Nitrofurantoin Side Effects Reduction by Using Royal Jelly Produced by Honey Bee Workers

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Abstract
Nitrofurantoin is a safe antimicrobial therapy, which is most effective in the treatment of Urinary Tract Infection (UTI) wide affecting on different types of E. coli. In this study, mice were grouped into 4 treatments, the first was used as control, whereas the 2nd, 3rd and 4th were treated with Royal Jelly RJ, Nitrofurantoin and with a mixture of royal jelly and Nitrofurantoin, respectively. There was a significant increase in the RJ group in each of the following parameters: sperm count, sperm viability, sperm motility, Luteinizing (LH), Follicle-Stimulating (FSH) and testosterone hormones. While the abnormal form of sperm was significantly reduced, and at in the Nitrofurantoin group was the opposite, where each of sperm count, viability and movement and Malondialdehyde (MDA) decreased, but at the abnormal sperm, FSH, LH and testosterone hormone as well as the level of CAT, SOA enzyme there was a significant increase. So finally, it can be concluded from this study that RJ can be used to reduce the harmful role of Nitrofurantoin on sperm properties, levels of sex hormones and levels of antioxidant enzymes.

Key words: Nitrofurantoin, ALT, FSH, royal jelly, Testosterone


Introduction
Nitrofurantoin, an antibiotic with a broad spectrum effect has been discovered since 1952. No major resistance has developed clinically during its use for more than four decades, as it appears with other commonly used antibiotics. This may be due to that it contains several activity sites and levels compared to antibiotics with a single (or two targets e.g. Ampicillin or Cotrimoxazole, respectively. Nitroreductase, a bacterial enzyme cleaves Nitrofurantoin into intermediates with a high reactive electrophilic activity. These can attack bacterial ribosomal proteins, non-specifically, which may be resulting in a complete inhibition in protein synthesis process (McOsker et al., 1994) and causing single-strand breakage in DNA (McCalla et al., 1970). Urinary tract infection (UTI) disease is a major health problem in most countries including India, particularly in females, which is mainly caused by Escherichia coli (E. coli) infection. Furthermore, the Nitrofurantoin is a safe antimicrobial drug according to modern scientific sources, and it has been found to be the most effective therapeutic drug for uncomplicated (UTI) treatment, even against Escherichia coli, which are resistant to many medicines. Thus, the Nitrofurantoin may be used as an experimental drug (UTI) even with if there are major errors in human organization as described in this study, where similar studies maybe proceeded to establish an appropriate experimental therapy for patients, due to the variable allergic form (Konar et al., 2016). Nitrofurantoin has been used as a drug for the (UTI) treatment since 1950s. The current uses of this drug are uncomplicated (UTI) treatment and prevention of patients exposed to recurrent (UTI). (Gary J., 2008). The drug mode of action includes causing disruption in the bacterial DNA, due to its low form is very interactive. This was facilitated by the rapid Nitrofurantoin reduction within the bacterial cell by Flavoprotein (nitrofuran reductase) to multiple reactive intermediates attacking
ribosomal proteins, DNA, enzymes contributing to pyruvate metabolism and respiration within the bacterial cell. It has been found that Nitrofurantoin had significant effects on bacteria greater than mammalian cells because the previous drug activates the drug quicker. The various mechanisms of action are likely to lower the resistance development against this medicine. (Tu and Macala, 1975).

Royal Jelly
Royal jelly (RJ) a compound produced by the pharyngeal glands and the lower jaw of honeybee workers, is nourished to the queen larvae and the younger, where RJ has been widely used in medical, commercial, cosmetic and health food products worldwide (Ramadan and Ghamdi 2012).

Materials and method of works
Pure soaked; chemicals or antibiotic additives free RJ, produced by the purest beehives was provided by the apiaries around Baghdad. To prepare the stock solution, the content of each RT capsule (1000 mg) was suspended in 10 ml of deionized water (W / V) to obtain 100 mg of RJ per ml concentration according to Abd-Allah in (2012b). RJ was eluted in deionized water and homogenized by a vibrator at 4°C, then mixture prepared was sterilized and stored at -20°C.

Experimental design
Thirty two male, white mice aged for 10-12 weeks with an average weight ranged (250 ± 5 g) were used in this study. The mice were divided into four groups, randomly, six mice each as follows: Group 1: untreated control, mice fed on 0.3 ml of intraoral isotonic saline solution only. The 2nd group of mice was treated with RJ treated mice at 100 mg / kg rate of the body weight. The 3rd group was Nitrofurantoin treated mice while the 4th was Nitrofurantoin and RJ treated mice. A standard diet and tap water were given to mice, where the experiment period was thirty five days after RJ and Nitrofurantoin treatment. At the experiment end, feeding was stopped for 24 h, and mice were killed under the effect of ether anesthesia. A fine needle was used to take blood samples from the heart. Blood samples were kept at room temperature for 30 min and centrifuged for 15 min at 3000 RPM then stored at -20°C until use. Fertility parameters including sperm motility, viability, abnormalities and count, LH, FSH, antioxidant enzyme and blood testosterone levels were estimated at the end of the treatment period.

Collect sperm and study the standard traits
The mice killed were dissected immediately, where testicles were removed and transferred in a disposable Petri dish containing 3 ml RPMI-1640 solution, at 37°C. Sperms were collected from the cauda epididymis each mouse, cut and placed in a petri dish containing 1 ml chopped RPMI-1640 using scissors and microsurgical equipment according to Bearden and Faquay in (1992).

Sperm motility
The sperm motility assessment was performed based on Bearden and Faquay method in (1992). Sperms was examined using light microscopy by placing about 50 μl of sperm suspension on a slide then covered with a cover slip. Several microscopic fields were tested to estimate the individual sperm motility percent.

Sperm viability & Abnormality
Dead and abnormal sperm percentages were estimated based on a method adapted by Bancroft and Stevenin (1982). One drop of sperm suspension was mixed with equal volume of Eosin-Nigrosin dye and the mixture was spread with another slide and dried at room temperature. Two hundreds sperm were calculated to estimate the sperm death and abnormality percentages.

Biochemical analysis
At the end of the experiment, blood was collected by heart puncture using disposed insulin syringes from all animals after killing them. Samples were placed in Eppendorf tubes and centrifuged for 10 minutes at 3000 RPM to separate the serum. The hormone level was tested using the immune detection method that used by I-CHROMA TM. FSH, according to the immune system through antibody interaction with antigen and Madantech technology described by Goldstein and Kosasain (1975). Finally, Testosterone level was measured according to (Friteetai, 2008) method by the solid phase method enzyme-linked immunosorbent assay [ELISA] following D’souza et al. in(2012). For assessment of some biochemical test by using Cobas C111 Biochemical Analyzer for AST, ALT and ALP measurement.

Result and Discussion

The effectiveness of royal jelly on sperm properties in Nitrofurantoin treated male mice

Data showed that there was a significant increase in sperm count, capability and percentage in the therapy group compared to the treatment group, while the percentage of sperm abnormalities were significantly reduced (Table 1). This result presented a good agreement with (Nawar et al., 2015; Dujaili et al., 2015) findings. Moreover, Nitrofurantoin treated mice showed sperm function parameter such as sperm count, sperm capability and sperm motility, decreased (Omidi et al., 2016). (Al-Azzawi and Askar, 2017) was observed during the study of the effect of the reproductive system in male mice and testicular tissue that, the using of Royal Jelly with Nitrofurantoin reduced the harmful effect of the Nitrofurantoin on function parameters of the sperms.

Table (1): Effectiveness of royal jelly on sperm properties in Nitrofurantoin treated male mice

<table>
<thead>
<tr>
<th>treatment</th>
<th>sperm count</th>
<th>sperm viability</th>
<th>sperm Abnormal</th>
<th>sperm motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>39</td>
<td>75.5</td>
<td>9.875</td>
<td>90.25</td>
</tr>
<tr>
<td>Rj</td>
<td>45.125</td>
<td>90.25</td>
<td>7.875</td>
<td>94.75</td>
</tr>
<tr>
<td>Nitrofurant</td>
<td>21.25</td>
<td>20.5</td>
<td>23.5</td>
<td>60.375</td>
</tr>
<tr>
<td>Rj+ Nitro.</td>
<td>34.375</td>
<td>64.875</td>
<td>15.625</td>
<td>73</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>1.133**</td>
<td>1.167**</td>
<td>2.099**</td>
<td>2.424**</td>
</tr>
</tbody>
</table>

The effectiveness of royal Jelly on male mice hormone levels treated with Nitrofurantoin

Results showed no significant increase in FSH and LH levels in the RJ treated group, whereas a significant increase in the hormonal level of testosterone was detected (Table 2). Mice group that treated with Nitrofurantoin showed there were a significant decrease in FSH and testosterone levels, but not with LH levels, while in RJ and Nitrofurantoin group no significant decrease was observed compared to the control group. Royal jelly can be used to decrease the inhibitory effect of Nitrofurantoin by increasing sex hormones using RJ, where it increases FSH, LH s and testosterone hormones levels and also causes DNA damage (Yong et al., 2012; Zahamtic et al., 2014).

Table (2): The effectiveness of RJ on male mice hormone levels that treated with Nitrofurantoin

<table>
<thead>
<tr>
<th>treatment</th>
<th>testosterone</th>
<th>FSH</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>3.40</td>
<td>2.21</td>
<td>1.75</td>
</tr>
</tbody>
</table>
The enzyme level data (Table 3) showed no significant increase of the MDA, Chloramphenical Acetyltransferase (CAT) and Superoxide Dismutases (SOD) enzymes in the Royal Jelly treated group. Royal Jelly can be used as a natural antioxidant to protect damages caused by the presence of free radicals (Khanm et al., 2004). Royal jelly has been found to be highly effective to eliminate free radicals and reduce the toxicity of chemical factors. As well as, Royal Jelly has been identified with the activities of MDA and SOD (Alvarez-Suarez, 2017), and Biochemical factors (SOD, CAT and MDA). Thus, the decline in oxidative stress caused by Royal Jelly increased anti-oxidative activities (Abdel Moneim, 2011).

Effect of royal Jelly on antioxidant enzyme levels Nitrofurantoin treated male mice

<table>
<thead>
<tr>
<th>treatment</th>
<th>CAT</th>
<th>SOD</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.601</td>
<td>1.769</td>
<td>1.054</td>
</tr>
<tr>
<td>Rj</td>
<td>0.603</td>
<td>1.070</td>
<td>1.200</td>
</tr>
<tr>
<td>Nitrofurant</td>
<td>0.268</td>
<td>2.294</td>
<td>3.768</td>
</tr>
<tr>
<td>Rj+ Nitro.</td>
<td>0.330</td>
<td>1.560</td>
<td>1.431</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>0.04319**</td>
<td>0.1802**</td>
<td>0.0841**</td>
</tr>
</tbody>
</table>

The effectiveness of royal Jelly on liver function enzyme levels of Nitrofurantoin treated male mice

Data in table-4 showed significant increase in the liver enzymes AST, ALT & ALP in nitrofurantin drug group compared to control. The nitrofurantoin cause of toxic liver damage (Aksamij et al., 2009).while the levels enzymes raised in RJ group compared to control group. Nitrofurantion +RJ group showed significant decrease compared to nitrofurantion group only. These show positive effect to RJ to liver enzymes activity which results in a slight improvement in liver function compared to the nitrofurantion alone.

Table 4: The effectiveness of royal Jelly on liver function enzyme levels of Nitrofurantoin treated male mice

<table>
<thead>
<tr>
<th>treatment</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>76.25</td>
<td>217.25</td>
<td>86.63</td>
</tr>
<tr>
<td>Rj</td>
<td>65.13</td>
<td>154.38</td>
<td>79.00</td>
</tr>
<tr>
<td>Nitrofurant</td>
<td>396.25</td>
<td>588.50</td>
<td>216.75</td>
</tr>
<tr>
<td>Rj+ Nitro.</td>
<td>171.88</td>
<td>308.38</td>
<td>97.13</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>5.718**</td>
<td>9.87**</td>
<td>18.93**</td>
</tr>
</tbody>
</table>

Conclusion
The treatment with Nitrofurantoin may inhibit the sperm form in the natural characteristics, but with using Royal Jelly, it is reduced or mitigating the side effect of the drug, and this has a qualitative benefit to the drug. On the other hand, RJ works to increase the FSH, LH, testosterone, which are inhibited by...
Nitrofurantoin, as well as RJ works to increase the antioxidant enzymes, including CAS, MDA, and SOD, which decreases due to the Nitrofurantoin drug.

Reference

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