of blood plasma.

## Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

## Acknowledgment

This research was a part of the Preclinical Test of Electro-Capacitive Cancer Therapy (ECCT) in Breast Tumor Model Rats funded by the Ministry of Research, Technology and Higher Education Indonesia through Hibah Pengembangan Teknologi Industri (The Industrial Technology Development Program Grant) in 2018. We would like to thank Prof. Michael K. Richardson for proofreading this manuscript prior submission.

## References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R. Siegel, L. Torre, and A. Jemal. (2018). Global Cancer Statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.

[2] E. D. Kirson *et al.* (2004). Disruption of cancer cell replication by alternating electric fields. *Cancer Res.*, *64*(9), 3288-3295. Doi: 10.1158/0008-5472.CAN-04-0083.

[3] F. Alamsyah, I. N. Ajrina, F. Nur, A. Dewi, S. A. Prabandari, and W. P. Taruno. (2015). Antiproliferative effect of electric fields on breast tumor cells in vitro and in vivo, *6*(3), 71-77.

[4] M. Giladi *et al.* (2015). Mitotic spindle disruption by alternating electric fields leads to improper chromosome segregation and mitotic catastrophe in cancer cells. *Sci. Rep.*, *5*, 1-16. Doi: 10.1038/srep18046.

[5] F. Alamsyah *et al.* (2021). Cytotoxic T cells response with decreased CD4/CD8 ratio during mammary tumors inhibition in rats induced by non-contact electric fields. *F1000Research*, *10*, 35. Doi: 10.12688/f1000research.27952.1.

[6] S. E. Abo-Neima, H. A. Motaweh, H. M. Tourk, and M. F. Ragab. (2016). Effects of extremely low frequency electromagnetic fields on kidney functions of albino rats in vivo study. *Am. J. Biomed. Sci.*, 247-258. Doi: 10.5099/aj160400247.

[7] L. I. Khayyat. (2011). The histopathological effects of an electromagnetic field on the kidney and testis of mice.*EurAsian J. Biosci.*, *109*, 103-109. Doi: 10.5053/ejobios.2011.5.0.12.

[8] A. K. Petri *et al.* (2017). Biological effects of exposure to static electric fields in humans and vertebrates: A systematic review. *Environ. Heal. A Glob. Access Sci. Source*, *16*(1), 1-23. Doi: 10.1186/s12940-017-0248-y.

[9] R. Pratiwi *et al.* (2020). CCL2 and IL18 expressions may associate with the anti-proliferative effect of noncontact electro capacitive cancer therapy in vivo. *F1000Research*, *8*, 1770. Doi: 10.12688/f1000research.20727.2.

[10] J. D. Bancroft and H. C. Cook. (1984). *Manual of Histological Techniques*. Singapore: Longman Singapore Publisher,

[11] K. N. Gibson-Corley, A. K. Olivier, and D. K. Meyerholz. (2013). Principles for Valid Histopathologic Scoring in Research, *50*(6), 1007-1015. Doi: 10.1177/0300985813485099.

[12] H. Gouda and B. Bastia. (2010). Acute renal failure following electrocution. *Indian J. Med. Sci.*, *64*(1), 45. Doi: 10.4103/0019-5359.92488.

[13] J. Payne-James, A. Busuttil, and W. Smock. (2003). *Forensic medicine: clinical and pathological aspects*. Cambridge University Press.

[14] E. D. Kirson *et al.* (2009). Alternating electric fields (TTFields) inhibit metastatic spread of solid tumors to the lungs. *Clin. Exp. Metastasis*, *26*(7), 633-640. Doi: 10.1007/s10585-009-9262-y.

[15] R. van Schilfgaarde, J. C. Stanley, P. van Brummelen, and E. H. Overbosch. (2012). *Clinical Aspects of Renovascular Hypertension*, *4*. Springer Science & Business Media.

[16] A. I. Dakrory, S. R. Fahmy, A. M. Soliman, A. S. Mohamed, and S. A. M. Amer. (2015). Protective and curative effects of the sea cucumber holothuria atra extract against DMBA-induced hepatorenal diseases in rats.

[17] S. Zare, S. Alivandi, and A. Ebadi. (2007). Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in guinea pig. *World Applied Sciences Journal*, *2*(5), 509–511.

[18] R. V Shippee-Rice, S. Fetzer, J. V Long, and A. Armitage. (2011). *Gerioperative nursing care: principles and practices of surgical care for the older adult*. Springer Publishing Company.

[19] V. Kumar, A. K. Abbas, N. Fausto, and R. Mitchell. (2007). *Robbins and cotran pathologic basis of disease*, 7th ed. Amsterdam: Elsevier Health Sciences.

[20] J. C. Jennette, V. D. D'Agati, J. L. Olson, and F. G. Silva. (2014). *Heptinstall's Pathology of the Kidney*. Lippincott Williams & Wilkins.

[21] E. Cote. (2010). *Clinical veterinary advisor-e-book: an excerpt from clinical veterinary advisor 2e-Reprint*. Amstermdam: Elsevier Health Sciences.

[22] B. A. Molitoris and M. C. Wagner. (1996). Surface membrane polarity of proximal tubular cells : Alterations as a basis for malfunction. *Kidney Int.*, *49*(6), 1592-1597. Doi: 10.1038/ki.1996.231.

[23] W. Haschek, C. Rousseaux, and M. Walling. (2010). *Fundamentals of toxicologic : pathology*. California: Academic Press Elsevier.

[24] B. Y. E. Ford and C. Huggins. (1963). From the Ben May Laboratory for Cancer Research, The University

of Chicago, Chicago, Hydrocarbon-Induced Damage of Testis . --Tuchmann and Demay (4) injected. *5*.
[25] R. C. Sills, G. A. Boorman, J. E. Neal, H. L. Hong, and T. R. Devereux. (1999). Mutations in ras genes in experimental tumours of rodents. *IARC Sci. Publ.*, *146*, 55-86.
[26] Ö. Lindhe, L. Granberg, and I. Brandt. (2002). Target cells for cytochrome P450-catalysed irreversible binding of 7,12-dimethylbenz[a]anthracene (DMBA) in rodent adrenal glands. *Arch. Toxicol.*, *76*(8), 460-466. Doi: 10.1007/s00204-002-0367-1.
[27] B. Di Iorio, S. Torraca, P. Gustaferro, G. Fazeli, and A. Heidland. (2012). High-frequency external muscle stimulation in acute kidney injury (AKI): potential shortening of its clinical course. *Clin. Nephrol.*, *79*, 37-45. Doi: 10.5414/CNX77S101.
[28] G. Thakral, J. Lafontaine, B. Najafi, T. K. Talal, P. Kim, and L. A. Lavery. (2013). Wound healing. *Diabet Foot Ankle*, *1*, 1-9. Doi: 10.3402/dfa.v4i0.22081.
[29] M. Brezis, S. Rosen, P. Silva, and F. H. Epstein. (1984). Renal ischemia: A new perspective. *Kidney Int.*, *26*(4), 375-383. Doi: 10.1038/ki.1984.185.
[30] B. Trump and S. Chang. (2001). Apoptosis, Oncosis, and Necrosis. *Cardiovasc, 146*(1), 3-15.
[31] R. J. Park, H. Son, K. Kim, S. Kim, and T. Oh. (2011). The effect of microcurrent electrical stimulation on the foot blood circulation and pain of diabetic neuropathy. *J. Phys. Ther. Sci.*, 23(3), 515-518. Doi: 10.1589/jpts.23.515.
[32] M. C. Moloney, G. M. Lyons, P. Breen, P. E. Burke, and P. A. Grace. (2006). Haemodynamic study examining the response of venous blood flow to electrical stimulation of the gastrocnemius muscle in patients with chronic venous disease. *Eur. J. Vasc. Endovasc. Surg.*, *31*(3), 300-305. Doi: 10.1016/j.ejvs.2005.08.003.
[33] T. M. Murad, J. Leibach, and E. Von Haam. (1973). Latent effect of DMBA on adrenal glands of Sprague-Dawley rats: an ultrastructural study. *Exp. Mol. Pathol.*, *18*(3), 305-15. http://www.ncbi.nlm.nih.gov/pubmed/4708311.
[34] C. L. Dai *et al.* (2001). Heat shock protein 72 normothermic ischemia, and the impact of congested portal blood reperfusion on rat liver. *World J. Gastroenterol.*, *7*(3), 415. Doi: 10.3748/wjg.v7.i3.415.
[35] R. Wyss, M.; Kaddurah-Daouk. (2000). Creatine and creatinine metabolism. *Physiol. Rev.*, *80*(3), 1107-1213.
[36] P. Sokal *et al.* (2013). Differences in blood urea and creatinine concentrations in earthed and unearthed subjects during cycling exercise and recovery. *Evidence-Based Complement. Altern. Med.* Doi: 10.1155/2013/382643.
[37] S. L. Delwatta *et al.* (2018). Reference values for selected hematological, biochemical and physiological parameters of Sprague-Dawley rats at the Animal House, Faculty of Medicine, University of Colombo, Sri Lanka. *Anim. Model. Exp. Med.*, *1*(4), 250-254. Doi: 10.1002/ame2.12041.
[38] R. V. Priyadarsini and S. Nagini. (2012). Quercetin suppresses cytochrome P450 mediated ROS generation and NFκB activation to inhibit the development of 7,12-dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinomas. *Free Radic. Res.*, *46*(1), 41-49. Doi: 10.3109/10715762.2011.637204.
[39] B. L. Wei, L. J. Bi, J. Z. Z. Qu, and C. Lin. (2015). Effect of long-term pulsed electromagnetic field exposure on hepatic and immunologic functions of rats, 959-962. Doi: 10.1007/s00508-015-0732-8.
[40] E. G. Kıvrak, K. K. Yurt, A. A. Kaplan, and G. Altun. (2019). Effects of electromagnetic fields exposure on the antioxidant defense system. *5*, 167-176. Doi: 10.1016/j.jmau.2017.07.003.
[41] Y. Mandel, R. Dalal, P. Huie, U. States, and J. Wang. (2013). Vasoconstriction by electrical stimulation : new approach to control. Doi: 10.1038/srep02111.
[42] C. C. Leung and K. K. Young. (2018). Clinical aspects of hepatic disease. *Anaesth. Intensive Care Med.*, *19*(1), 4-6. Doi: 10.1016/j.mpaic.2017.10.009.
[43] C. Petterino and A. Argentino-Storino. (2006). Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. *Exp. Toxicol. Pathol.*, *57*(3), 213-219. Doi: 10.1016/j.etp.2005.10.002.
[44] M. Jamal, O. Worsfold, T. McCormac, and E. Dempsey. (2009). A stable and selective electrochemical biosensor for the liver enzyme alanine aminotransferase (ALT). *Biosens. Bioelectron.*, *24*(9), 2926-2930. Doi: 10.1016/j.bios.2009.02.032.
[45] P. Y. Kwo, S. M. Cohen, and J. K. Lim. (2017). ACG clinical guideline: evaluation of abnormal liver chemistries. *Am. J. Gastroenterol.*, *112*(1), 18-35. Doi: 10.1038/ajg.2016.517.
[46] X. Gao, Y. Zeng, S. Liu, and S. Wang. (2013). Acute stress show great influences on liver function and the expression of hepatic genes associated with lipid metabolism in rats. *Lipids Health Dis.*, *12*, 118. Doi: 10.1186/1476-511X-12-118.
[47] B. Jansson. (2019). Potassium, sodium, and cancer: a review. *J. Environ. Pathol. Toxicol. Oncol.*, *15*(2-4), 65-73. http://europepmc.org/abstract/med/9216787.
[48] M. Barisic and H. Maiato. (2016). The mitotic spindle. *Encyclopedia of Cell Biology*, 637-648.
[49] C. Cebra, R. J. Van Saun, D. E. Anderson, A. Tibary, and L. R. W. Johnson. (2013). *Llama and Alpaca Care: Medicine, Surgery, Reproduction, Nutrition, and Herd Health: First Edition*.