Abstract
Brain neuronal disorders have the least outcomes to therapeutic response due to their complex and delicate integration and lack of drug response upon damage in addition to difficult surgical approach to CNS. These properties of brain cortical diseases elaborate more trials of finding an effective and safe alternative treatment. Making a full use of the high responsiveness of neurons to the electrical currents induced with magnetic fields allows an appreciable therapeutic approach to be evaluated. In a trial of assessment of the neuronal repair effects of a time varying external magnetic field, rabbit model of stroke was arranged with 10 minutes occluding the left middle cerebral artery of the anesthetized rabbit. A follow up with right hind limb spasticity, EEG and eosin histopathological assessment of neuronal reconnections in response to applying a 1000 Hz and 1000 gauss magnetic flux density provided by an external solenoid magnet in comparison with untreated control group along 30 days of daily applying the field on the left frontal lobe over the scalp for 30 minutes. There was a significant reduction of spasticity score from 2.76 +/- 0.1 to 1.33 +/- 0.2 obtained with extracranial magnetic stimulation ECM; P< 0.05 as compared with untreated group. Similar improvements in EEG criteria were also recorded in ECM treated group in form of an increase in EEG amplitude from 4 microV to 32 microV whereas brain frequency over the affected area had significantly increased from 2 Hz to 9 Hz at P< 0.05. Histopathological assessment of the number of neuronal reconnections revealed 355 +/- 21 per high power field in comparison with just 105 +/- 19 in untreated group. In conclusion there was a promising neuronal repair enhancement by applying a suitable extracranial magnetic field that could improve the therapeutic outcomes of different treatments.

Keywords: Stroke, extracranial magnetic stimulation ECM, neurogenesis
Introduction

Magnetic field had long been verified to induce energy in different materials. Conductive silks had widely been applied in interchanging magnetic and electrical energy. Living system has a special form of electrochemical circuits especially in excitable tissues like neurons [1]. These cells being connected in the brain in form of biological integrated circuits that are composed of connectors (the axonal and dendritic processes), capacitors (the lipid bilayer of cell membranes), resistors (the chemical resistance of cellular and extracellular fluids) in addition to electrical generators which are the electrolytes pumps in cell membranes [2]. When an external alternating magnetic field is applied on neurons it will interfere with their electrochemical properties. Magnetism generates a voltage in tissue according to the equation:

\[ V = n \times a \times \frac{dB}{dt} \]

where:
- \( V \) = Voltage
- \( n \) = number of turns in the electromagnetic coil
- \( a \) = area of the loop
- \( \frac{dB}{dt} \) = the rate of change of magnetic field with respect to time
- \( B \) = the strength of the magnetic field (in Teslas) [3].

According to right hand rule anions like Cl, HCO\(_3\) ions and free electrons will respond to that magnetic field in an alternative dissociations from protons, Na, K, Ca or magnesium cations [4]. Variations of these electrolytes across the excitable neuronal cell membrane will trigger a phenomenon called cell membrane plasticity which become more noticeable even histologically if exposed for prolong time depending on the magnetic field intensity and frequency [5,6]. Plasticity is characterized by rearrangement of neuronal membrane components [7] with contribution of elastin, actin, spectrin and myosin structural proteins in addition to changing gene expression of ion channels like Na and Ca channels and enzyme activity like proteases [8] lipases that will change level of neuronal membrane sphingolipids and cholesterol [9]. These modulations in neurons lead to evagination of the neuronal membrane and according to the period of applied magnetic field processing will continue toward the direction of the applied field until a new complete neuronal process is formed. This process is called dendrogenesis if dendrocyte is synthesized or axonogenesis in case of axonal connection. These responses are controlled and induced form of functional neurogenesis otherwise neurogenesis is the main intended mechanism upon treating any neuronal damage like ischemic stroke [10].

Materials and Methods

Instrumentation included

1- Electroencephalograph of (Physiograph EEG MK III. US version) set at 10 microVolts/small square sensitivity, 1cm/min. with 4 channels.
2- Dissecting set
3- Microtome and histopathological set with eosin stain technique.
4- Computerized extracranial magnetic stimulator.

The research had been conducted in pharmacology laboratory of College of Pharmacy in Kufa University.
Animals
Eighteen local domestic rabbits were divided into 3 groups, N = 6 for each. They were males aging 3-4 months and 1-1.5 kg average body weights. All rabbits were bred in standard breeding cages with supplement of water and standard oxoid diet freely. Under general anesthesia with intraperitoneal phenobarbital 100 mg/kg, two of these groups were prepared for induction of stroke. The skull bones over the left frontal lobes were incised to expose the meninges and brain through which the origin of the middle cerebral artery is clamped with a small forceps for 10 minutes a period during which an ischemic stroke is established [11,12]. The skull bone is then ligated with 4 small steel clips whereas scalps is ligated with silk stitches. This process was completely done under aseptic condition with fucidic acid and 20% iodine. Spasticity of fore and hind limbs were obvious by examination one day after induction of stroke.
The electroencephalography EEG was monitored for the left FC portion (the wave of the stoked brain area) soon after induction to measure the frequency (in Hz) and amplitude (in microV) as a prognostic indicator of the neurogenesis process after neuronal insult with the induced ischemia and once weekly measured then after with concomitant assessment of the clinical severity of right hind limb spasticity [13,14].
The later sign was graded to normal = 0, mild = 1, moderate = 2 and severe = 3.
Extracranial alternating magnetic ECM field was applied over the ischemic left frontal motor gyri of the brain for 30 minutes once daily for 30 days. It was guided under computer program for provision of higher frequencies of alternation and adjusted to 1000 guass of 1000 Hz from a solenoid magnet and amplifier. The magnetic probe was set directly over the scalp with heat monitoring. Control group of rabbits were giving nothing apart from clinical, EEG and histopathological assessment.
After the 30 days of treatment with ECM all rabbits were submitted to autopsy histopathological sectioning and examination. The staining used to evaluate neuronal connections was silver impregnation procedure to reveal the dendrites and axons under high power field light microscopy.

Figure 1 the complete set of extracranial magetic stimulator with output computerized signal generator.
**Results**

Table 1 the mean spasticity score of the rabbits left hind limbs. A comparism between the untreated group and ECM treated group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>The mean rabbits hind limb spasticity score of clinical assessment</th>
<th>Statistical test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 30</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Induced untreated</td>
<td>2.86 +/- 0.1</td>
<td>2.66 +/- 0.2</td>
</tr>
<tr>
<td>Magnetic field treated</td>
<td>2.86 +/- 0.2</td>
<td>1.33 +/- 0.3</td>
</tr>
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A significant decrease in spasticity score was noticed upon treating stroked rabbits with 1000 Hz, 1000 gauss solenoid magnet extracranially for 30 days at P< 0.05.

![Figure 2](attachment:image.png) the EEG wave amplitude response of treatment with magnet. There was a significant increase in amplitude from 4 microV to 32 microV when stroke rabbits were treated with ECM for 30 days.
A significant increase in the treated group from 2 Hz theta wave to 9 Hz alpha wave at the left frontal gyri was obtained.

Table 2: the mean number of neuronal connections estimated per high power field among the magnetic field treated and the control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>The mean number of neuronal connections per high power field at the left frontal gyrus after autopsy histopathological examination at day 31</th>
<th>Statistical t test of significance at P&lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy group</td>
<td>1025 +/- 130</td>
<td>-</td>
</tr>
<tr>
<td>Untreated group</td>
<td>105 +/- 19</td>
<td>-</td>
</tr>
<tr>
<td>ECM treated</td>
<td>355 +/- 21</td>
<td>significant</td>
</tr>
</tbody>
</table>
Figure 4 the number of neuronal connections estimated under HPF with silver impregnation as an indicator of neuronal repair mechanism enhanced with ECM. There was a significant reconnection upon treatment with ECM 355 +/- 21 as compared with control untreated 105 at P< 0.05.

Discussion

Central neuronal damages whether arise from stroke, tumor, distress, toxin, trauma or infection, all are characterized by distortion of the highly integrative neuronal functions and structures that interventions with conventional antiplatelet, vasodilator or thrombolytic drugs have unreliable curative outcomes [15] at the same time surgery has just a narrow space of application except in subarachnoid hemorrhages and tumors. On the other hand neurons although not practically replicating, they have latent capacity of dandrogenesis and axonogenesis over the impaired region to replace 20-40% or even more of the damaged area [16].
Moreover, this neurogenesis is highly responding to any electrical activity so that the rate of repair will be significantly accelerated upon an interneuronal electric currents induced from external electric or magnetic fields [17]. This principle was considered in many studies to treat many higher CNS lost functions like autism, hearing loss, dysphasia, hemiplagia and even schizoaffective disorders by applying a time varying (alternative) magnetic field with promising improvements [18,19] and safety. Many studies have shown acceptable assurance regarding mutagenicity and side effects of externally applied magnetic field [20] to the level that different magnets become commonly applied in the orthopedics wards to promote bone fractures healing. However, in CNS, different frequencies (upto hundreds of kilohertz) and intensities (10 gauss to fraction of tesla) are required to achieve effectiveness and safety [21]. Other requirements of application include magnets direction and duration of stimulation. The most common forms of treatment with magnetic field include setting of a specific form (mainly solenoid measured in henry) magnet extracranially over the affected region with power supply of specific frequency[22].

In this current study, different parameters of motor neuronal damage were considered. Spasticity reflect the sufficiency of cortical inhibitory neuronal function that will be lost upon stress [23] giving rise to lead pipe spasticity and could be scored to mild, moderate and severe in order to quantitatively assess the curative activity of different treatments [24]. There was a significant reduction in spasticity score upon treatment with 30 minutes of 1000 gauss solenoid magnet at 1000 Hz applied on the left frontal region of the stroked rabbits in comparison with untreated control group at P< 0.05 (t test ) that decreased spasticity score from mean of 2.66 +/- 0.2 to 1.33 +/- 0.2 after 30 days of treatment similar findings regarding the improvement of spasticity score were obtained in other study on 38 patients with multiple sclerosis[25].

In regard to EEG records, the infarcted damaged neurons lose their electrical wave characters in EEG records so that frequency mode will decrease from beta or alpha to theta or delta at about 4 hertz as well as, amplitude will also decrease down to few micrVolts. Magnetic field treated group showed a significantly regaining brain frequency from 2 Hz to 9 Hz in comparison with the untreated stroked rabbits P< 0.05; figure (2). Similar findings were obtained in evaluating the EEG amplitude which have been significantly rised from 4 microV to 32 microV at the end of extracranial magnetic ECM stimulation at P< 0.05 (standard t test); figure (3). One study that correlated the EEG findings in human exposed to transcranial magnetic stimulation has disagreed with EEG results of this study in that slowing of EEG waves have been obtained from magnetic stimulation of human motor cortex, however, this contradiction may be attributed to application on normal human in comparism with stroked rabbits in this study [26]. The most reliable definite finding of ECM treatment is the significant increase in number of reconnections (dandrogenesis and axonogenesis) estimated after silver impregnation staining of the autopsy histopathological slides for the damaged left frontal areas in comparison with the healthy and control untreated groups at P< 0.05 table (2), that ECM stimulation enhance neuronal reconnections 355 +/- 21 in the ECM treated group as...
compared with 105 +/- 19 connection in the untreated group. A study on estimating the rat supraventricular zone neurogenesis stimulation with magnetic field stimulation have showed that there was a significant neuroproliferative effect in the group exposed to magnetic stimulation [27] a result nearby rabbit cortical neurogenesis finding of this study.

**Conclusion**
Extracranial time varying magnetic stimulation is a beneficial therapeutic alternative of treating different neuronal damages that could be applied specifically as an adjuvant treatment for many CNS diseases in human.

**Recommendation**
A further evaluation of effectiveness and safety of different intensities, frequencies and wave forms of magnetic field is to be tried in different neuronal and systemic human diseases.

**References**
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