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Antifertility Effect of Polyaniline with Iron α-Fe_2O_3 on Epididymal Sperm of Mice

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Abstract

Chemical polymerization produces polyaniline in two concentrations (0.02,0.05g) at room temperature. The reaction of polymerization was exothermal in nature. The characterization peak was observed in the FTIR range between 1470 and 1560cm^{-1}which defined benzenoid and quinoid ring. To find out the effects Fe_2O_3, this investigation was carried out with two weights of Fe_2O_3 (0.05 and 0.02 g). In the experiment part, 32 mice were separated into eight groups, as indicated. The experiment lasted for 35 days. The results showed a significant ($P < 0.05$) decrease in sperm motility, concentration, percentage of live sperm and testosterone concentration with a significant ($P < 0.05$) increase in the percentage of abnormal sperm in group treated with PANI-Fe_2O_3-Cys without magnetic field in comparison with control group. The results also showed a significant ($P < 0.05$) increase in sperm motility, Relative sperm count of live sperm and testosterone concentration, with a significant ($P < 0.05$) decrease in the percentage of abnormal sperm in group treated with PANI-Cys and PANI-Fe_2O_3-Cys exposed to magnetic field in comparison with the samples without magnetic field. The group treated with PANI alone showed an improvement in all parameters studied except the percentage of abnormal sperms which showed a significant ($P < 0.05$) decrease. Using the alternating magnetic field, it was found that it has a obvious effect on the work of the material PANI- Fe_2O_3-Cys and PAN-Cys. Thus, it affects sperm motility
Article Info.

Keywords: Conductive polymer, Fe_2O_3/PANI, nanoparticle composite, cancel activities, Morphological properties, sperm.

التأثير المضاد للخصوبة لبولي أنيلين مع الحديد α−Fe_2O على الحيوانات المنوية البربخية للفئران

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الخلاصه

تنتج البلمرة الكيميائية البولي أنيلين بتركيزين (0.02.0.05) عند درجة حرارة الغرفة الطبيعة حيث كان تفاعل من نوع الطاردة للحرارة لمركبات النانو. تمت ملاحظة منحني الاواصر المميزة للبولي أنيلين في نطاق FTIRبين 1470 و1560. لمعرفة تأثيراتFe_2O_3 ، تم إجراء هذا البحث (0.05 و0.02 جم). في جزء التجربة، تم فصل 32 فأرًا إلى ثماني مجموعات، كما هو موضح. استمرت التجربة لمدة 35 يومًا. أظهرت

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النتائج انخفاضًا معنويًا (P <0.05) في حركة الحيوانات المنوية وتركيزها ونسبة الحيوانات المنوية الحية وتركيز هرمون التستوستيرون مع زيادة معنوية (P <0.05) في نسبة الحيوانات المنوية غير الطبيعية في المجموعة المعالجة بـ $\alpha- Fe_2O_3$ بدون مجال مغناطيسي في. مقارنة مع المجموعة الضابطة. كما أظهرت النتائج زيادة معنوية (P <0.05) في حركة الحيوانات المنوية وتركيزها ونسبة الحيوانات المنوية الحية وتركيز هرمون التستوستيرون مع انخفاض معنوي (P <0.05) في نسبة الحيوانات المنوية غير الطبيعية في المجموعة المعالجة بـ .PANI و $PANI - \alpha- Fe_2O_3$ المجال المغناطيسي المكشوف بالمقارنة مع المجموعة $\alpha- Fe_2O_3$ بدون مجال مغناطيسي. أظهرت المجموعة التي عولجت بـ PANI وحده تحسناً في جميع العوامل المدروسة باستثناء النسبة المئوية للحيوانات المنوية غير الطبيعية التي أظهرت انخفاضاً معنويا (P <0.05). باستخدام المجال المغناطيسي المتناوب، وجد أن له تأثير واضح على عمل المادة –PANI Fe_2O_3–Cys و PAN–Cys. وبالتالي، فإنه يؤثر على حركة الحيوانات المنوية

1. Introduction

To combat cancer, bacterial infection, inflammatory illnesses, and so on, hyperthermia (also known as thermotherapy or thermal therapy) is used to expose the bodily tissue to higher temperatures in order to destroy disease cells or pathogens by denaturation of proteins and membrane breakdown [1]. Conventional techniques of hyperthermia include the use of heated saline through catheters, sitting in a hot room, or wrapped in hot blankets. Other methods include microwave heating, ultrasonic heating, and so on. Most of these treatments are invasive and can damage the normal tissue. To address the limitations of traditional hyperthermia, noninvasive techniques involving near-infrared (NIR) light, radiofrequencies, or inductively linked magnetic fields have been used [2]. As therapy drugs' inherent toxicity is unavoidable, the development of multifunctional therapeutic nanoplatforms, particularly those with targeted specificity, is considered crucial for future application in clinical cancer treatment32. 32−34 Additionally, Haam and colleagues in 2014 produced one sort of targeted gadolinium-enriched PANI NPs via modification of cetuximab, where they accomplished simultaneous diagnostic imaging and PTT for epithelial carcinoma though promising results have been achieved, the preparation process is somewhat complicated [3].

Since the 1970s, the usage of polymers in biological applications has risen significantly. Tissue engineering has taken a keen interest in conducting polymers and their derivatives since it was discovered that CPs might regulate cellular activity by stimulating it electrically, such as cell growth and migration. There are several similarities between CPs and ordinary polymers, such as their ease of synthesis and processing compared to metals [4]. An example of CPs is polyaniline (PANI), which has been the subject of numerous research. PANI is a well-known conducting polymer that exhibits a wide range of structural shapes, excellent environmental stability, and straightforward ability to carry electrical charges [5]. Belmonte et al. [6] were the first to show that this polymer is biocompatible both in vitro and in vivo. There are two major categories of PANI biocompatibility studies that have been reported. In-vivo testing is the primary emphasis of one group, while in vitro proliferation and differentiation of cells on PANI surfaces are the subject of the other group [7].

In this article, a new process to synthesize the composite of /PANI- α- Fe_2O_3 Cysteine was developed. Study of the effect of the concentration of Fe_2O_3 in polyaniline and the effect of magnetic field on cancer cells in sperm of mice was studied.

2. Experimental work

Reagents and materials

Hybrid polyaniline with iron ferric nanocomposite was created using following materials. All of the compounds employed here were of the highest purity possible, and the firms that supplied them are noted in Table (1).

Table 1: show the materials used in preparing the hybrid nanocomposites

NO.	Chemical Materials	Purity	Supplier
1	Aniline ($C_6H_5.NH_2$)	99%	Hopkin Williams
2	Ammonium Persulphate $(NH_4)_2S_2O_8$	98%	Himedia-India
3	L-Cysteine ($C_6H_{12}N_2O_4S_2$)	98%	Himedia-India
4	Iron II (αFe_2O_3)	98%	Himedia-India (particle size 20-30 nm density 5.6 g/cc)
5	Dimethyl Sulfoxide (DMSO) $(CH_3)_2SO$	99.6%	BDH-England (Conductivity 10 µm/cm)

3.Synthesis of PANI-Fe_2O_3-Cys

0.45ml of aniline was weighed using an electronic balance and then diluted with 50ml of deionized water. A concentration of one mole was achieved in this manner. The mixture was placed in a flask, kept under steady stirring. This mixture was divided into two and different weights of Fe_2O_3 of 0.02g and 0.05 g were added; Meanwhile, another solution of 1M Ammonium Persulphate (APS) was prepared; 4.5g of this substance was dissolved in 50 ml of distilled water. This solution adding to aniline to complete polymerization. The reaction was kept in an ice bath at 3°C for 15 minutes with constant stirring this condition were used to keep this solution stable. The APS solution was poured dropwise into the aniline solution. Dark green tinted granules appeared in the solution, which had previously been clear, as shown in Figure 1. A digital thermometer was dipped in the solution to monitor its temperature. The temperature increased from 3°C to 40°C in less than 10 minutes and then began to fall. The polymerization process was allowed to proceed for five hours at a temperature of 3°C with continuous stirring. The solution was allowed to sit for a further 20 hours.

This mixture was filtered under vacuum after 24h. In the meantime, the mixture was washed with distilled water. Filtration produced a dried product, which was dried in an oven at 80°C for four hours. The product powder (PANI-Fe_2O_3) was grinded with mortars into a fine powder for further diagnostic analysis after making sure it was free of dampness. In order to convert the product powder from hydrophobic to hydrophilic, the polymer PANI-Fe2O3 must be immersed for a one day (24h) in (1M) of L-cysteine amine solution

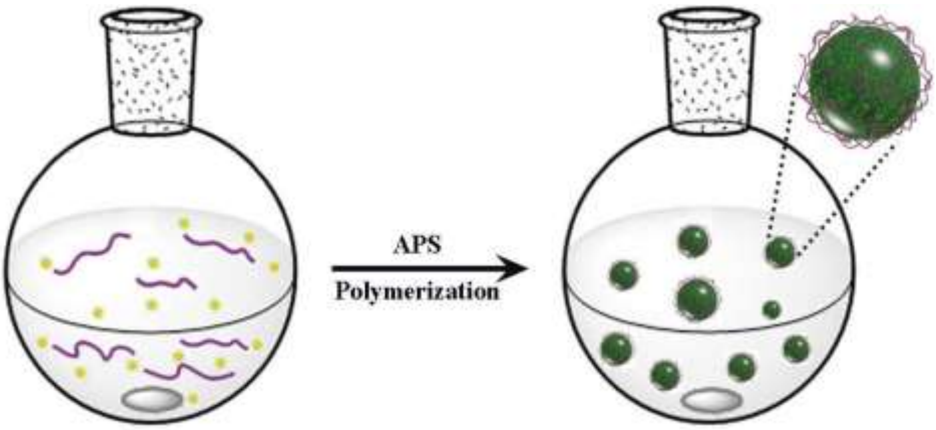

Figure 1: Preparation of PANI- Fe_2O_3-Cys NPs (counterions are omitted for clarity) [8] L

3. Preparing the in vivo sample and mechanism of Alternating Magnetic Field (AMF) therapy

For the purpose of studying the effect of the magnetic field on the sample (PANI-Fe_2O_3-Cys), an alternating magnetic field (AMF) was used (the magnetic induction B (flux density) = 0.2 tesla, measured by TD 8620 flax meter, and the period time was 2 min) for preheating the PANI-Fe_2O_3-Cys) inside the animal body (sperm cells) even become the temperature from (50-65C), where this temperature was the cause of in the preferential death and changes in affected sperm cells. The changes in the sperm (shape, deformation and motility) were observed using a microscope. Figure 2 shows the diagram of circuit used for alternating magnetic field (AMF) therapy and Figure 3 shows a mouse inside the magnetic field.

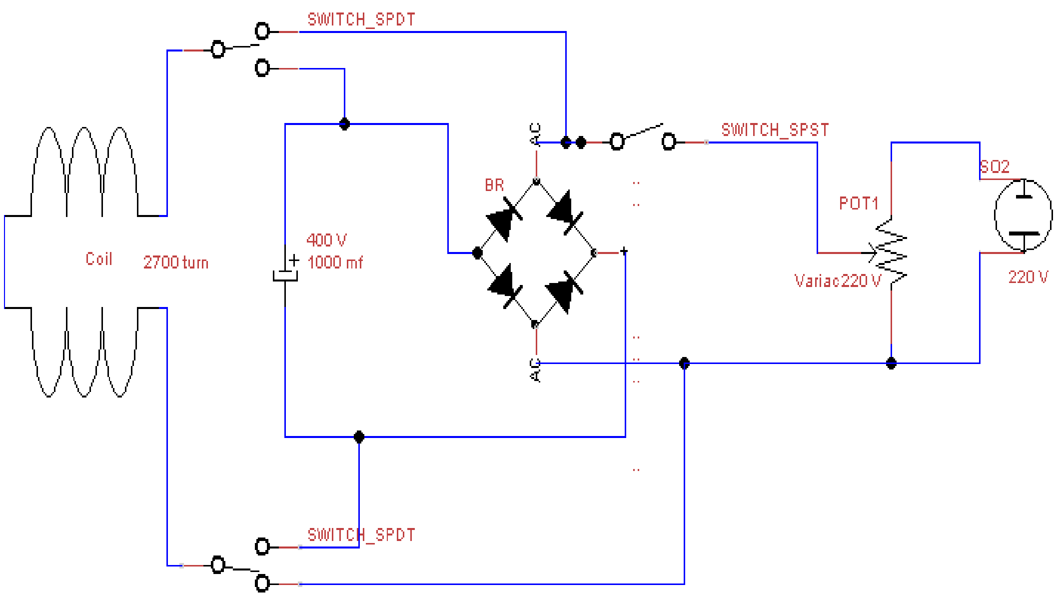

Figure 2: The circuit diagram of Alternating Magnetic Field (AMF) therapy

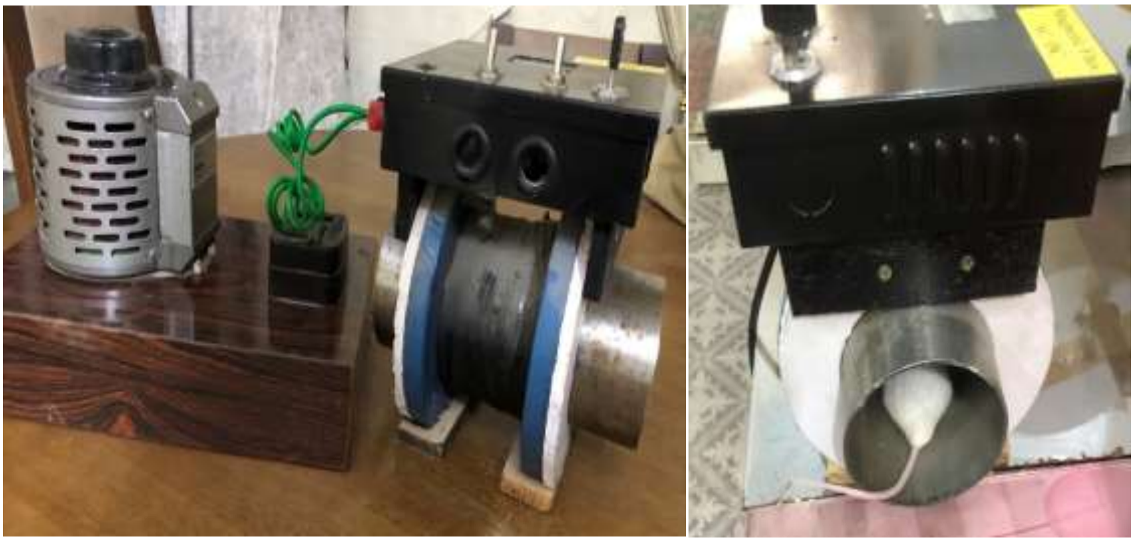

Figure 3: show a mouse inside the alternating magnetic field (AMF)

5. Experimental Animals:

In this study, 32 white Swiss mice aged 10-8 weeks with an average weight of 25-30 g were used. The mice were infected with cancer type methotrexate (MTX). They were placed in plastic cages spread with sawdust, and the mice were divided into eight groups, 4 in each group, as show in Figure (4). The mice groups were as follows:

First group: The mice injected with water only (negative control group).

Second group: The mice injected with MTX only (positive control group).

Third group: The mice injected with MTX and subjected to AMF.

Fourth group: The mice injected with PANI-Fe_2O_3-Cys with Fe_2O_3 of (0.02g).

Fifth group: The mice injected with PANI-Fe_2O_3-Cys with Fe_2O_3(0.05g).

Sixth group: The mice injected with PANI-Fe_2O_3-Cys (Fe_2O_3= 0.02g) and subjected to AMF.

Seventh group: The mice injected with PANI-Fe_2O_3-Cys at (Fe_2O_3=0.05g) and subjected to AMF.

Eighth group: The mice injected with PANI-Hybrid Composite only and subjected to AMF.

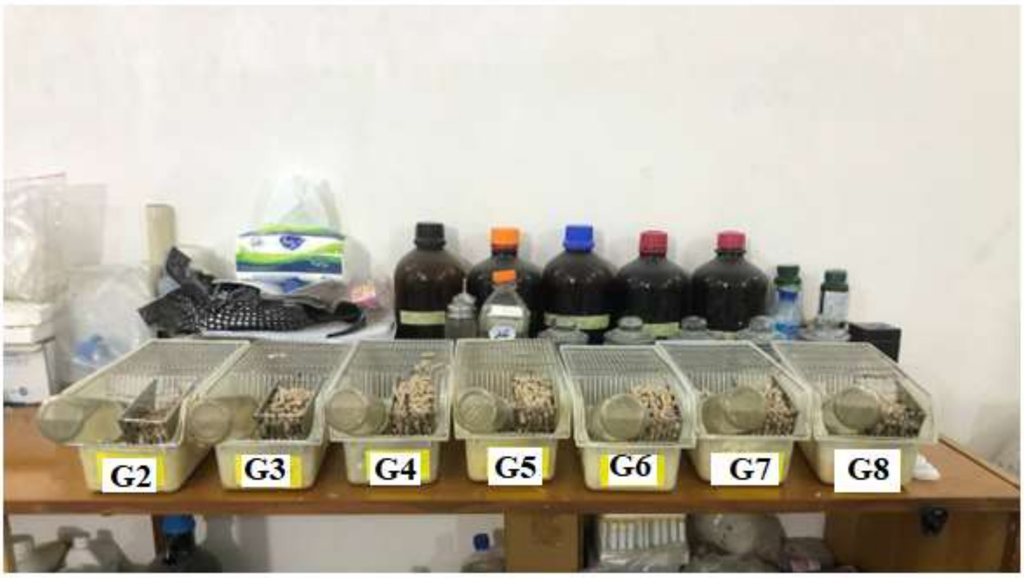

Figure 4: The eight groups of mice.

After taking a blood sample, the animals were sacrificed by cervical dislocation by pulling and the animals were dissected by opening the abdominal cavity in an inverted T shape. The epididymis was excised, the fatty tissue attached to it was removed, dried with filter paper and placed in physiological saline solution to calculate the following:

motility and density of sperm

Using 0.2 ml of physiological saline, a known quantity of cauda epididymis was extracted for assessing sperm motility and density. Five minutes after sacrifice, one drop of an evenly mixed sample was applied to a glass slide under a cover glass. Spermatozoa per unit area were counted to determine the proportion of motile spermatozoa. The density (count) of sperm in the cauda epididymis was determined using standard procedures and expressed in million/mm^3 of suspension [9].

$$\text{Number of sperms /ml} = \frac{n*400*200}{80*0.1} \qquad \text{……. (1) [10]}$$

n: number of live sperm

Percentage of live and dead sperm

A drop of the sperm mixture was placed on the side of a glass slide and placed on a hot plate at a temperature of 37°C; next to the drop, drops of necrosing-eosin mixture the size of the first drop itself were placed. The mixture on the slide was left to dry at room temperature and then examined under 40X magnification. The percentage of live and dead sperm was calculated, as well as knowing the percentages of abnormality sperm through the following equation:

$$\text{Percentage of live and dead sperm} = \frac{\text{Dead/live or abnormal sperm count}}{\text{total sperm count}} * 100\% \qquad \text{…… (2)[10]}$$

Measurement of Testosterone concentration

Blood samples were taken using a 1 ml syringe by the heart stab procedure before the animal was sacrificed. The blood samples were centrifuged at 2000 rpm for 10 minutes to separate the serum, which was then frozen at -20 °C till the hormone concentration was tested using a device (Male Hormone panel).

6. Characterization of chemical structure

Using the Fourier Transformation IR Analysis (FTIR), the PANI-Fe_2O_3-Cys were analyzed in the wave number range 400-4000 cm^{-1}. Most samples were analyzed with a mid-IR scope; however, data can also be gleaned from far- and near-IR bands.

7. Result and discussion

Figure 5 shows the relation between reaction temperature and time of polymerization a typical cycle of polymerization practically recorded in the lab. there are three distinct stages; the temperature remains virtually constant in the first stage which is called the induction period. In stage 2, polymerization commences and the temperature of the reaction mixture increases and the color of the solution has been changed to dark black color after being transparent with the appearance of granules which preceded the composition of oligomeric intermediates. the cycle passes through a maximum after the reaction is finished, Post polymerization stage which is stage 3 begins when the temperature drops and the reaction enters its saturation stage and the medium cools down. Polymerizations using aniline concentrations over 1 M, especially when carried out in large volumes (over 0.5 L), can result in the overheating of the system, followed by an explosion [11]. Such reaction conditions should be avoided.

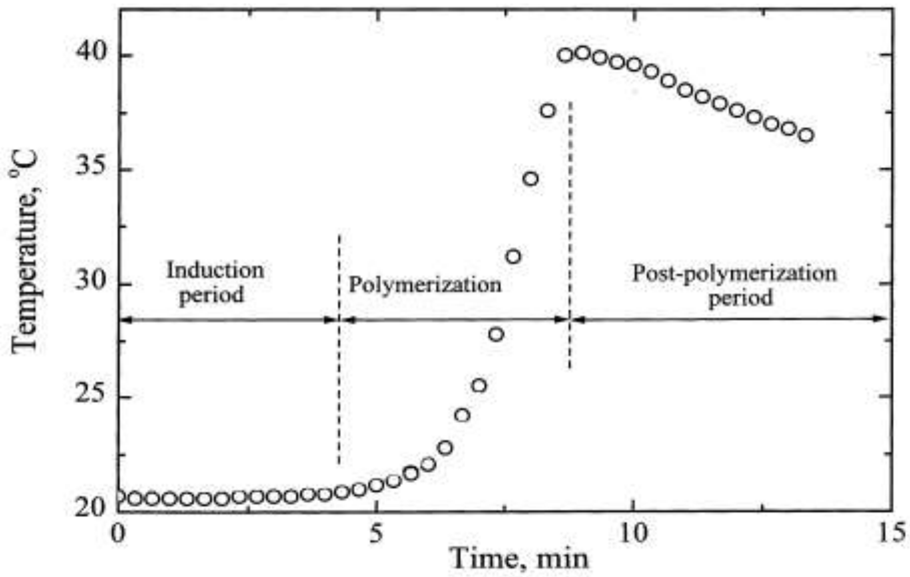

Figure 5: The polymerization process as a function of time [11].

Fourier transform infrared (FTIR) spectroscopy validated the chemical structure of PANI and PANI-Fe_2O_3 of (0.02gm and 0.05 g weight) hybrid nanocomposites. PANI FTIR spectra (Figure 6) shows the C-H out of plane bend on the aromatic ring at the 1, 4 position in the intrinsic PANI, as well as the carbon skeleton peak at 1121 cm^{-1} and the C-N stretching peak at 1292 cm^{-1} [12]. Benzenoid ring C=C stretching is responsible for the characteristic peak at 1470 cm^{-1}, whereas the high doping degree of the PANI is shown by the peak at 1559 cm^{-1}. N-H stretching vibrations can be seen at 3428 cm^{-1} [13]. PANI's creation has been confirmed by all of the distinctive peaks that have been found.

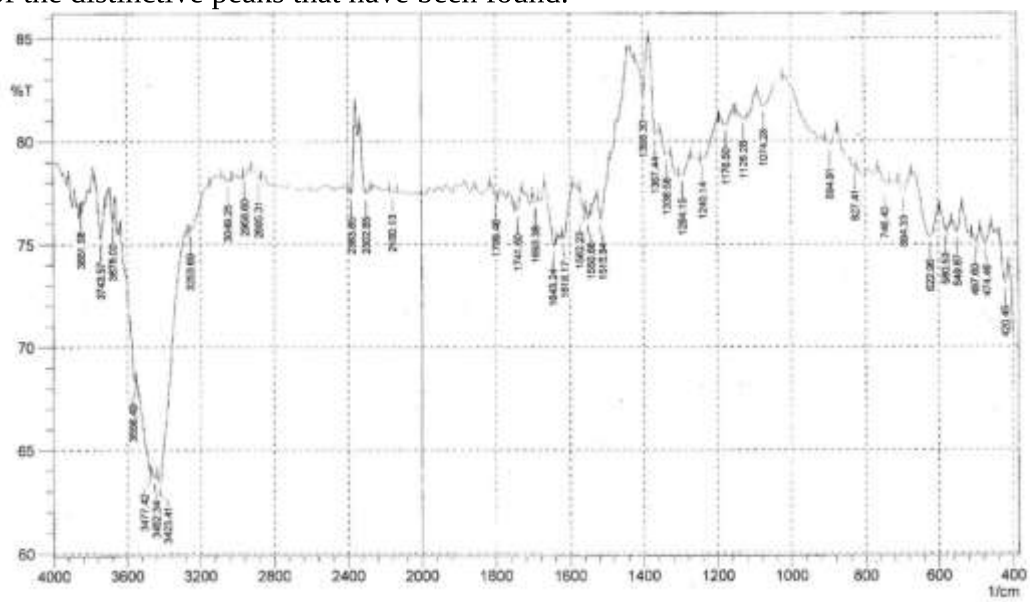

Figure 6: FTIR spectra of PANI.

PANI-Fe_2O_3-Cys (0.02,0.05g) FTIR spectra (Figure 7) show that the characteristic peaks of pure PANI at 801 cm^{-1} shifted to 822 cm^{-1}, 1121 cm^{-1} shifted to 1132 cm^{-1}, 1292 cm^{-1} shifted to 1294 cm^{-1} and 1470 cm^{-1} shifted to 1489 cm^{-1} [14]. The electronic interaction between PANI and nano Fe_2O_3 may be the cause of such a shift in wave number. Iron oxide

IS involved in the polymer matrix, as seen by the peak at 561 cm^{-1}, which represents the stretching vibration mode of the Fe-O molecule. Although PANI- Fe_2O_3 (0.02,0.05) hybrid nanocomposites have characteristic peaks at 1294 cm^{-1}, 1489 cm^{-1}, and 1576 cm^{-1}, which are attributed to the benzenoid (NH-B-NH) and quinonoid rings of PANI. The fact that the iron in PANI tends to form a coordination complex with nitrogen atoms is indicated by a shift in peak height from 1121 $cm^{-1}$to 1132 cm^{-1}. It also indicates that the amine nitrogen is more involved in the interaction, as the characteristic peak shift from 1559 $cm^{-1}$to 1576 cm^{-1} [15]. The -CH stretching vibrations are thought to be responsible for the broad peak at 2915 cm^{-1}. The polymer hybrid composite is clearly visible in all of these characteristic peaks [16].

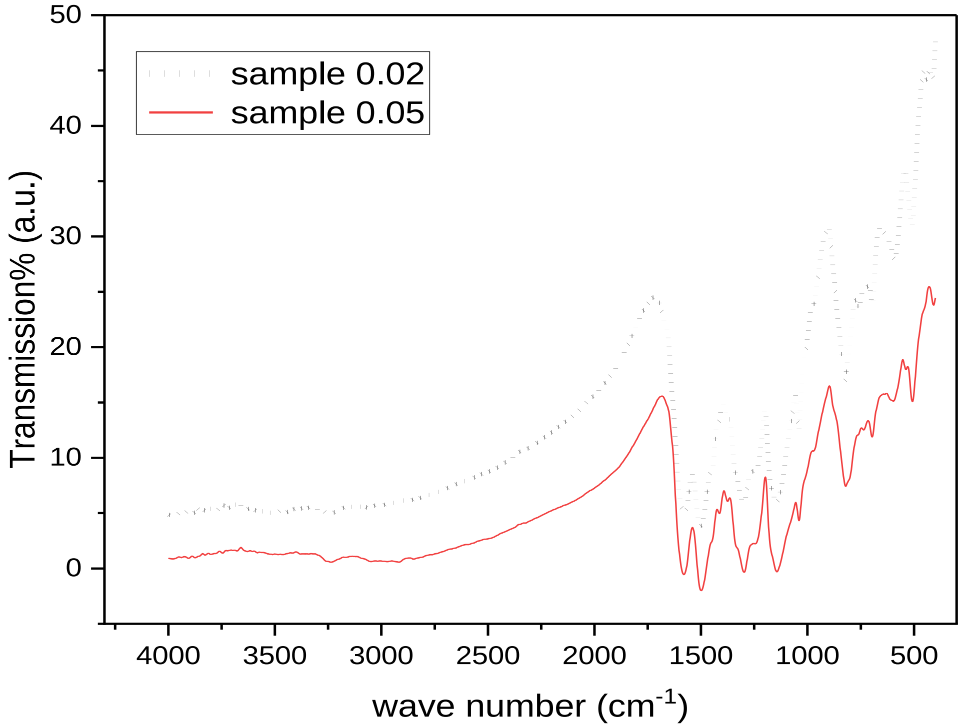

Figure 7: The Fe_2O_3PANI-Cys FTIR spectral areas**.**

Figure 8-a shows FE-SEM images of PANI-Fe_2O_3-Cys at (0.02g) and Figure 8-b for PANI- Fe_2O_3-Cys at (0.05g). This figure illustrates the growth of the PANI- Fe_2O_3-Cys microgranules/microparticles with a diameter less than (80.1-90 nm). These rang are randomly. Distributed and separated by different voids. These microgranules have irregular shapes. The PANI- Fe_2O_3-Cys microgranules have a rough the final shape that were ovular flower. This result explain that the interface procedure can be used to control the polymerization reaction with homogenously dispersed nucleation centers. This complicated structure that fabricated in this work using simple, low cost, and massive production method can be used in different applications including catalysts, sensors, and biomedical applications. This is highly expected because of the huge surface to volume ratio [11].

This fact demonstrates that the composite is highly microporous and can increase the liquid–solid interfacial area. An FE-SEM analysis of Jaque et al. [17] corroborated the material's highly porous structure and clumped spherical form. And the electrostatic interaction between the amino groups (-NH2) of PANI-Cys and the carboxylate group from Fe_2O_3could be attributed to the decreased PANI-Cyst size, as seen in Field emission SEM for PANI- Fe_2O_3-Cys [18].

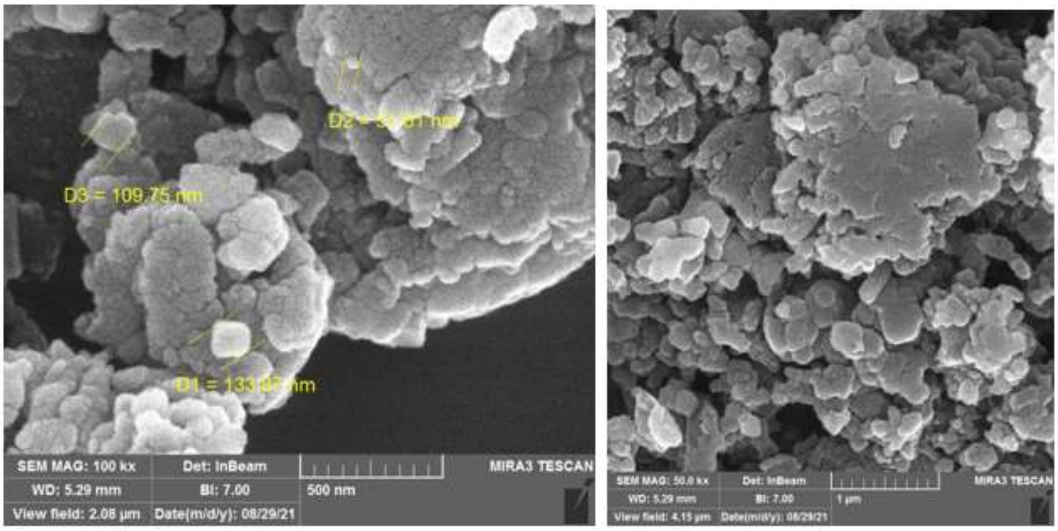

Figure 8 a: The FE-SEM micrographs of PANI-Fe_2O_3-Cys (0.02g)

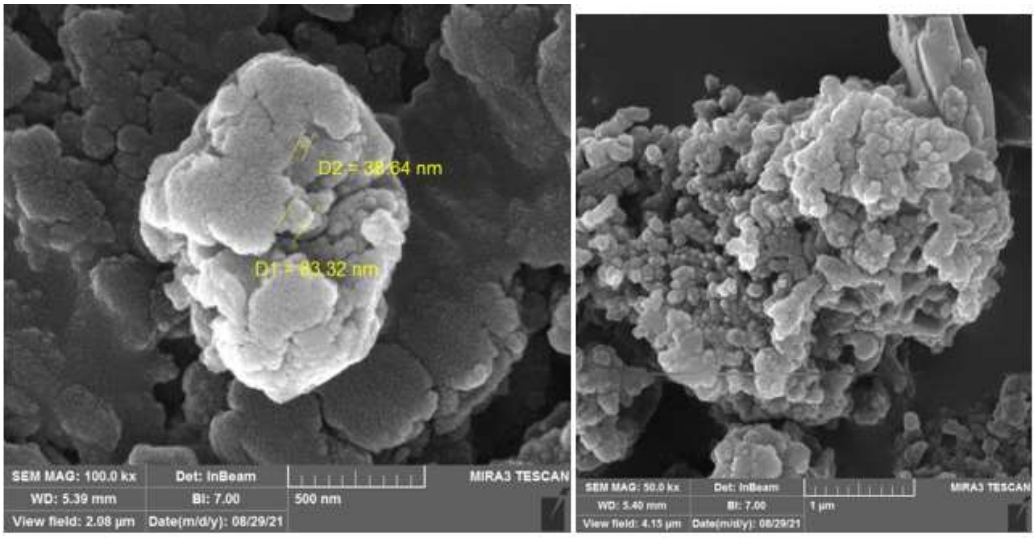

Figure 8 b: The FE-SEM micrographs of PANI-Fe_2O_3-Cys (0.05g)

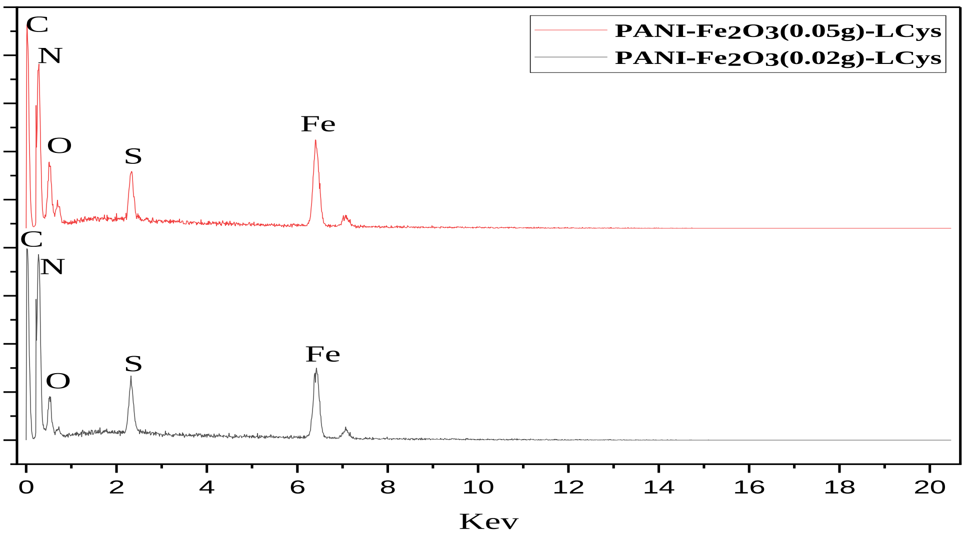

Figure 9: EDX analysis for Fe_2O_3 PANI-Cysteine

From the elemental analysis of Figure (9) for PANI-Fe_2O_3-Cys at (0.02g and 0.05g), observed many peaks corresponding to carbon (C), sulfur (S) and sodium (N) are observed, which corresponds to the characteristic materials of the composite powder of PANI and L-Cystine. The ratio of weight percentage (wt%) and atomic percentage (A%) were shown in Table (2)[19]. PANI- Fe_2O_3-Cys for 0.02g and 0.05g Fe_2O_3, nano-composite consist of exact elementals composition of specific element like Fe, O, C. The observed elemental composition is Fe=19.6%, O=10.44%, C=35.19%, N=25% and S= 10%.

Table 2: a EDX analysis for PANI-Fe_2O_3-Cys powder (0.05g)

Element	Series	AN	W%	A%
Carbon	Kα	16	35.19	58.39
Nitrogen	Kα	7	25	15.33
Oxygen	Kα	8	10.44	19.38
Sulfur	Kα, Kβ	16	10.14	1.06
Iron	Kα, Lα	26	19.23	5.84
Sum:			100%	100%

Table 2 b: EDX analysis for PANI-Fe_2O_3-Cys powder (0.02g)

Element	Series	AN	W%	A%
Carbon	Kα	16	38.19	58.39
Nitrogen	Kα	7	29	15.33
Oxygen	Kα	8	11.44	19.38
Sulfur	Kα, Kβ	16	11.14	1.06
Iron	Kα, Lα	26	10.60	5.85
Sum:			100%	100%

8. The results of sperm test

Table 3 shows the number of dead sperm, motility and abnormal sperms of these groups.

Percentage of Sperm Motility and Live Sperms

A significant decrease ($P < 0.05$) in the % sperm motility, % live sperm and sperm concentration for the PANI-Fe_2O_3-Cys treated group compared to the control group was noted. There was also a significant increase ($P < 0.05$) in sperm motility and live sperms count close to that of the control group in the pure PANI treated group and in the PANI-Fe_2O_3 of 0.05g treated group compared to the PANI- Fe_2O_3-Cys of 0.02 g treated group. The PANI-treated group showed an improvement in the percentage of sperm motility [20].

Percentage of abnormal sperm

The group of mice treated with PANI-Fe_2O_3 showed a significant I increase I ($P < 0.05$) inI the percentage of I deformed sperms compared to I the control group. I As for the group treated I with pure PANI and treated with PANI-Fe_2O_3 (0.05g), there was a significant decrease I ($P < 0.05$) in the percentage of deformed I sperms compared to the I group of mice treated with PANI-Fe_2O_3 (0.02g). The group treated with pure PANI I showed levels close to that of the control group.

Table 3: The sperm motility, dead and abnormalities sperm

Parameter I s Groups I	Motility I % (mean±SD) I	Dead I % (mean±SD)	Abnormalities I % (mean±SD) I	I Count ×10⁷ I (mean±SD)
Neg.control	A 89+3.61	F 13.667+1.528	F 14+2	A 32.33+3.06
MTX	D 57.33+2.52	A 38+2.65	A 27.33+2.52	E 16+2
MTX+AFM	C 65.33+5.51	B 28.33+2.08	AB 25+2	E 17+1
MTX+Polymar 0.02	C 68.67+3.06	B C25+2	BC 22.667+1.528	E 17.667+1.155
MTX+Polymar 0.05	C 70+2	CD 22+2	CD 20.333+1.528	CD 23.333+1.528
MTX+Polymar (0.02) + AFM	B 79.67+4.51	DE 20.33+2.52	D 19+2.65	D 21.67+2.08
MTX+Polymar (0.05)+ AFM	A 86.67+2.89	DE 19+2	DE 17.667+1.528	BC 25.333+1.528
MTX+Polymarpure+ AFM	A 89+3.61	E 18+2	EF 15+2	BC 27.67+3.06
LSD	6.260	3.671	3.480	3.568
P-value	0.0002	0.00011	0.00006	0.00003

Note: Different lettering (A, B, C, D) on the same columns indicate significant differences among groups (P <0.005), Values are mean of four animals ± S.D.; data analyzed by Student

Figure (10) shows deformed (with abnormal tails, coil & folded) and dead sperms stained by eosin dye; while live sperm and normal sperm do not stain with the dye.

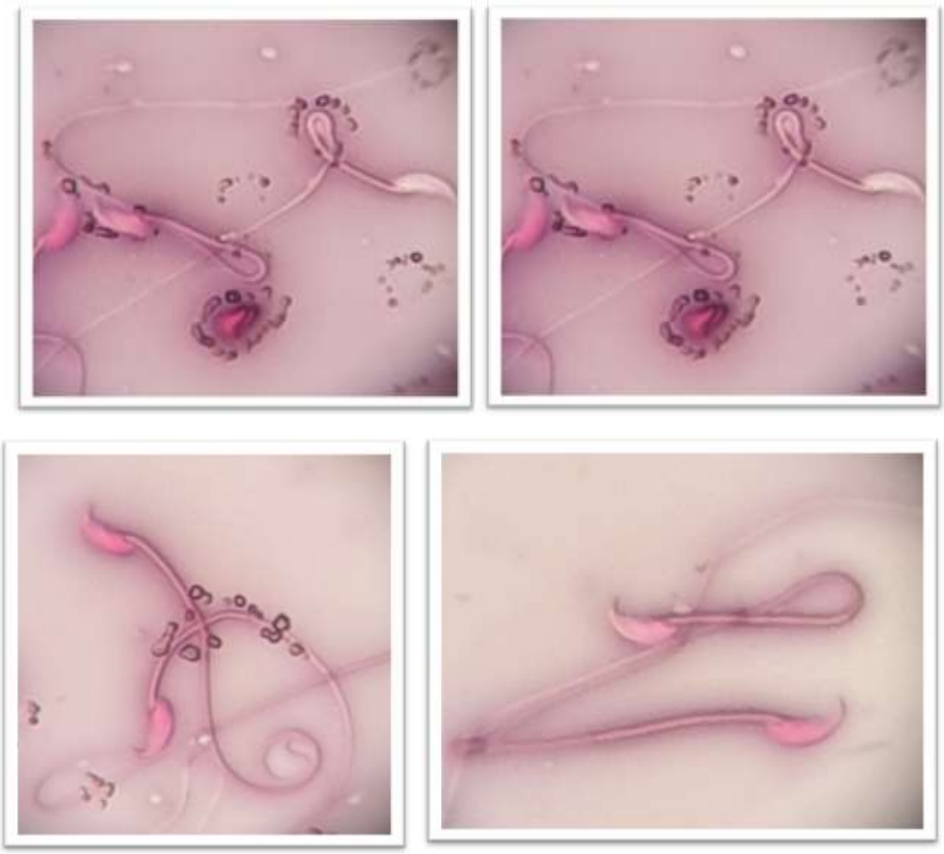

Figure 10: Abnormal sperm under microscope (magnification force of x100)

Testosterone Concentration

Table (4) shows the I concentration I of testosterone for the eight mice groups. It is noticed that there was a I significant decrease I ($P < 0.05$) in the I concentration I of testosterone I in the I group exposed to PANI-Fe_2O_3-Cys compared I to the control group. I The results of the group treated with PANI-Cys and treated with PANI-Fe_2O_3-Cys (0.05g Fe_2O_3) indicated a significant increase ($P < 0.05$) in the average testosterone I concentration compared to the group of mice treated with PANI-Fe_2O_3-Cys (0.02g Fe_2O_3). The group of mice treated with PANI-Cys and PANI- Fe_2O_3 (0.05) showed an increase in testosterone at a higher level than its level in the control group.

Table 4: The sperm Testosterone hormone

Parameters Groups	Testosterone ng/ml (mean±SD)	FSH mIU/ml (mean±SD)	LH mIu/ml (mean±SD)
Neg.control	A 5.0552+0.1333	E 3.0927+0.1698	D 2.707+0.346
MTX	F 3.719+0.206	A 5.664+0.399	A 4.062+0.315
MTX+Lasser	E 3.9726+0.1077	B 5.066+0.202	AB 3.551+0.367
MTX+Polymar 0.02	DE 4.0222+0.1069	C 4.5575+0.1575	BC 3.284+0.234
MTX+Polymar 0.05	DE 4.1486+0.0952	C 4.408+0.205	BCD 3.04+0.24
MTX+Polymar 0.02+Lasser	CD 4.216+0.1148	D 3.945+0.337	CD 2.884+0.234
MTX+Polymar 0.05+Lasser	CD 4.432+0.181	D 3.81+0.202	CD 2.907+0.481
MTX+Polymarpure+Lasser	B 4.8223+0.0675	D 3.6308+0.137	CD 2.796+0.336
LSD	**0.231**	**0.418**	**0.569**
P-value	**0.00012**	**0.00005**	**0.00007**

9. Discuss sperm results

The results of the current study showed that the treatment of PANI-Fe_2O_3-Cys mice led to a significant decrease in the percentage of sperm motility and sperm concentration rate. One of the studies indicated that exposure to PANI- Fe_2O_3-Cys for a period of more than a month caused a decrease in the rate of sperm concentration and movement. PANI- Fe_2O_3-Cys causes oxidative stress in the semen and in the sperm, which leads to an imbalance between the active types of oxygen and antioxidants. Fe_2O_3 also causes a decrease in the effectiveness of antioxidant enzymes, including Superoxide dismutase and Catalase, and then the death of the sperm and the decrease in the movement of the live sperm. [21]. Another study indicated that exposure of male mice to a dose of 0.05 mg/kg of PANI- Fe_2O_3-Cys causes a decrease in sperm motility and their number in the epididymis.

The study showed that iron oxide interacting with polyaniline works to generate active types of oxygen in the testes of male mice, which leads to an increase in fat oxidation and then a decrease in the number of sperms with an increase in sperm abnormalities in the treated mice [22].

The current study showed that the treatment of hybrid PANI-Cys animals caused a significant increase in the percentage of sperm motility. It was noted that calcium and L-Cysteine increases the effectiveness of antioxidant enzymes, including glutathione peroxidase, and the important role of glutathione peroxidase was noted in the correct and good formation of the midsection of the sperm, as it was noted in the mitochondria and in the nucleus of the sperm, and it has a role in protecting the sperm from effective types of oxygen [23]. A relationship was also observed between the level of PANI, the activity of glutathione peroxidase and the quality of the sperm. As the deficiency of PANI causes a defect in the mid-section of the sperm, and then causes a defect in the movement of the sperm and a weakness in the process of sperm formation [24]

One of the studies showed the occurrence of sperm abnormalities, especially the head area of the sperm, and other abnormalities in the neck, mid-section and tail of the sperm, and abnormalities in the sperm chromosomes of male mice that suffer from a deficiency in calcium, as the calcium present in hybrid polyaniline has a role in the process of formation The sperm in mice ,and the magnetic field works to disperse the active substance and improve the properties of the hybrid polyaniline [25], as Glutathione peroxidase has a key role in protecting the plasma membrane of cells from oxidative damage, and PANI, in the presence of the hybrid substance included in its composition, is necessary in the formation of the sperm flagellum, during the process of sperm transformation through its role in the formation of the envelope surrounding the axial filaments of the flagellum. And that the deficiency of PANI causes the lysis of the lysosomal cells of the mothers of spermatozoa during the spermatid transition, so the lysosomal enzymes present inside the lysosomes lead to the breakdown of the tail membrane of the spermatozoa [26].

However, Regarding the role of the magnetic field there were no differences between the groups, which suggests that the magnetic field used does not interfere with spermatogenesis in such a way that it would increase sperm head abnormalities. The present study did not provide evidence of adverse effects of magnetic field on sperm counts in cauda epididymis or testis.
The magnetic field may influence sperm motility This is for reasons mentioned the movement of hemoglobin increases in the blood vessels, which leads to a reduction in the proportion of calcium, cholesterol, and even waste suspended on the surfaces of the blood vessels, which removes high blood pressure and relieves the work of the heart. hormones. The movement of blood and lymph activates, so that all nutrients reach the cells adequately. Sperm cell motility is an important indicator of fertility [27]. and Increased sperm motility is an interesting finding that needs to be confirmed in further studies.

10. Conclusion

1- The PANI-Cys and PANI-Fe_2O_3-Cys nanocomposites were successfully synthesized via in situ chemical oxidative polymerization of polyaniline in the presence of different weight percentages of Fe_2O_3NPs.
2- The relation was exothermic between reaction temperature and polymerization time. It was found that adding Fe_2O_3 NPs to the aniline before polymerization raises the temperature of the reaction and accelerates the process of polymerization.
3- In vivo application show, no adverse effects on fertility indicators were observed in mice exposed to PANI-Cys. The body weight development were not affected by the PANI-Cys used, and no exposure-related differences were observed in sperm counts or sperm head abnormalities. While we noticed obvious side effects on sperm movement, deformation and other abnormalities in the sperm in PANI-Fe_2O_3-Cys. Increased sperm motility is an interesting finding that needs to be confirmed in further studies.
4- In terms of the effect of the alternating magnetic field (AMF), it was found that it has a clear effect on the work of the material PANI-Fe_2O_3-Cys and PAN-Cys.

References

[1] J. Mancilla-Galindo and N. Galindo-Sevilla, "Exploring the rationale for thermotherapy in cancer," *International Journal of Hyperthermia*, vol. 38, no. 1, pp. 202-2121, 2021.

[2] J. G. Short, P. F. Turner, "Physical hyperthermia and cancer therapy," *Proceedings of the IEEE*, vol. 68, no. 1, pp. 133-142, 2012

[3] Lu Tang, Shun He, et al. "Combination of nanomaterials in cell-based drug delivery systems for cancer treatment," *National Center Biotechnology information*, vol. 3, no. 11, p. 1888, 2021.

[4] M. Ates, T. Karazehir and A. Sezai Sarac, "Conducting Polymers and Their Applications," *Current Physical Chemistry*, vol. 2, pp. 224-240, 2012.

[5] D. Mahnoush, Suraya Abdul Rashid, C.W. Lee, and S.H. Lee, "Synthesis and Properties of Photo Acid Generators from Conducting polyaniline," *Polymer Preprints MDPI*, vol. 45, pp. 273-284, 2021.

[6] Taimoor H Qazi, Ranjana Rai. Aldo R Boccaccini, "Tissue engineering of electrically responsive tissues using polyaniline-based polymers: A review," *Biomaterials*, vol. 35, no. 33, pp. 9068-9086, 2014.

[7] X.Du, Z. Zhang, W. Liu, Y. Deng, "Nanocellulose-based conductive materials and their emerging applications in energy devices- a REVIEW," *Nano Energy*, vol. 35, pp. 299–320,2017.

[8] [8] T. Bashir, A. Shakoor, E, Ahmad, M. Saeed, N. A. Niaz, and S. K. Tirmizi, T., "Structural, morphological, and electrical properties of polyaniline Fe_2O_3 nanocomposites," *Polymer Science Series B*, vol. 57, pp. 257-263, 2015.

[9] D. Behne, H. Weiler, A. Kyriakopoulos, "Effects of selenium deficiency on testicular morphology and function in rats," *J. Reproduction and Fertility*, vol. 106, no. 2, pp. 291-297, 1996.

[10] A. H. Hussain, "The Effect of Sodium Selenite on Fertility of Albino Male Mice Treated with Hexavalent Chromium," *Baghdad Science Journal*, vol. 11, no. 4, pp. 1434-1440, 2014.

[11] J. Stejskal, R. G. Gilbert, "Polyaniline. Preparation of a conducting polymer". *Pure and applied chemistry*, vol. 74, no. 5, pp. 857–867, 2002.

[12] D.K. Bandgar, S.T. Navale, S.A. Vanalkar, "Synthesis, structural, morphological, compositional and electrical transport properties of polyaniline/α-Fe_2O_3 hybrid nanocomposites". *Synthetic metals*, vol. 195, pp. 350-358, 2014.

[13] B. Yazdani, L. Nikzad, M.R. Vaezi. "Synthesis of $CoFe_2O_4$-polyaniline nanocomposite and evaluation of its magnetic properties". *International Journal of Engineering*, vol. 22, no. 4, pp. 381-386, 2009.

[14] A. Sarkar, P. Ghosh, A. K. Meikap, et al. "Electrical-transport properties of iodine-doped conducting polyaniline". *Journal of applied polymer science*, vol. 108, no. 4, pp. 2312-2320, 2014.

[15] W. Yan, X. Shuangxi, J. Shengyu, Z. Teili, and Z. Chun, "Preparation of polyaniline/Fe_2O_3 composite dispersions in the presence of dodecylbenzene sulfonic acid. *e-Polymers*, vol. 7, no. 1, 2007.

[16] S. Umare, B. H. Shambharkar. "Synthesis, characterization, and corrosion inhibition study of polyaniline-α-Fe_2O_3 nanocomposite". *Journal of applied polymer science*, vol. 127, no. 5, pp. 3349-3355, 2013.

[17] S. L Patil, M. A Chougule, et al., ″ Fabrication of polyaniline-ZnO nanocomposite gas sensor″. *Sensors & Transducers Journal*, vol. 134, no. 11, pp. 120-13, 2011.

[18] F. Tudoract, M. Grtgora, "Study of polyaniline-iron oxides composites using for gas detection". *Optoelectronic. Adv. Mater*, vol. 4, pp. 43-47, 2010.

[19] M. Ghavami, M. Z. Kassaee, R. Mohammadi, M. Koohi, B. N. Haerizadeh, "Polyaniline nanotubes coated with TiO2γ-Fe2O3graphene oxide as a novel and effective visible light photocatalyst for removal of rhodamine B from water," *Solid state sciences*, vol. 38, pp. 143-149, 2014.

[20] K. Kumari, M Capstick, A M Cassara, M Herrala., et al. "Effects of intermediate frequency magnetic fields on male fertility indicators in mice". *Environmental research*, vol. 157, pp. 64-70, 2017

[21] F. A Quinteros, A. Poliandri, Leticia., et al. “In Vivo and in Vitro effects of chromium VI on anterior pituitary hormone release and cell viability”. *Toxicology and applied pharmacology,* vol. 218, no. 1, pp. 79-87, 2007.
[22] T Watanabe, A Endo, Akira. “Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice”. *Mutation Research Letters*, vol. 262, no. 2, pp. 93-99, 2000
[23] H. W. Chen, T. Chiang, C. Y. Wang, C. K. Lii, et al. “Inhibition of tert-butyl hydroperoxide-induced cell membrane bleb formation by α-tocopherol and glutathione”. *Food and chemical toxicology,* vol. 38, no. 12, pp. 1089-1096, 2000
[24] Edith I. Yslas, Pablo Cavallo, Diego F. Acevedo, César “Cysteine modified polyaniline films improve biocompatibility for two cell lines”, *Materials Science and Engineering*, vol. 51, pp. 51-56, 2015.
[25] Henkel, Ralf; Samanta, Luna; “Oxidants, antioxidants, and impact of the oxidative status in male reproduction”. Academic Press, 2018. Copyright © 2018 Elsevier Inc. All rights reserved.
[26] Watanabe, Toshiaki; Akira. “Effects of selenium deficiency on sperm morphology and spermatocyte chromosomes in mice”. *Mutation Research Letters*, vol. 262, no. 2, pp. 93-99, 2010.
[27] E. Ernst, J, P. Bonde, Jens Peter. “Sex hormones and epididymal sperm parameters in rats following sub-chronic treatment with hexavalent chromium”. *Human & experimental toxicology*, vol.11, no. 4, pp. 255-258, 2014.