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Effectiveness of Fe2O3 nanoparticles more than magnetic field against the destructive effect of colchicine on *Paramecium caudatum*

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Abstract

Background and Objective: Colchicine depolymerizes microtubules and reduces their diamagnetic capacity. We investigated the effect of a static magnetic field (SMF) and the Fe2O3 NPs on the *Paramecium caudatum* exposed to colchicine.

Materials and Methods: The samples were collected from temporary water sources and after identifying the species, were cultured in the laboratory. They were also sub-cultured at regular intervals (7-10 days) for purification. From pure culture, several samples were placed in the laboratory or under SMF with an intensity of 61 mT for 3 days. To evaluate the effect of materials in comparison with distilled water (control), a sample (0.1 ml) of each medium was placed on a slide and exposed to 1 µl of distilled water or volume-concentrations of colchicine (0.05 to 25 µg/µl) or Fe2O3 NPs (0.05 to 3 µg/µl). The effect of different concentrations of Fe2O3 NPs in accompany with the field was also investigated. The movement of the animal was examined for 30 sec under a constant view and the sample was fixed for staining and study at the cellular level. Data were analyzed using analysis of variance.

Results: Colchicine at high concentrations (15 and 25 µg/µl) significantly reduced the motility of *Paramecium caudatum*. Magnetic field, but not NPs, alone reduced cell motility. Co-exposure to particles completely improved cell motility due to exposure to colchicine, however, in animals initially housed in the magnetic field, the healing effect of the particles was impaired.

Conclusion: The protective effects of NPs may depend on the diamagnetic capacity of the microtubule.

Keywords: *Paramecium caudatum*, Colchicine, Static magnetic field, Fe2O3 nanoparticles

1. Introduction

Parasitic diseases such as malaria, especially in tropical and subtropical regions, affect human health and survival, and the main way to control most of them is to use antiparasitic drugs. However, these treatments are ineffective in many cases and in some cases the condition gets worse due to drug resistance (1).

Microtubules are the major part of the cytoskeleton in almost all eukaryotic cells. In addition, they are the target of many drugs, especially anti-cancer drugs (2). According to recent findings, the microtubule network is a key component of insulin secretion from pancreatic beta cells, which is essential for blood glucose homeostasis (3).

We are familiar with these structures (microtubules) from the past. They play the most important role in the

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formation of mitotic spindles during cell division. The spindle consists of microtubules, microtubule-related proteins, and motor proteins (4). These are some of the findings, but we are more surprised by recent discoveries.

Microtubule inhibitors (such as colchicine) have long been used for chemotherapy, but today they are restricted and used only in the treatment of diseases such as Mediterranean fever, gout, and Behcet's disease (5). Reducing damage to microtubule structures will certainly help us accelerate the development of low-risk therapies. One of these low-risk methods is the use of electromagnetic fields (5), which is our goal. Some authors have written that non-invasive frequencies of electromagnetic waves can improve benign prostate cancer (6).

In this laboratory, the effect of colchicine on the motor system of *Paramecium caudatum* has been investigated and the destructive effect of this substance on its microtubule complex has already been shown. The aim of this study was to investigate the effect of magnetic field with or without Fe2O3 nanoparticles (NPs) on damaged microtubules due to colchicine neurotoxin. A comparison was made between control samples treated with distilled water which is not harmful and samples treated with colchicine.

2. Materials and Methods

2.1. Tested organisms

The tested organisms in this study were *Paramecium caudatum*. This single cell animal was collected from temporary water sources on the campus of Shahed University located on the Persian Gulf Highway. It was specifically identified and then placed in a suitable culture medium (hay-infusion) and passaged weekly. The cells were fed and grown in this medium and after about 3-4 months (due to successive passages for purification), a pure culture medium rich in *Paramecia* was achieved and used to continue the research.

2.2. Materials used

Material used in this study: Colchicine (Merck - Germany) in doses of 0.05, 1, 5, 15 and 25 micrograms, Fe2O3 nanoparticles (donated by Dr. Hajnorouzi- Department of Physics, Shahed University) in doses of 0.05, 0.1, 1 and 3 micrograms. Silver Nitrate (Merck - Germany), and Hematoxylin (Farzaneh Arman Co, Tehran, Iran) were also used in staining.

2.3. Synthesis and characterization of Fe2O3 nanoparticle

The sonoelectrochemical method, which is a common method for the production of pure metal nanopowders, was used to synthesize nanoparticles (7). Also, nanoparticle identification analyses were performed by powder X-ray diffraction (XRD) and field emission scanning electron microscopy (FESEM) (8).

2.4. Preparation of the natural culture medium (hay-infusion)

First, about 10 grams of hay was poured into 500 ml of boiling water and heated for about 5 min. The resulting culture medium was then placed in smaller glass jars and allowed to cool. Then, with the help of Pasteur pipette, the desired size of single cell samples was inoculated into fresh culture medium. The expected time for the optimal growth rate of single cells in each culture medium was about 7 to 10 days (based on growth curve). Finally, the *Paramecia*-rich culture medium was provided and used for the experiments.

2.5. Behavioral studies (locomotor activity measurement) of *Paramecia*

Because *Paramecia* has several fixed movement patterns, we measured motor activity as a criterion for studying behavior. One of these patterns is the S-shaped motion that was considered and counted. The movements were counted at 5-sec intervals over a period of 30 sec.

2.6. Counting of complete helical (S-shaped) motion

Using a Pasteur's pipette, a volume (0.1 ml) of the single cell-rich culture medium was transferred to the slide and studied under a light microscope. After adjusting the field of view by a fixed objective lens, the number of the helical movements was counted at 5-sec intervals and recorded for 30 sec (for both control samples receiving 1 µl of distilled water and experimental samples receiving 1 µl volume-concentrations of materials).

2.7. Evaluation of the behavior of *Paramecia* after inoculation of specific doses of colchicine and nanoparticles (NPs)

The samples were divided into two groups to evaluate the effect of the drugs used in this experiment (colchicine and NPs): the samples without exposure to magnetic field received colchicine alone or colchicine and NPs (Fe2O3). We also provided samples that were first exposed to a magnetic field and then, received either colchicine alone or a combination of NPs and colchicine. For sampling a constant volume (0.1 ml) of medium rich in *Paramecium caudatum* was placed on the slide and distilled water (1 µl) or volume-concentration of material was added to study the result. Control (the distilled water) or each dose of the substance was studied 25 times. In the case of Fe2O3 NPs, due to the magnetic nature of these

particles, after preparing the desired concentration, the mixture was first ultrasonicated (10 min) to prevent adhesion of the NPs and then immediately added to the sample. Simultaneously with inoculation of the drugs, spiral movements and other performances were recorded at intervals of 5 sec for 30 sec.

2.8. Investigation of the behavior of *Paramecia* under exposure to static magnetic field (SMF) and nanoparticles (NPs)

In this section, a sample of a single-celled organism medium that was first exposed to the field for 3 consecutive days was tested by NPs to investigate the kinematic behavior of *Paramecia* exposed to NPs. Field-tested specimens were also exposed to NPs and colchicine simultaneously. Sampling was performed in the same manner as in other experiments, and spiral motions were recorded at 5-sec intervals for 30 sec.

2.9. Magnetic field used for treatment

The fixed magnetic field used in the experiment consists of two permanent ring-like magnets with an outer diameter of 12 cm, an inner diameter of 6 cm and a thickness of 2 cm. When the two rings were placed on top of each other, a magnetic flux (60 mTesla) was established between them, which was probed using a Teslameter.

2.10. Dry silver nitrate staining of silver-line system

The classic method is called dry silver nitrate because the sample is first impregnated with silver nitrate and then decolorized with sunlight after placing the slide on a porcelain plate. In this method, the sample exposed to the dye should be examined regularly under a microscope to ensure differentiation.

2.11. Hematoxylin staining

The slides (covered by a 0.1 mL sample) were first dried in the laboratory and washed with tap water. They were then exposed to 96%, 70% and 50% alcohol solutions, respectively, and then rapidly passed through distilled water. The slides were then immersed in hematoxylin dye (diluted 1: 4 with distilled water) for 15 min and washed with tap water and placed in 50%, 70% and 96% alcohols, respectively. The slides were then placed in xylol and immediately mounted by Entellan (Merck, Germany) and covered with coverslip. After drying, the slides were examined under a light photomicroscope.

2.12. Statistical Analysis

Raw data were analyzed using SPSS software (version 19). In order to increase statistical accuracy and reduce deviation, each experiment was done repeatedly (at least 25 times). All data were first analyzed by normality test. The variance was calculated and compared and a *post hoc* test was performed to show the differences between the groups.

3. Results

3.1. Size and shape of Fe2O3 nanoparticles (NPs)

The size range of NPs was 33-45 nm and their shape was round according to the field emission electron microscope (FESEM) image. (Fig. 1).

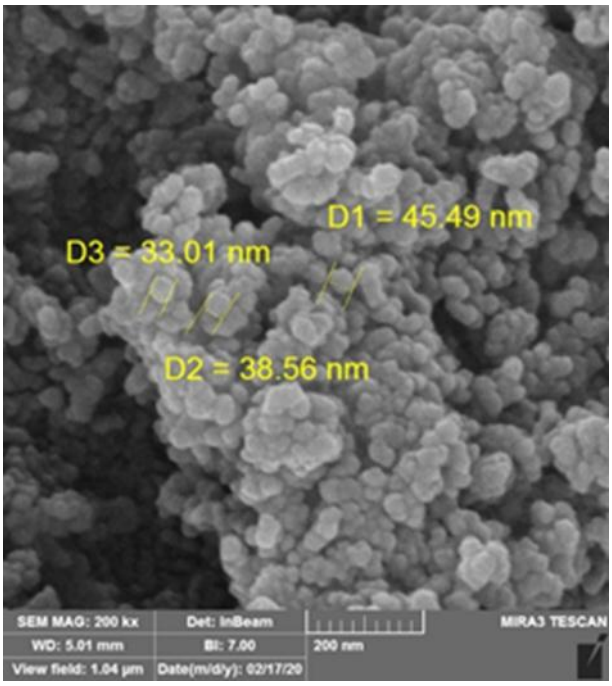

Fig. 1. Image of nanoparticles (NPs). The field emission electron microscope (FESEM) image of Fe2O3 NPs is visible. As shown, the particle size is between 33 and 45 nm and its shape is round.

3.2. Confirmation of the *Paramecium* species

This species has several unique characteristics such as the presence of two nuclei, bean-shaped macronucleus and compact micronucleus, long caudal cilia with a length of more than 170 µm, as well as the forward spiral movement (Fig. 2).

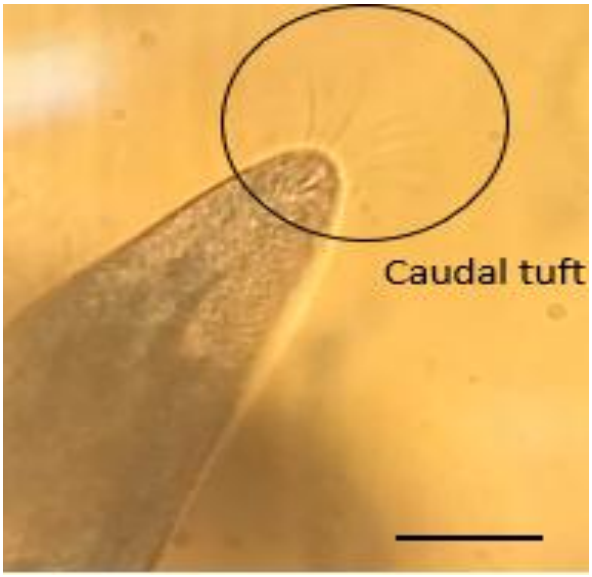

Fig 2. Caudal tuft of cilia. This image shows a key character (longer caudal tuft of cilia) that is crucial to identifying the animal (Paramecium caudatum). Line shows 25 µ.

3.3. Movement of *Paramecium caudatum*

The effect of static magnetic field (SMF) on motor activity of *Paramecia* was not significant in comparison with laboratory conditions and in both cases motor activity decreased due to exposure to different doses of colchicine (0.05, 1, 5, 15 and 25 µg/µl), especially at higher concentrations (15 and 25 µg/µl) (Fig. 3). However, due to the presence of NPs (0.05 to 3 µg/µl), this activity improved, , although these particles had a weaker healing effect in the presence of the field, indicating a correlation between the protective effects of nanoparticles and the diamagnetic capacity of microtubules.

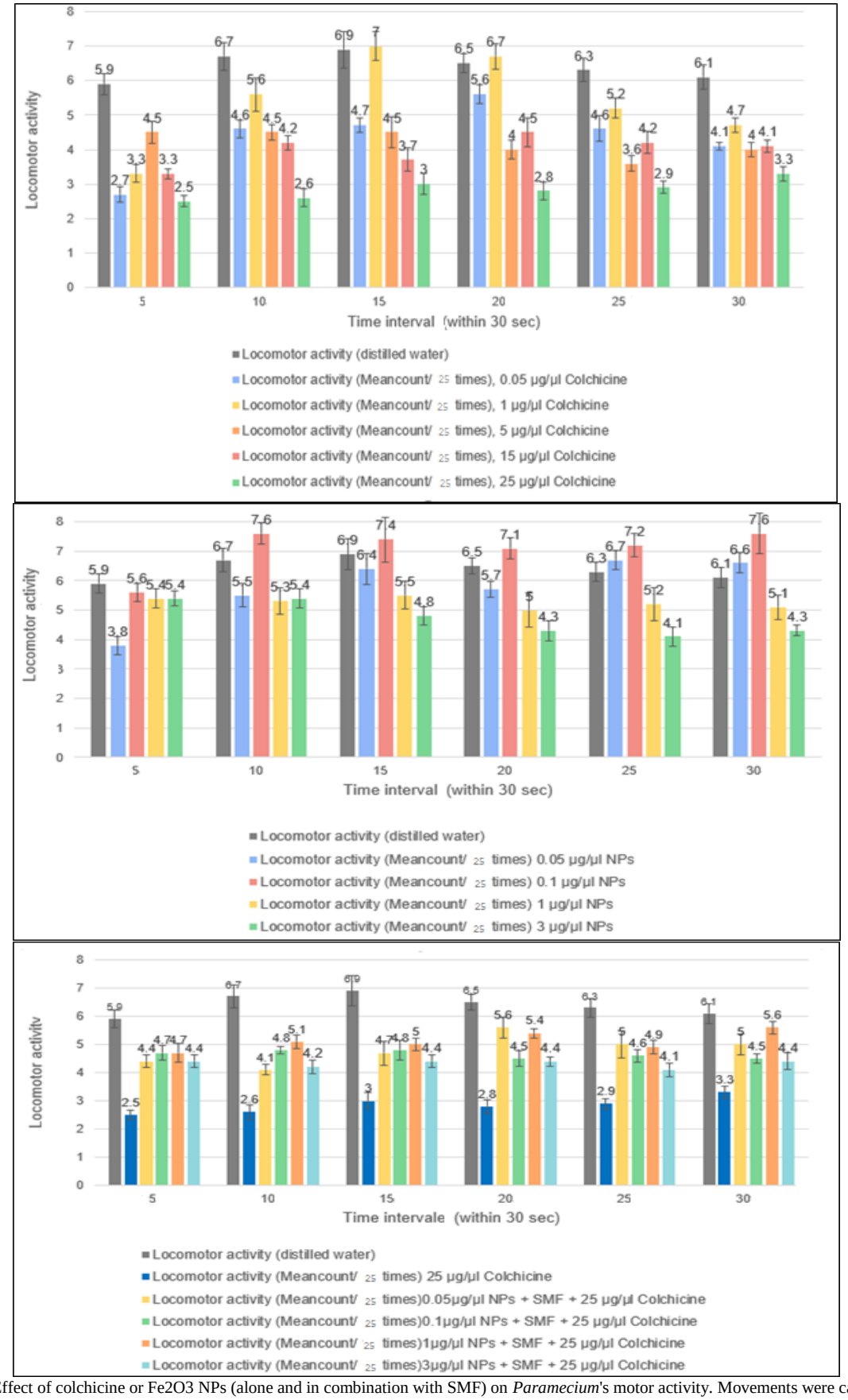

Fig 3. Effect of colchicine or Fe2O3 NPs (alone and in combination with SMF) on *Paramecium*'s motor activity. Movements were calculated in 25 experiments for 30 sec at 5-sec intervals. There is a significant reduction effect of colchicine on motor activity (up). The NPs alone increased the kinetic activity (middle) and also partially improved the weak activity due to colchicine (down) in the presence of colchicine and the magnetic field.

3.4. Neuromotor system staining with silver nitrate

Neuromotor system in seven groups (control, field, different doses of colchicine, different doses of NPs, NPs + colchicine and NPs + field + colchicine) was stained with dry silver nitrate method and the percentage of degradation was calculated in each case: In the control sample, 7% of the population showed degradation, under different doses of colchicine, different rates of degradation were observed, indicating a correlation with doses of colchicine (up to 27.41% at higher doses). NP alone had a destructive effect (Up to 12% at the highest dose) but the field alone showed little effect, however it neutralized the colchicine effect in the presence of NPs (Fig. 4).

Fig 4. Motor system degradation. This image shows the effect on the structure of the *Paramecium* locomotor engine. There is a significant destructive effect by colchicine: the sample treated with colchicine (right) is compared with the control (left). Line is 25 µm.

3.5. Nuclear system staining with hematoxylin

No significant changes were observed in any of the experiments except for the colchicine (Fig. 5).

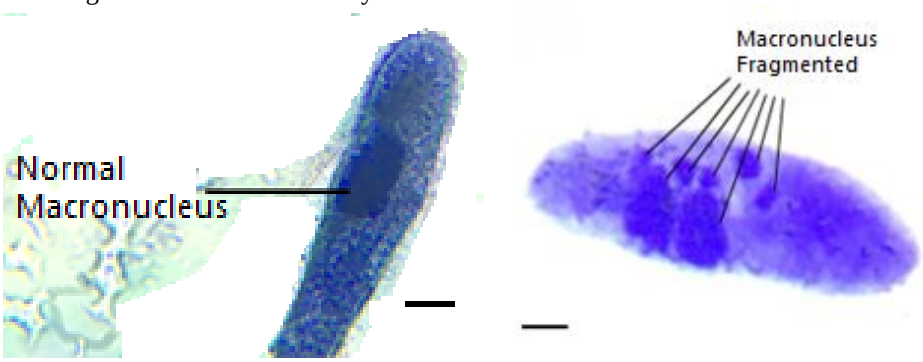

Fig 5. Nucleus fragmentation. This image shows the effect on the nucleus of *Paramecium*. There is a significant effect by colchicine on this structure: colchicine-treated case (right) is compared with control (left). Line is 25 µ.

4. Discussion

This animal model was exposed to Fe2O3 NPs and a magnetic field to investigate a possible interaction with colchicine for the first time. The study was conducted in several stages. In the first stage, *Paramecia* were observed live and their free movements were recorded. In the second step, the treated samples were dried and stained with silver nitrate and hematoxylin. In the next step, the interaction effect of magnetic field and NPs on colchicine-treated *Paramecia* was studied and more innovative data were presented.Colchicine is a well-known alkaloid substance that has been used for many years to treat inflammation (since 1763). It has also been introduced in 1972 as an effective drug to treat the symptoms of gout and Mediterranean fever (9).

It has long been used in clinics, but it has recently been found that colchicine C-ring is highly toxic and prevents microtubule polymerization. In addition, mescaline, a methoxyphenyl analogue of colchicine A-ring, stops the accumulation of microtubules, therefore, the use of colchicine is very limited due to damage to the cytoskeleton (10).

It is true that *Paramecium* is a single-celled organism, but due to the multi-complex microtubules formed in

different parts of its body (many cilia, cytoskeleton, and spindle) and the similarity of its microtubule structure to the axoplasmic microtubule, it can be adapted to show the harmful effects of colchicine to neural structures. Many studies have been completed so far on the effect of colchicine and other substances on *Paramecium*. There are reports that a small amount of colchicine has a positive effect on growth and division of this animal (11). But another group of experiments have introduced colchicine as an antiparasitic drug due to its destructive effect on protozoa (12). So, it seems that the alkaloid colchicine can have different effects on the structure and life of these organisms. In addition, we know that this freely living animal, *Paramecium*, reacts to electric and magnetic fields by showing alignment in the field direction (13). Therefore, this organism can be modeled to study the destructive effect of colchicine on neuronal structures in the presence of therapeutic candidates such as nanoparticles and magnetic field.

In this study, we showed that the velocity of the *Paramecium* in the face of the magnetic field was significantly reduced compared to the control sample. Considering the magnetic anisotropy of the microtubules and the bonding of the tubulin subunits to form the tubular structure (14), we may assume that magnetic waves can amplify this property and thus protect the tubulin structure against colchicine. However, current data suggest that placing the animal in a magnetic field prior to colchicine exposure does not prevent colchicicne-induced immobilization. Therefore, another goal was considered: to use NPs together with the field.

NPs are of great importance in medical science due to their special structural properties and are used as vectors for proteins and DNA fragments as well as in targeted drug delivery in the treatment of cancer (15).

The magnetic susceptibility of NPs increases with the presence of a magnetic field, and the same property is used in the destruction and treatment of tumors (16).

Paramecium caudatum responds quickly to environmental stimuli because it is very sensitive to ecological changes of any kind (heat, cold and chemicals) and this feature makes it the best tool for assessing the destructive effects of colchicine on cell movements alike the nervous system under live state. Although the *Paramecium* changes its motor behavior abruptly at the first moment of inoculation (shows avoidance), it adapts to the disturbances in a little fraction of the time and then over a period of time (e.g. 30 sec) shows the definitive response. However, in order to increase accuracy, a large number of *Paramecia* should be tested in a sample.

We hypothesized that the diamagnetic properties of Fe2O3 NPs would increase due to the organism's previous exposure to the magnetic field, and that these particles would be more effective against the destructive effect of colchicine on *Paramecium caudatum*. Therefore, we first exposed a group of animals to a magnetic field in the presence of NPs for a few days and then exposed them to colchicine, but surprisingly, the protective effect of the particles diminished.

In summary, the protective effects of nanoparticles may depend on the diamagnetic capacity of the microtubule, and that these particles alone are more useful than the field.

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Conflict of interests

There is no conflict of interests.

References

1. Fennell B, Naughton JA, Barlow J, Brennan GP. Microtubules as antiparasitic drug targets. Expert Opinion on Drug Discovery 2008;3(5):501-18.
2. Trogden KP, Zhu X, Lee JS, Wright CVE, Gu G, Kaverina I. Regulation of glucose-dependent golgi-derived microtubules by cAMP/EPAC2 promotes secretory vesicle biogenesis in pancreatic b cells. Current Biology 2019;29:2339-50.
3. Prosser, SL, Pelletier L. Mitotic spindle assembly in animal cells: a fine balancing act. Nature Reviews Molecular Cell Biology 2017;18:187-201.
4. Qiang L, Sun X, Austin TO, Liu M, Yu W, Baas WP. Tau does not stabilize axonal microtubules but rather enables them to have long labile domains. Current Biology 2018;28:1-9.
5. Lu Y, Chen J, Xiao M, Li W, Miller DD. An overview of tubulin inhibitors that interact with the colchicine binding site. Pharmaceutical Research 2012;29(11):2943-71.
6. Tenuta M, Tarsitano MG, Mazzotta P, Lucchini L, Sesti F, Fattorini G, et al. Therapeutic use of pulsed electromagnetic field therapy reduces prostate volume and lower urinary tract symptoms in benign prostatic hyperplasia. Andrology 2020; 8(5):1076-1085. DOI: 10.1111/andr.12775.
7. Sáez V, Mason TJ. Sonoelectrochemical synthesis of nanoparticles. Molecules 2009;14:4284-99. doi:10.3390/molecules14104284.

8. Kumar Yadav V , Ali D, Heena Khan S , Gnanamoorthy G , Choudhary N , Kumar Yadav K, et al. Synthesis and characterization of amorphous iron oxide nanoparticles by the sonochemical method and their application for the remediation of heavy metals from wastewater. Nanomaterials 2020;10;1551. doi:10.3390/nano10081551.
9. Leung YY, Hui LLY, Kraus VB. Colchicine--Update on mechanisms of action and therapeutic uses. Semin Arthritis Rheum 2015;45(3):341-50.
10. Bhattacharyya B, Panda D, Gupta S, Banerjee M. Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin. Medicinal Research Reviews 2008;28(1):155-83.
11. Gray R, Gray A, Fite JL, Jordan R, Stark S, Naylor K. Simple microscopy assay to teach the processes of phagocytosis and exocytosis. CBE: Life Sciences Education 2012;11(2):180-86.
12. Fennell BJ, Naughton JA, Barlow J, Brennan G, Fairweather I, Hoey E, McFerran N, Trudgett A, Bell A. Microtubules as antiparasitic drug targets. Expert Opinion on Drug Discovery 2008;3(5):501–18.
13. Guevorkian K, Valles JM. Aligning *Paramecium caudatum* with static magnetic fields. Biophysical Journal 2006;90(8):3004–11.
14. Guevorkian K, VallesJr JM. Aligning *Paramecium caudatum* with static magnetic fields. Biophysical Journal 2006;90(8):3004-11.
15. Wang YXJ, Hussain SM, Krestin GP. Superparamagnetic iron oxide contrast agents: Physicochemical characteristics and applications in MR imaging. European Radiology 2001;11(11):2319–31.
16. Schwaminger SP, Fraga-García P, Eigenfeld M, Becker TM, Berensmeier S. Magnetic separation in bioprocessing beyond the analytical scale: From biotechnology to the food industry. Frontiers in Bioengineering and Biotechnology 2019 Doi.org/10.3389/fbioe.2019.00233.