The study of biogenic iron oxide nanoparticles effects on iron status in male rabbits infected with T. evansi

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Abstract

The present study aimed to evaluate the effects of propolis-iron oxide nanoparticles in eliminating the T. evansi parasite and rehome stasis of deleterious iron status in experimentally infected rabbits. Twenty male rabbits were divided into equal four groups (n=5). the 1st group as Control negative, 2nd control positive, 3rd trypanosomiasis and treated with propolis iron oxide nanoparticles, and 4th trypanosomiasis and treated with diminazene,2nd,3rd, and 4th groups were inoculated with T. evansi, and were checked for the onset of parasitemia. After 15 of the onset of parasitemia 3rd group was treated with propolis-iron oxide nanoparticles 30 mg iron /kg BW, and 4th group was treated with diminazene drug with a single dose 3.5 mg/kg BW. The result showed that experimentally infection with T. evansi caused a significant decrease of serum iron and ferritin and a significant increase in total iron-binding capacity and unsaturation iron-binding capacity, as well erythrocytes fragility, bilirubin totally and partially. Treatment with propolis-iron oxide nanoparticles improved iron status parameters to semi-normal values much better than diminazene drug, in addition, reduced the total bilirubin concentration and osmotic fragility of erythrocytes toward a normal state. It can be concluded that the propolis-iron oxide nanoparticles proved successfully rebalancing iron status and eliminating the parasite and making iron available.

Introduction

Trypanosomosis is a worldwide diseases caused by infection of living organisms by trypanosomes parasites, it is an endemic disease in many parts of the world especially in tropical countries like Iraq. Trypanosomiasis caused by the hemoflagelate protozoa that infect a broad types of mammalian (1) and even gees (2).

Anemia is one of the most common symptoms of trypanosomiasis, results from various factors among them the most important is the iron homeostasis dysregulation, however the molecular mechanism is not yet explained (3). T. evansi cause anemia defined by reduction in total serum iron, ferritin, and transferrin saturation (4).

Nanobiotechnology, a unique therapeutic alternative through the repositioning of existing medications and directed drug delivery in treatment of trypanosomiasis (5,6). The propolis, the resin compound, the third product of bees for protection of and sealing the beehive (7), with high contents of phenolic groups act as antitrypanosomal agent (8).

The application of propolis is free from hepatic or kidney side effects (9). The Propolis mediated IONPs biosynthesis improved iron homeostasis in iron deficiency anemia (10).

Only few drugs have been approved for the treatment of trypanosomiasis (11). Depending on the hypothesis that the large parasite needs iron, there is now a new strategy to treat infections with the parasite by decrease iron availability to
parasite, using iron chelating agent leading to parasite iron deprivation consider as treatment (4) Hereby, the Propolis – iron oxide nanoparticles could be considered as a dual target use, 1st for treatment of anemia, 2nd for killing the trypanosome.

The application of iron oxide nanoparticles (IONPs) as a drug form to treat anemia is a novel drug delivery system, the Propolis mediated IONPs biosynthesis improved iron homeostasis in iron deficiency anemia (10).

Accordingly, the present study designed to explore the possibility of using Propolis iron oxide nanoparticles (Pr-IONPs) as a drug to control the growth and proliferation of the parasite in a condition used to treat iron deficiency anemia caused by the experimentally trypanosomiasis in rabbits.

**Material and methods**

**Biosynthesis of Iron Oxide Nanoparticles (IONPs)**

IONPs prepared by using aqueous macerated propolis solution as reducing agent for the mixture of ferric and ferrous chloride following the procedure of Al-Hussain and Al-Qayim M.2017 (12). The prepared nanoparticles (IONPs) were sterilized by autoclave.

**Isolation of *T. evansi* parasite**

The parasite was isolate from +ve infected camel at Al-Najaf city- Iraq, the most endemic area for trypanosomiasis. Presence of the parasite in infected camels confirmed as by the study of blood film stained with Giemsa staining technique. O.3 ml of infected camel’s blood was inoculated via i/p rout to mice for activation and preservation of the parasite.

**Experimental design**

Twenty local breeds, male rabbits with age range between 3-5 months were divided into four equal groups, 1st Control Negative for trypanosomiasis (C-ve), 2nd Group Control Positive for trypanosomiasis (C+ve), 3rd Group trypanosomiasis and Pr-IONPs (Tr-Pr-IONP), and 4th Group trypanosomiasis and diminazene (Tr-Dim group).

Trypanosomiasis experimentally induced in rabbits of 2nd, 3rd, 4th groups by intra peritoneal (i/p) injection of 0.3 ml from the infected mice blood with parasitemia of 10^3/s/ml. Treatment of trypanosomiasis: after 5, 10, 15 days of *T. evansi* infection infected rabbits were examined for conformation of parasitemia via blood film giemsa stained.

Treatment start after 15 days of infection, 3rd group was given Pr-IONPs via i/p a double dosages of iron (30mg /kg) by 7days interval (10), 4th group was treated by single dosage diminazene drug (3.5mg/kg). Giemsa stained blood film were performed weekly to confirm the presence of the parasitemia. Blood samples were collected at the 30th days of experiment by cardiac puncture.

Body weight changes estimated by subtracting final body weight at 30th day from initial weight at 1st day of experiment.

**Iron Status measurements**

Concentration of total serum iron (TSI) µg/dl, Total iron binding capacity (TIBC) µg/dl, ferritin, and Bilirubin measured by spectrophotometric method using enzymatic assay kit (human, Germany).

Unsaturated iron binding capacity (UIBC) µg /dl calculated from the following equation (13): UIBC (µg/dl) =Total serum Iron concentration (µg/dl) -TIBC (µg/dl)

Equation 1 Transferrin saturation % (TfS): Transferrin saturation calculated by equation: TfS (%) = Total serum iron - Total iron binding capacity X 100 Equation 2.

**Osmotic Fragility test of RBCs**

Osmotic fragility test is done to estimate the membrane integrity of red blood cells (RBCs), measure the ability of RBCs to resistance the hemolysis when exposed to hypotonic solution with different concentration.

Calculated the percentage of hemolysis of red blood cells (RBCs), according to the following equation: RBCs hemolysis (%) = absorbance of sample at 540 nm absorbance of blank (NaCl)×100% Equation 1.

**Bilirubin**

Serum Total Serum Bilirubin (TSB) and direct Bilirubin concentration measured by spectrophotometric method by using specialized kit (human, Germany).

**Results**

**Clinical signs and iron status**

Weekly blood tests can help to track infection rates and monitor the effectiveness of treatment in eliminating parasites. *Trypanosoma evansi* infected rabbits showed reduced appetite or varying degrees of emaciation, stress and fatigue like behavior represented by less movement, always tend to recombinant, with aggressive behavior. These clinical signs were gradually toward normal state after treatment of each group, coincided with the parasite disappearance in blood film specially when treated with Iron Oxide Nanoparticles compared with diminazene drug. Results in Figure 1 showed significant reduction in body weight of C+ve, and Tr. Dim groups. Treatment with Pr-IONPs lead to no significant improvement in body weight, in compare with C-ve group.

Iron status measurements values summarized in Table 1. Results revealed significant (P<0.05) decreased in total Serum iron (TSI), Transferrin saturation, Ferritin concentration and significant (P<0.05) increased in total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) of group +ve positive when compare with C-ve.
Figure 1: Effects of IONPs treatment on body weight changes in experimentally trypanosomiasis compared with Diminazene drug.

Table 1: Changes in some peripheral and central iron status parameters

<table>
<thead>
<tr>
<th>Groups treatment</th>
<th>S.I. (µg/dl)</th>
<th>TIBC (µg/dl)</th>
<th>U.I.B.C. (µg/dl)</th>
<th>T.S. (%)</th>
<th>Ferritin (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C–ve</td>
<td>126.12 ± 2.45A</td>
<td>250.23 ± 3.62C</td>
<td>130.11 ± 4.92C</td>
<td>49.27 ± 1.33A</td>
<td>963.23 ± 32.83AB</td>
</tr>
<tr>
<td>C+ve</td>
<td>95.81 ± 2.75C</td>
<td>303.39 ± 3.63A</td>
<td>207.57 ± 5.83A</td>
<td>31.62 ± 1.18C</td>
<td>599.79 ± 25.44C</td>
</tr>
<tr>
<td>Tr. Pr-IONPs</td>
<td>125.67 ± 2.85A</td>
<td>248.07 ± 4.04C</td>
<td>122.40 ± 6.27C</td>
<td>50.76 ± 1.80A</td>
<td>1040.43 ± 30.0A</td>
</tr>
<tr>
<td>Tr. Dimi</td>
<td>117.23 ± 5.14B</td>
<td>272.40 ± 4.71B</td>
<td>152.16 ± 7.87B</td>
<td>43.49 ± 2.15B</td>
<td>894.08 ± 42.13B</td>
</tr>
<tr>
<td>LSD</td>
<td>8.602</td>
<td>14.214</td>
<td>18.327</td>
<td>4.329</td>
<td>96.77</td>
</tr>
</tbody>
</table>

Different letters denoted significant differences at (P<0.05).

Table 2: Effects of Pr-IONP S treatment on total, conjugated, and unconjugated Bilirubin (mg/dL)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Bilirubin</th>
<th>Conjugated Bilirubin</th>
<th>Un-conj. Bilirubin</th>
</tr>
</thead>
<tbody>
<tr>
<td>C–ve</td>
<td>1.45 ± 0.05B</td>
<td>0.91 ± 0.05B</td>
<td>0.54 ± 0.05B</td>
</tr>
<tr>
<td>C+ve</td>
<td>2.14 ± 0.07A</td>
<td>1.20 ± 0.03A</td>
<td>0.94 ± 0.05A</td>
</tr>
<tr>
<td>Tr. Pr-IONPs</td>
<td>1.41 ± 0.04B</td>
<td>0.84 ± 0.03C</td>
<td>0.66 ± 0.04B</td>
</tr>
<tr>
<td>Tr. Dimi</td>
<td>1.62 ± 0.03AB</td>
<td>0.92 ± 0.03B</td>
<td>0.66 ± 0.04B</td>
</tr>
<tr>
<td>LSD</td>
<td>0.149</td>
<td>0.131</td>
<td>0.150</td>
</tr>
</tbody>
</table>

Different letters denoted significant differences at (P<0.05).

Table 3: Protective role of IONPs and diminazene drug in RBCs hemolysis resistance against T. evansi effects

<table>
<thead>
<tr>
<th>NaCl Con. %</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>Tr. Pr-IONPS Group</th>
<th>Tr. Dimi Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>64.00 ± 1.77B</td>
<td>68.75 ± 2.25A</td>
<td>62.25 ± 1.18B</td>
<td>65.00 ± 1.41AB</td>
</tr>
<tr>
<td>0.1</td>
<td>59.00 ± 1.47C</td>
<td>66.25 ± 2.21A</td>
<td>57.50 ± 0.86C</td>
<td>60.25 ± 1.03B</td>
</tr>
<tr>
<td>0.3</td>
<td>56.00 ± 1.77C</td>
<td>68.25 ± 2.49A</td>
<td>56.75 ± 2.46C</td>
<td>61.00 ± 1.95B</td>
</tr>
<tr>
<td>0.5</td>
<td>53.25 ± 1.70D</td>
<td>66.25 ± 1.65B</td>
<td>53.75 ± 1.37D</td>
<td>58.25 ± 2.86C</td>
</tr>
<tr>
<td>0.7</td>
<td>11.75 ± 1.18L</td>
<td>18.25 ± 1.37K</td>
<td>6.95 ± 2.31M</td>
<td>9.72 ± 3.06L</td>
</tr>
<tr>
<td>0.9</td>
<td>4.50 ± 0.28N</td>
<td>8.00 ± 0.40M</td>
<td>3.50 ± 0.28N</td>
<td>4.50 ± 0.64N</td>
</tr>
</tbody>
</table>

Different letters denoted significant differences at (P<0.05). N=5. (LSD: 4.59).
Discussion

Anemia is one of the main symptoms caused by trypanosomiasis (3,14,15), therefore, stress, weakness, hair loss, fatigue, and low body weight in affected rabbits were clear as a result of iron deficiency anemia. The protective role of Pr-IONPS synthesized by propolis extracts was clearly demonstrated by gradually absent the clinical signs and the body weight back toward normal and showed an improvement in their health condition during the period of treatment, as a result of potential replacement of iron and efficient enough to homeostasis iron to normal level (10). Intramuscular dose of a diminazene drug was shown to be highly active against trypanosome, but when compared with Try-Pr-IONPs group, the results of present study cleared the effects of Pr-IONPS is more efficient than diminazene drug as a treatment to reward the body weight and removed the clinical signs of diseases from the animals. Host with trypanosomiasis suffering from anemia may directly or indirectly suppress host erythropoiesis (16,17). The treatment of infected rabbits with Pr-IONPs turn two directions, the first is to eliminate the parasite and the second restore homeostasis iron status parameters to the natural environment. There are many trials for treatment and control parasitic diseases by new strategies, here applying nanotechnology is the best candidate. Improvements are needed in drug administration and formulations to treat parasitic infections and with less toxicity to the host. The trypanocidal effects of Pr-IONPs used in the present study could be attributed to parasite necrosis caused by iron elements, as have been noticed in using silver nanoparticles (18).

Trypanosomiasis infection causes dysregulation in oxidant / antioxidant status of infected animals, result in lipid peroxidation of RBCs membrane (17,19). On this regard, the *T. evansi* caused the increase of osmotic fragility and injury of the erythrocytes. Increased bilirubin in circulation demonstrated increased red blood cells hemolysis. Infected mammals such as buffalo, dogs, rats and rabbits with *T. evansi* suffered from increased total bilirubin, conjugated and unconjugated bilirubin in the serum (20). Propolis used in the biosynthesis of IONPs is rich in polyphenols compound exert antioxidant activities (21). These abnormal changes in RBCs caused anemia denoted in the present study (22). Taken together, data obtained from the present study suggest that Pr-IONPS has anti-trypanosoma as well as diminazene did (23), furthermore it’s act as antioxidant thus removing and treating the main cause of the red blood cells hemolysis and rises total bilirubin level Using radial immunodiffusion (RID) plates for the quantitation of immunoglobulins IgG determination can be used in cases of parasitic diseases diagnosis. The reduction of IgG in Pr-IONPs treated rabbits considering as marker for the immunomodulatory role, similar hyper immunoglobulinemia were noticed in naturally infected camels, in both acute and chronic stages of the disease (7,24).

Conclusion

The present results showed that Pr-IONPs are safe, have anti-trypanosoma activity and reverse iron status, red cell membrane integrity, and immunomodulatory markers associated with *T. evansi* experimentally infection

Acknowledgments

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Conflict of Interests

The manuscript has been approved by all the co-authors and declare that there is no conflict with any other

References

دراسة تأثيرات الجسيمات النانوية الحيوية لأوكسيد الحديد على وضع الحديد في كولر الأرانب المصابة بمثلثات إيفنساي

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زراعة بغداد، بغداد، العراق

الخلاصة

هتَدَثت الدراسة الحالية لتقني تأثير الجسيمات النانوية لأوكسيد الحديد
- العكير في التخلص من مثلثات إيفنساي وإعادة وضع الحديد المختل في
الأرانب المصابة بعنقودية معنويات إيفنساي. عذرو أن أربعة قمسموا
إلى أربع مجاعم متشابهة (عدد=5) المجموعة الأولى غدت سطوة المجايع
المحتلة بال nhiêuات والمجاعم الثانية غدت مجموعة سطوة موجبة والثالثة تم إصطبها
بالประโยمات وما أنعمت بجسيمات أوكسيد الحديد النانوية - العكير والرابعة
أصبحت الملاحظات ومت تعلمناها بجسيمات أوكسيد الحديد النانوية - العكير والرابعة
أصبحت المعالجات ومت تعلمناها دامنزين، أصبت المجايع الثانية والثالثة
والرابعة بمثلثات إيفنساي وحقتنا تألق الإصابة من خلال ظهور الطفيلي
في الدم و بعد خمسة شهر وما تم تفعيلة المجايع الثالثة بجسيمات
أوكسيد الحديد النانوية - العكير 30 ملم محتل كغم، والمجموع الراجع
بجرعة واحدة واحده كغم كنيانز 5 ملم/كغم. أظهرت النتائج أن
الإعصار الجذري بنفاذيات إيفنساي سبب انخفاض في تركز الحديد في
مصل الدم، مبتاع الفرزف، والفرزف، وزيادة معنوية بين الحديد الكلي
المتخبك و الحديد غير المتخبك كذاك تكسر الخلايا الحمراء والبلوئين
الكلي والجزيئي. ان العلاج بالأوكسيد الحديد النانوي - العكير وعشق تركز
الجسيمات النانوية في ردود الفعل بالتمثث والحمراء والبلوئين
الكلي والجزيئي. إن الفعل الصرف بل تعريف الحديد النانوي - العكير، ويؤثر
على الجسيمات النانوية في ردود الفعل بالتمثث والحمراء والبلوئين
الكلي والجزيئي. يمكن الاستنتاج بأن أوكسيد الحديد النانوي المصنوع
بعثة نجاح و إعادة توزيع الحديد المختل والتشكل من الطفيلي وتوفر الحديد
للحيوان.