Nanoparticle based modulation of microwave radiation induced alterations in reproductive parameters of male Wistar rats

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Abstract
Microwaves (MW) are a type of non-ionizing electromagnetic radiation with various applications. It causes oxidative stress in testes and may affect fertility potential of male reproductive system. BSA conjugated Manganese dioxide nanoparticles (MNP*) have antioxidant mimicking activity that may decrease or prevent the oxidative stress in cells. In the present study male Wistar rats were exposed to MW and MNP* and their affect was studied on the testes and sperm parameters. We observed that MNP* reduced the oxidative stress induced damage by MW. Hence, MNP* function as a potential modulator of MW induced oxidative damage.

1 Introduction
Microwaves (MW) are electromagnetic waves that range in the frequency range of 300 MHz to 300 GHz. They are used widely in broadcasting, radar, mobile phones, their base stations, Bluetooth devices and Wi-Fi [1]. Reactive oxygen species (ROS) are highly reactive, unstable oxygen containing atom or molecule. Overproduction of ROS or their precursors generate oxidative stress which leads to lipid, protein and DNA damage in cells. To combat these ROS, cell produces enzymes called antioxidant enzymes, that protect cells by scavenging ROS. MW has been shown to alter male reproductive parameters and the alteration has been reported to be oxidative stress mediated [2]. BSA conjugated Manganese dioxide nanoparticle (MNP*) has been shown to mimic the activities of antioxidant enzymes [3]. Hence, we hypothesized that MNP* can play a role in reducing the ROS levels and thus decreasing the oxidative stress mediated damages in the reproductive parameters of male rats.

2 Methodology
In vivo exposure
Wistar rats (male) were exposed to MW radiation in the exposure chamber as shown in Figure 1. Rats were placed in individual plexiglas cages and the cages were then placed in an anachoic chamber of dimension 150cm (l) × 115cm (w) × 130cm (h). Plexiglas cages were ventilated with 1 mm diameter holes. The frequency of 2 GHz and power of 7.5 dBm was set in the signal generator. During exposure period, the signal generator was switched on at the mentioned frequency and power. The calculated power density was 4.25 mW/cm². Whole body specific absorption rate (SAR) was 1.2 W/kg.

Figure 2 shows the Scanning electron microscope (SEM) image of the synthesized MNP*.
Ten weeks old male Wistar rats were divided into four groups(n=6): Control (rats that did not receive MW or MNP* exposure), MNP* exposed animals were administered with 12.5 mg/kg MNP* once a week for eight weeks. MW exposed group animals were exposed to 2 GHz for 2 hrs/day, 6 days a week, for eight weeks and MW+MNP* treatment animals were exposed to both MW and MNP*.
3 Results and Discussion

Effect of MNP* and MW on reproductive hormone

![Graphs showing testosterone, luteinizing hormone, and follicle stimulating hormone levels](image)

**Figure 3:** Effect of MW and MNP* on (A) Testosterone (B) Luteinizing hormone (C) Follicle stimulating hormone. Results are expressed as mean±SEM (n=6). Significance test was done by one-way ANOVA (Analysis of variance) followed by Tukey post hoc test. Significance between different treatment groups are represented as letters a (versus control), b (versus 12.5 mg/kg MNP*), c (versus MW). Level of significance are represented as *p<0.05, **p<0.01, ***p<0.001.

The serum of rats was assayed for reproductive hormones testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair. LH causes the testicles to make testosterone, which is important for producing sperm. FSH stimulates testicular growth. The results show that MW exposed rats had a significant decline in testosterone levels as compared to control (p<0.05) and MNP* (p<0.01) exposed rats (Figure 3 A). It is evident that the combined treatment of rats with MW and MNP*, prevented the alteration in testosterone levels as compared to the control. LH and FSH also decreased insignificantly in MW exposed rats which was reverted back in MW+MNP* exposed rats.

**Effect of MNP* and MW on antioxidant/oxidant status**

In the present study, the three important antioxidant enzymes viz, superoxide dismutase (SOD), Catalase and Glutathione peroxidase (GPx) were found to significantly decrease in MW exposed rats as compared to the control and MNP* treated rats (Figure 4A, 4B, 4C). These enzymes scavenge ROS produced in tissues. But, excessive production of ROS disrupts the antioxidant/oxidant balance in tissues exposed to MW. Cells utilize the versatility of sulfhydryl or thiol groups to protect itself from the cellular oxidants. Thus, we measured the total sulfhydryl group in this study. The results showed that the sulfhydryl group decreased insignificantly in MW exposed rats as compared to control (4D). Ferric reducing ability of plasma (FRAP) activity or total antioxidant activity of testis homogenate also decreased significantly in the MW exposed rats as compared to control and MNP* groups (Figure 4E). Malondialdehyde (MDA) is one of the products of polyunsaturated fatty acid (PUFA) peroxidation in cells. MDA concentration indicates the extent of lipid damage in cellular membrane. We found that the level of MDA was increased significantly in MW exposed rats as compared to control and MNP* exposed groups (Figure 4E). However, the MNP* modulated the effect on the antioxidant enzyme and MDA level in MW exposed rats. The group that received both the MW and MNP* showed a significant increase in the level of SOD, Catalase and GPx as compared to MW exposure alone. This may be due to the antioxidant activity of MNP*. MNP* was also able to significantly increase the total antioxidant capacity of rats in MW+MNP* group as compared to only MW exposed group. Due to an increased antioxidant status in the MW+MNP* rats, the level of lipid peroxidation decreased, as indicated by a significant decrease in MDA. The present study thus confirms that MW induces oxidative stress and lipid damage whereas MNP* shows protective effect on MW induced alterations in testis of rats.
In order to assess the mitochondrial activity of sperms, it was stained with DAB (3,3-diaminobenzidine). DAB gets oxidized by the cytochrome c complex of mitochondria and is polymerized and deposited at the complex. The percentage staining was classified as 100% stain (full sperm midpiece stained), >50% (more than half of midpiece stained), <50% (less than half of midpiece stained) and 0% (unstained midpiece). In the present study, the percentage of sperms with 0% as well as <50% stain was significantly high in MW exposed rats. In the MW+MNP* treated rats, the percentage of sperm with 100% stain increased as compared to control group. Thus, a decreased amount of sperm midpiece staining in MW exposed rats shows a decreased mitochondrial activity. However, the activity was increased in MW+MNP* treated rats.

**Effect of MW and MNP* on sperm parameters**

Table 1: Sperm parameters of different groups

<table>
<thead>
<tr>
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<th>Control</th>
<th>MNP*</th>
<th>MW</th>
<th>MW+MNP*</th>
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<tr>
<td>Sperm count (milli on/ml)</td>
<td>64.16±2.93</td>
<td>78±2.42</td>
<td>43.33±1.5</td>
<td>55.16±2.3</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>76.16±2.82</td>
<td>77.3±1.96</td>
<td>58.33±2.6</td>
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<td>HOS Test (%)</td>
<td>69.66±1.76</td>
<td>72±1.32</td>
<td>46.83±4.8</td>
<td>67.33±1.4</td>
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<tbody>
<tr>
<td>Amorphous</td>
<td>1.11</td>
<td>0.44</td>
<td>2.83</td>
<td>1.44</td>
</tr>
<tr>
<td>Hookless</td>
<td>0.61</td>
<td>0.61</td>
<td>1.55</td>
<td>0.66</td>
</tr>
<tr>
<td>Banana</td>
<td>1.94</td>
<td>1.44</td>
<td>5.16</td>
<td>1.38</td>
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<tr>
<td>Bent head</td>
<td>0.61</td>
<td>1.22</td>
<td>2.22</td>
<td>0.5</td>
</tr>
<tr>
<td>Total abnormally (%)</td>
<td>4.27</td>
<td>3.72</td>
<td>11.77±4.8</td>
<td>4.33±4.3</td>
</tr>
</tbody>
</table>

**Note:** Data expressed as mean±SEM (n=6). Significance test was done by ANOVA (Analysis of variance) followed by Tukey post hoc test. Significance between different treatment groups are represented as letters a (versus control), b (versus MNP*), c (versus MW). Level of significance are represented as *p<0.05, **p<0.01, ***p<0.001. 

Table 1 shows the values of the quantitative assessment of sperm parameters in control and different treatment groups. The sperm count in MW exposed rats decreased significantly (p<0.001) as compared to control and MNP*.
exposed rats. When treated with MNP* i.e., in MW+MNP* group the sperm count increased significantly (p<0.05) as compared to MW exposed group. Percentage viability of sperm was evaluated by staining the sperms with Eosin Y. The percentage viability of sperms was significantly (p<0.01) decreased in the MW exposed rats. In the MW+MNP* exposure group, the percentage viability increased significantly (p<0.01) as compared to MW group. To assess the effect of MW and MNP* in sperm membrane integrity, HOS (Hypo osmotic swelling) test was done. The results showed that the membrane integrity of sperms of MW exposed rats decreased significantly (p<0.001) in the HOS test, when compared to control and MNP* treated groups of rats. Upon combined treatment of MW and MNP* there was a significant increase (p<0.001) in membrane integrity. The alteration in morphology of sperm head was classified into four different groups viz, amorphous, hookless, banana shaped and bent head (Figure 6).

![Figure 6: Sperm morphology; arrow shows normal hook shaped sperm (a), amorphous head (b), hookless (c), banana headed sperm (d) and bent head sperm (e) [4]](image)

The average number of these deformities observed in each group is shown in table 1. Total percentage deformity found was significantly higher (p<0.01) in MW exposed rats as compared to control and MNP* exposed rats. MNP*+MW exposed rats showed a significantly lesser (p<0.01) percentage of total deformity than the MW exposed rats.

4 Conclusion:

The present study validates that the MW induced alterations in male reproductive parameters are oxidative stress mediated, as the alterations were reduced by administering antioxidant mimicking particles. It also shows the potential therapeutic activity of these particles (MNP*) against MW induced alterations in male reproductive system of rats.

In the present study reproductive hormone, antioxidant status of rat testis and the sperm parameters were observed to be affected by MW. The sperm count was significantly decreased by MW exposure. This might be due to the low level of testosterone which is essential for spermatogenesis. Sperm exposed to MW also showed a reduced viability and membrane integrity. Percentage abnormality in sperm morphology also increased in MW exposed rats. MW generates mitochondrial ROS that form adducts by interacting with mitochondrial proteins that take part in the electron transport chain. The reaction goes on till an equilibrium is reached and an intrinsic apoptotic cascade is initiated. This correlates with the present study that shows a decrease in percentage mitochondrial activity in the MW exposed group. The lipid peroxidation of testis (MDA levels) increased due to decrease in total antioxidant status in MW exposed rats. On the other hand, MNP* treatment did not show any undesirable effect on sperm parameters at the administered dose (12.5 mg/kg). When MW and MNP* was administered in combination, the testis and sperm parameters improved.

The modulatory role of MNP* in the MW induced alterations can be attributed to its antioxidant capacity that increased the activity of antioxidant enzymes, thereby decreasing the ROS mediated damage by MW. Hence it may be concluded that MNP* at dose 12.5 mg/kg protect male reproductive system of rats from MW induced alterations.

5 Acknowledgements

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6 References


