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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.

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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 trypanosomiaisis (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 ($IONP_S$) 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-$IONP_S$) 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 10^5s/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 aftre tratment 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.

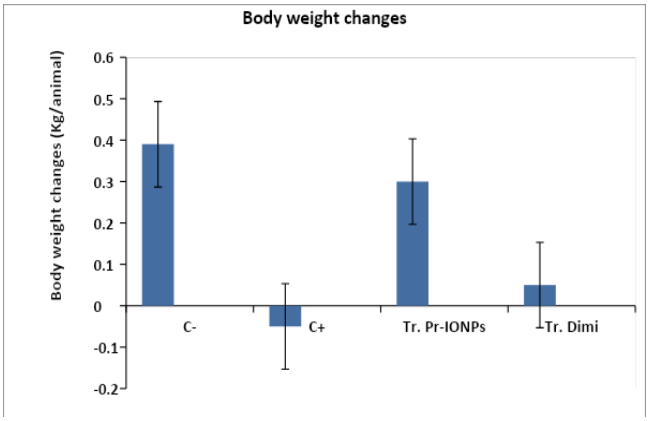

Figure 1: Effects of IONPs treatment on body weight changes in experimentally trypanosomiasis compared with Diminazean drug.

Bilirubin Concentration (mg/dl) and Erythrocyte Osmotic Fragility

Total, conjugated and unconjugated Bilirubin increased significantly (P<0.05) in C+ve group as shown in Table 2. Results showed treatment of both groups, Tr. Pr-IONPs & Tr. Dimi, reduced bilirubin to semi normal as in C-ve.

Osmotic fragility in erythrocytes reflects the effects of the parasites (Trypanosoma) on cell wall integrity of erythrocytes (Table 3). There was significant increase in RBCs hemolysis of the C +ve group in 0.1, 0.3, and 0.5 % NaCl solutions, compared with C-ve this ratio of hemolysis was reduced in PR-IONPs treated animals particularly at 0.1 & 0.3 % Pr-IONPs keep hemolysis ratio in normal values in spite of the infection. At 0.9% NaCl or normal saline Pr-IONPs showed the lowest RBCs hemolysis ratio.

Immunological evaluation of $IONP_S$ treatment in IgG protein in experimentally trypanosomiasis compared with Diminazene Drug, IgG concentration (mg/ml) as shown in Figure 2.

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

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

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)

Groups	Total Bilirubin	Conjugated Bilirubin	Un-conj. Bilirubin
C–ve	1.45 ± 0.05^{B}	0.91 ± 0.05^{B}	0.54 ± 0.05^{B}
C+ve	2.14 ± 0.07^{A}	1.20 ± 0.03^{A}	0.94 ± 0.05^{A}
Tr. Pr-IONPs	1.41 ± 0.04^{B}	0.84 ± 0.03^{C}	0.66 ± 0.04^{B}
Tr. Dimi	1.62 ± 0.03^{AB}	0.92 ± 0.03^{B}	0.66 ± 0.04^{B}
LSD	0.149	0.131	0.150

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

Table 3: Protective role of $IONP_S$ and diminazene drug in RBC_S hemolysis resistance against *T. evansi* effects

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

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

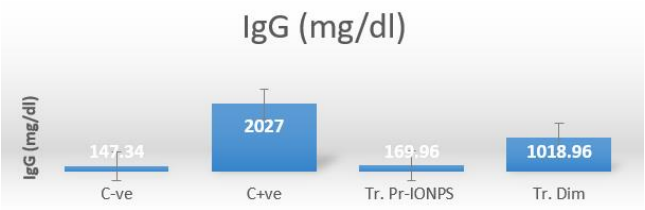

Figure 2: Effects of Pr-IONPS treatment on IgG protein of experimentally trypanosomiasis compared with diminazean drug.

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 results 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 the 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 considered 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

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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

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دراسة تأثيرات الجسيمات النانوية الحيوية لأوكسيد الحديد على وضع الحديد في كولر الأرانب المصابة بمثقبات ايفنساي

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الخلاصة

هدفت الدراسة الحالية لتقييم تأثير الجسيمات النانوية لأوكسيد الحديد -العكبر في التخلص من مثقبات ايفنساي وإعادة وضع الحديد المختل في الأرانب المصابة تجريبيا بمثقبات ايفنساي. عشرون أرنبا بالغا قسموا إلى أربع مجاميع متساوية (عدد=٥) المجموعة الأولى عدت سيطرة، المجاميع الثانية عدت مجموعة سيطرة موجبة والثالثة تم إصابتها بالمثقبات وتم معاملتها بجسيمات أوكسيد الحديد النانوية - العكبر والرابعة أصيبت بالمثقبات وتم معاملتها دايمنزين، أصيبت المجاميع الثانية والثالثة والرابعة بمثقبات ايفنساي ودققت لتأكيد الإصابة من خلال ظهور الطفيلي في الدم و بعد خمسة عشر يوماً تم معالجة المجموعة الثالثة بجسيمات أوكسيد الحديد النانوية - العكبر ٣٠ ملغم حديد/كغم، والمجموعة الرابعة بجرعة واحدة من عقار دايمنزين ٣,٥ملغم/ كغم. أظهرت النتائج أن الإصابة التجريبية بمثقبات ايفنساي سبب انخفاض في تركيز الحديد في مصل الدم، تشبع الترانسفرين، والفرتين وزيادة معنوية بين الحديد الكلي المرتبط والحديد غير المرتبط كذلك تكسر الخلايا الحمراء، والبلروبين الكلي والجزئي. إن العلاج بالأوكسيد الحديد النانوي - العكبر رفع تركيز الحديد إلى المستويات الطبيعية بالمقارنة مع الدايمزن، إضافة إلى تقليل تكسر كريات الدم الحمراء والبلروبين الكلي والجزئي لتصل إلى التراكيز الطبيعية. يمكن الاستنتاج بان أوكسيد الحديد النانوي المصنعة بالعكبر حسنت بنجاح وإعادة توازن الحديد والتخلص من الطفيلي وتوفير الحديد للحيوان.