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POSSIBLE THERAPEUTIC EFFECTS OF THE EXTREMELY LOW-FREQUENCY ELECTROMAGNETIC FIELD AND BROCCOLI EXTRACT IN THE TREATMENT OF PHZ-INDUCED COLON CANCER IN RATS

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ABSTRACT:

Colon cancer is the second-most dangerous type of cancer, affecting both men and women worldwide. Nowadays, scientists aim to increase the effectiveness of existing cancer treatments. The application of electromagnetic magnetic fields (EMF) in the treatment of different kinds of cancer whether alone or in combination with other treatments seems to be a promising therapy that has drawn attention during the last decades. Therefore, the current study aimed to investigate the potential beneficial effects of extremely low-frequency electromagnetic field (ELF-EMF) application alone and in combination with broccoli extract in the treatment of colon cancer in an animal model . 25 male Albino rats (*Rattus rattus*) were randomly divided into five groups:first group was control group, the second group was exposed to ELF-EMF (50 Hz, 0.9 mT) daily for 75 mins for 4 weeks. the third group was injected intraperitoneally with phenyl hydrazine(PHZ) (90mg/Kg) only twice a week for 4 weeks, the fourth group was injected intraperitoneally with PHZ (90mg/Kg) only twice a week for 4 weeks, then exposed to ELF-EMF daily for 75 mins for another 4 weeks. And the last group was injected intraperitoneally with PHZ (90mg/Kg) only twice a week for 4 weeks then exposed to ELF-EMF daily for 75 mins and was given broccoli extract orally (300 mg/kg) twice/week regulatory for 4 weeks. The results revealed that PHZ injection was able to induce carcinogenicity in the intestinal tissues of rats. While the treatment by ELF-EMF application mitigated the effects of PHZ and succeeded in suppressing tumor progression, especially when combined with broccoli extract.

INTRODUCTION:

Cancer constitutes one of the most serious causes of death worldwide. It accounted for 7.6 million deaths (around 13% of all deaths) in 2008 (WHO, 2011). Colorectal (CRC), in particular, is the 4th commonest cancer in the world (Metwally *et al.*, 2018). Colon cancer is the second-most dangerous type of cancer, affecting both men and women (Kuppusamy *et al.*, 2014). Certain types of cancer, due to their complexity, require a combination of treatments which eventually aim to increase the effectiveness of the existing treatments, eliminate the side effects and to improve as much as possible the quality of life of a patient. Several studies revealed that Electromagnetic fields (EMFs) have a vital role as adjunctive therapy or even a primary role in certain forms of cancer (Cameron *et al.*, 2007; Tatarov *et al.*, 2011).

In addition, recently, the usage of cruciferous vegetables has drawn a great deal of attention in cancer research because of their antioxidant and potential protective properties (Kim & Park, 2009). So, the current study aimed to apply a combination of EMFs and broccoli extract for the treatment of colon cancer in male rats.

MATERIAL AND METHODS

CHEMICALS:

- Phenyl hydrazine (Sigma Chemical Co.).

-All other chemicals from sigma-Aldrich-USA.

ANIMALS:

The study was conducted on 25 male Albino rats (Rattus rattus) about 8-12 weeks old with average weight ranged between 120-160 gm. Animals were obtained from the animal house in the Faculty of Science, Assuit University, Egypt. They were housed in stainless cages at room temperature, five rats each. They were fed ad libitum a commercial pelleted ration, and drunk tap water. Rats were also subjected to a photoperiod of 12 hrs light/day.

ELECTROMAGNETIC FIELD APPARATUS:

A cycle composed of a direct current (DC) and alternating current (AC) magnetic fields, which can increase the mobility of specific ions near receptor sites and/or through ion channels according to Liboff (1985). The AC was 26.6 mv in the bottom, 44 mv in the center beside the wall box, the intensity was .9 mT, the distance between the two wires was 11.5 cm and frequency was 50Hz. The DC was 450 m amb. and 0.75 mT in the center.

BROCCOLI:

500g of broccoli was washed with distilled water, mixed with electric mixer using 50 ml of bi-distilled water, and then the mixture was separated with a white, clean, sterilized transparent cloth. After separation , the solid part was withdrawal and the aqueous part was taken and used in LD50 of 300 mg/kg according to (Adly. 2013)

EXPERIMENTAL DESIGN:

After two weeks of acclimation. rats were randomly allocated into five groups, as follows:

1. **Group1(G1) (n=5): Served as a negative control group only provided with water and feeding for 4 weeks.**
2. **Group2 (G2) (n=5):** Rats were **subjected to ELF-EMF for 75 mins daily for 4 weeks.**
3. **Group3 (G3) (n=5):** Rats were **injected intraperitoneally with PHZ (90mg/kg) twice a week regularly for 4 weeks according to Berger & Josef et al. (2007).**
4. **Group4 (G4) (n=5):** Rats were **injected intraperitoneally with PHZ (90mg/kg) twice a week regularly for 4 weeks according to Berger & Josef et al. (2007), and then exposed to ELF-EMF (50 Hz,.9 T) daily for 75 mins.**
5. **Group5 (G5) (n=5):** Rats were **injected intraperitoneally with PHZ (90mg/kg) twice a week regularly for 4 weeks according to Berger & Josef et al. (2007), and then exposed to ELF-EMF (50 Hz,.9 T) daily fo75 mins. In addition, they were given broccoli extract (300mg/kg) by oral gavage twice a week regularly for 4 weeks.**

Collection of samples:

Large intestines were quickly removed and washed in physiological saline (0.9%Nacl) solution, blotted on filter paper,

and fixed in 10% neutral buffered formalin and routinely processed. After fixation, intestines were washed and dehydrated in ascending series of absolute alcohol (50%,70%,90%,100% and incubated for 20 mins in toluene. The samples were then embedded three times in paraffin for 6 hrs/each at 60°C. Paraffin blocks were cut at 5 Mm thick rated on glass slides by a microtome. Then sections of intestinal tissues from different groups were mounted on slides and dried overnight at 37°C. Sections were deparaffinized by xylene, followed by hydration through descending ethanol series(100%, 90%, 70% and 50%), and stained with hematoxylin (H), washed by water, then stained with eosin (E), followed by dehydration through ascending ethanol series (50%,70%,90% and100%) then clearing in xylene and mounting DPX (Axio Lab. Al ,Carl ZEISS ,Germany) (Drury & Wallington, 1980). Finally, the slides were examined under a light microscope (Axio Lab. Al., Carl ZEISS, Germany).

RESULTS:

Clinical signs show dark blue colour in outer hair appearance of perioral region of PHZ- injected rats group (Fig .1B) compared with control and treated groups (Fig .1A). There was clear abnormal gross anatomy in PHZ- injected rats (G3) which exhibited by swelling in some area of large intestine and revealed alarge amount of light yellowish ascitit liquid in the abdomen, kidnies were darker than control animals and abnormal coagulate tumor appearancecause in abdominal organs (Fig 1D). The gross anatomy of intestine from G5 (PHZ-injection then broccoli treatment) was slightly improved that swlling disappear, PHZ remark injured and yellow ascitic liquid reduced .

There was no marked difference between the negative control and positive control in the histological structure of large intestine of G1 Fig. (2-A&B) and G2 Fig. (3-C&D) that normal histological architecture in ELF-EMF exposed rats was shown , no changes or abnormalities were detected in the crypts or villi. However, there were pronounced abnormalities in the intestinal epithelial cells and their nuclei of G3 which injected with PHZ that have high-grade dysplastic lesions were considered as carcinoma-in-situ. The abnormal crypts were not in the same focal plane as the surrounding normal crypts Fig. (4- E), additionally, they could be observed as a group of altered crypts that appeared to form a single unit with stained nuclei of different sizes, shapes, different mitotic figures were observed and intestinal wall of crypt and villi lost their normal architecture Fig. (4-F).

In G4, sections showed improved intestinal wall with well-ameliorated villi and crypt which re-excepting their normal architecture Fig.(5-G). The mitotic figures were also observed at lower rates and the majority of nuclei maintain their normal basal pattern Fig.(5-H).The intestinal sections from G5 showed considerable improvement when compared with sections from G4 since there were a marked amelioration and improvement in the intestinal wall architecture appearance of crypt cell masses and the majority of nuclei restore their normal basal pattern Fig. (6-I&J). At the end of experiment ,rate of death was 40% in (G-3,5) ,20% in G4 and there were no dead rats in (G-6,7).

DISCUSSION:

The present results indicated that ELF-EMF (50 Hz, 0.9 mT) had no manifested side effects on the histological features of the rats' large intestine and there were no abnormalities detected when the intestinal sections from G2 were compared with that of control G1. This observation agreed with Tatarov et al. (2011) who reported that mice exposed directly to 100 mT, 1-Hz, half-sine-wave unipolar magnetic fields for 60, 180, or 360 mins daily for 4 weeks did not display any signs of clinical disease and/or weight. Additionally, their lung, liver, or skin (mammary gland) tissues collected at necropsy did not display any gross or histopathological abnormalities. On the contrary, Semnani et al. (2018) have reported a different finding that exposure of adult male Wistar rats to ELF-EMF (50 Hz, 1 mT) for 4 weeks caused focal hepatocitolysis and mild to moderate portal inflammation in the rats' liver tissue. These conflicting data might be returned to the fact that the biological effects of ELF-EMF vary with the difference in frequency, intensity, duration, and also a certain type of cell lines (Vianale et al., 2008).

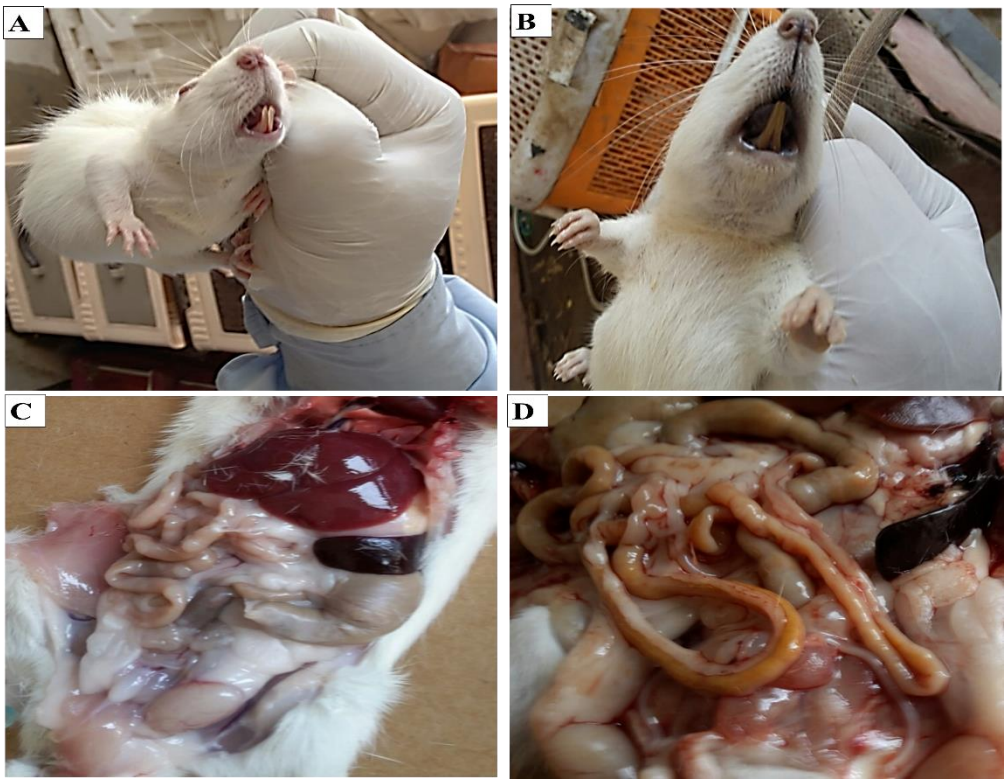
In the current study, the histopathological investigation by H&E of large intestinal sections from rats injected by PHZ reflected a pathological state in the intestinal tissues ranging between low-grade dysplasia and high-grade dysplasia. that revealed a wide variety of changes regarding the nucleus and the architecture of the intestinal wall that could be described as high-grade dysplasia These abnormalities included: the crypt architecture was no more preserved in some parts of the intestinal sections because of extensive divisions of the lining epithelial cells, which render the crypt size bigger, nuclei were enlarged, hyperchromatic and mitotic figures were also increased and atypical giving unknown large masses Thrse results indicated that PHZ could be a potential inducer of colorectal cancer. The possibility that PHZ-oral administration may develop cancer was consistent with Berger, (2007) who showed that PHZ is carcinogenic in mice following oral dosing, including tumors of the vascular system. PHZ carcinogenicity mechanism was discussed by Pandey et al. (2014) who reported that PHZ is absorbed and causes oxidative stress and generates reactive oxygen species.

Rats of G4 undergone injection by PHZ then treated only by exposure to ELF-EMF show a marked improvement in the histopathological investigation by H&E stains, enhancement in the crypt architecture which was lined by columnar epithelium with minor size abnormalities. However, the nucleus was crowded, hyperchromatic and confined to the basal half of the epithelial cells. In addition, mitotic divisions may be distinguished, but atypical mitotic figures appeared to be few. This description can be labeled as low-grade dysplasia The current results revealed that the treatment only by ELF-EMF application mitigated the PHZ damaging effects and succeeded in suppressing tumor progression. This result agreed with Tatarov *et al*. (2011) who speculated that the cell death and necrosis observed in the tumors of mice inoculated with breast cancer cells and then exposed to magnetic fields may be due to the combined effect of suppression of angiogenesis, blood supply blockage to tumor tissues, and cell death

due to apoptosis. Another viewpoint by Lai & Singh, (2010) who hypothesized that cancer cells are more responsive to magnetic fields than normal cells since magnetic fields have a selective cytotoxic effect on cancer cells, the tumor aggressiveness is positively correlated with the concentration of cell surface transferrin receptor of its cells, for example, breast cancer cells have 5-15 times of transferrin receptors on their cell surface than normal breast cells, and they do take up more iron than normal breast cells according to Reizenstein (1991) and Shterman *et al.* (1991).

As for intestinal sections from G5 that have been treated by both broccoli and exposure to ELF-EMF in parallel exhibited a marked improvement in tissue sections, which observed may be attributed to the synergistic suppression effect of ELF-EMF when combined with broccoli bioactive components. Sulforaphane (SNF) is an isothiocyanate, isolated from glucoraphanin in broccoli and other cruciferous vegetables which suppresses proliferation and induces apoptosis by cell cycle arrest in various cancer cells (lee & kim, 2011). *Choi et al.* (2007) reported that SFN suppresses proliferation of cancer cells by causing apoptosis but the mechanism of cell death is not fully understood.

Fig. (1). (A,B) show pathological signs in G1,G3. (C,D) show gross anatomy of G1,G3.



G1

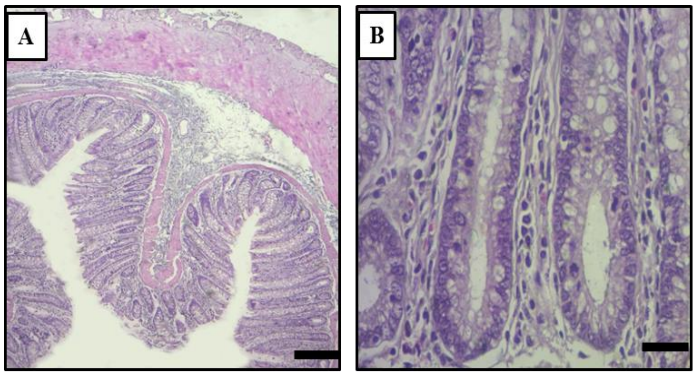


Fig. (2) Photomicrographs of transverse section from the intestinal wall of rats in G1. A=10µ, B=40µ.

G2

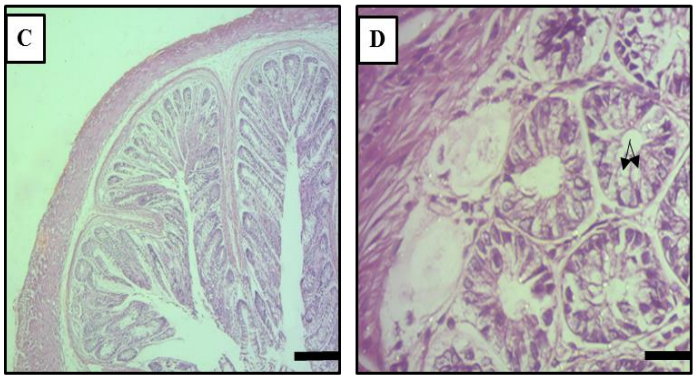


Fig. (3) Photomicrographs of transverse section from the intestinal wall of rats in G2.show the effect of ELF-EMF. C=10µ, D=40µ.

G3

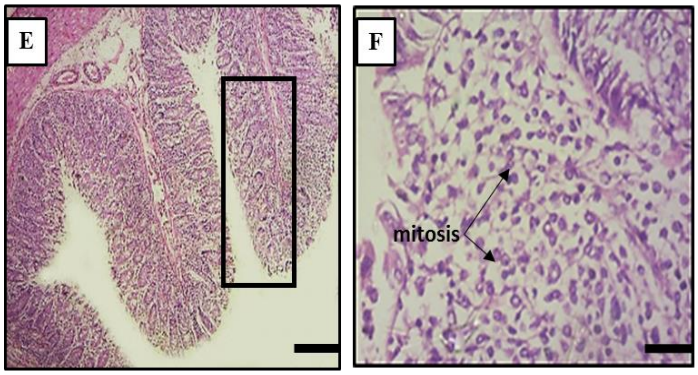


Fig. (4) Photomicrographs of transverse section from the intestinal wall of rats in G3.show the effect of PHZ. E=10µ, F=40µ.

G4

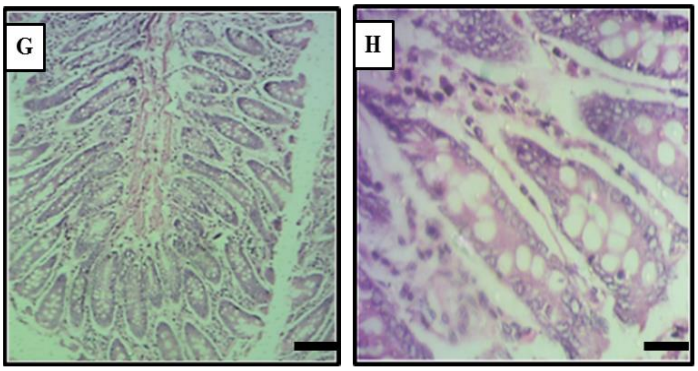


Fig. (5) Photomicrographs of transverse section from the intestinal wall of rats in G4.show the effect of ELF-EMF on PHZ injection. G=10µ, H=40µ.

G5

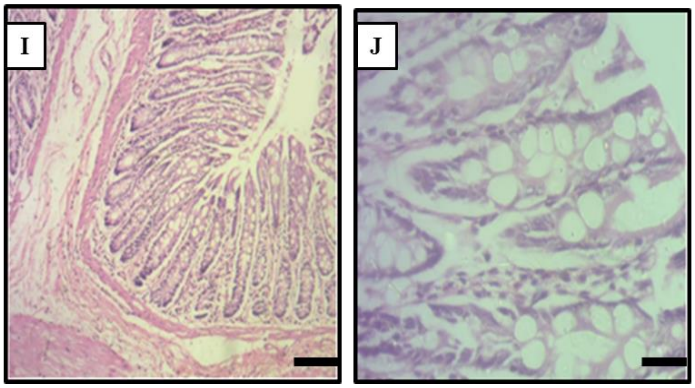


Fig. (6) Photomicrographs of transverse section from the intestinal wall of rats in G5.show the effect of ELF-EMF and broccoli extract on PHZ injection I=10µ, J=40

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التأثيرات العلاجية المحتملة للميدان الكهرومغناطيسي المنخفض التردد واستخراج البروكولي في علاج سرطان القولون الذي يصيب PHZ في الجرذان

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الملخص العربي

يعتبر سرطان القولون ثاني أخطر أنواع السرطان التي تصيب الرجال والنساء في جميع أنحاء العالم. يهدف العلماء في الوقت الحاضر إلى زيادة فعالية علاجات السرطان الموجودة بالفعل. ويعد استخدام المجالات الكهرومغناطيسية في علاج أنواع مختلفة من السرطان سواء استخدم بمفرده أو بالاقتران مع علاجات أخرى ، هو علاج واعد جذب الانتباه خلال العقود الماضية. لذلك، تهدف الدراسة الحالية إلى استكشاف الآثار المفيدة المحتملة لتطبيق المجال الكهرومغناطيسي ذو التردد شديد الانخفاض وحده وبالاقتران مع مستخلص البروكلي في علاج سرطان القولون في نموذج حيواني. تم تقسيم ٢٥ من الفئران الذكور بشكل عشوائي إلى خمس مجموعات: المجموعة الاولي هي المجموعة الضابطة ، المجموعة الثانية تعرضت لـ ٥٠ هرتز، ٠.٩ مللي تسلا) يوميًا لتطبيق المجال الكهرومغناطيسي ذو التردد شديد الانخفاض لمدة ٧٥ دقيقة لمدة ٤ أسابيع. المجموعة الثالثة تم حقنها باستخدام الفينيل هيدرازين ٩٠ (مجم/كجم) مرتين فقط في الأسبوع لمدة ٤ أسابيع والمجموعة الرابعة تم حقنها باستخدام الفينيل هيدرازين ٩٠(مجم/ كجم) مرتين فقط في الأسبوع لمدة ٤ أسابيع، ثم تعرضت للمجال الكهرومغناطيسي ذو التردد شديد الانخفاض (٥٠ هرتز، ٠.٩ مللي تسلا) يوميًا لمدة ٧٥ دقيقة لمدة ٤ أسابيع أخرى. وتم حقن المجموعة الأخيرة باستخدام الفينيل هيدرا زين (٩٠مجم / كجم)مرتين فقط في الأسبوع لمدة ٤ أسابيع، ثم تتعرض للمجال الكهرومغناطيسي ذو التردد شديد الانخفاض يوميًا لمدة ٧٥ دقيقة وتم إعطاؤها مستخلص البروكللي عن طريق الفم (٣٠٠ مجم/كجم) مرتين أسبوعيًا لمدة (٤) أسابيع .

كشفت النتائج أن حقن الفينيل هيدرازين كان قادرًا على إحداث التسرطن في الأنسجة المعوية للفئران. في حين أن العلاج بواسطة تطبيق المجال الكهرومغناطيسي ذو التردد شديد الانخفاض خفف من آثار الفينيل هيدرازين ونجح في ايقاف تطور الورم ، خاصةً عندما يكون مستخدما مع مستخلص البروكلي.