

# In vivo Study the Effectiveness of Novel Biogenic Pr- IONPs in Rehomeostasis iron mechanisms In Iron Deficiency anemia

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## Abstract

Of the most important health problems that disrupt public health, whether in human or animal is the Iron deficiency anemia (IDA). The biggest problem is the changes in the elements of iron metabolism that follow iron deficiency. Here, finding a quick and effective solution has become a target for professionals. There for the present study aimed to biosynthesis anew IONPS, with small size, low harmful, less expensive and highly effective, to rehomeostasis of iron regulatory mechanisms in iron deficiency anemia. Fifty weaning age female rats were divided into five groups, the 1<sup>st</sup>, control group, fed on IAD (40 mg iron /kg diet), the other groups fed on IDD(5-10 mg iron / kg diet) for two months, the IDD group then divided in to 4 groups and treated as following , 2<sup>nd</sup> group Iron deficiency anemia group (IDA) ,3<sup>rd</sup> group, Iron deficient anemia rats treated I/P with two dosages of Pr-IONPs (iron:30mg /kg B.W) at 10 days interval,(IDA2X), 4<sup>th</sup> group Iron deficient anemia rats treated I/P with single double dosage of Pr-IONPs (iron: 60mg / kg B.W) magnetic nanoparticles.,(IDAX<sup>2</sup>), 5<sup>th</sup> group Iron deficient anemia rats treated with multiple I/P injection (IDAD) of Iron Dextran (iron:30mg /kg B.W) each 72 hours. Results revealed that after 30 days of treatment anemia with the new Pr-IONPs, a statistically significant increase in total iron, and serum ferritin near normal to the semi-normal value and significantly decreased in TBIC and UBIC in IDA2X, IDAX<sup>2</sup> and IDAD. Transferrin saturation and serum soluble transferrin receptor 1(sTfR1) were increased significantly in IDA2X, IDAX<sup>2</sup> and IDA compared with IAD. Molecular evaluation of the transcription of Hpcidin and DMT1 were positively regulated in IDA2X, IDAX<sup>2</sup>, to extent significantly higher than IDAD, this was correlated with significantly increased of serum Hpcidin In conclusion, results refers to the effectiveness of this novel, less coast ,and health friendly propolis – IONPs in rehomeostasis the iron mechanism and could be consider the appropriate solution to the problem of iron deficiency anemia when compared with iron dextrin, which requires several injections.

**Key word:** iron oxide nanoparticle, iron deficiency anemia, hepcidin, divalent metal transporter.

## INTRODUCTION

Iron deficiency anemia is a condition which describes the insufficient oxygen delivery to the tissues. It is a global health problems caused impact on psychological, behavior, physical development, and work performance [1]. Iron deficiency is a state of no enough iron supplied to meet the requirement of the body. This occurs when storage iron is inadequate for the demands [2], which may be takes place due to reduced iron intake, reduced iron absorption by gut and increased iron loss [3]. Reduced bioavailability of dietary iron, increased needs for iron, obesity is a significantly associated with iron deficiency [4], chronic disease [5] and chronic blood loss especially in dogs and cats [6]. The biological importance of iron in many processes such as respiration [7], energy production, DNA synthesis and cell proliferation [8]. In the body the iron whether intracellular or extracellular regulates the presence of high-precision level. The body depends on the mechanism of iron preservation has in several ways, including the less excretion and the recycling of iron after the breakdown of red blood cells [9]. This critical regulation of blood iron is achieved by iron regulatory mechanisms are controlled by special proteins called the iron regulatory proteins [10, 11]. On the brush border of enterocytes, various iron import proteins are present, and specific pathways of absorption have been described for the two ionic forms of iron (Fe<sup>+2</sup> and Fe<sup>+3</sup>) both being non heme iron and heme molecules is present [12]. Iron transported by the proton-dependent ferrous iron transporter divalent metal transporter1 (DMT1), which located on the apical side of the intestinal cells and rapidly catabolized by an enzyme called heme oxygenase and release iron. Non-heme iron is associated with various storage proteins, including ferritin. Iron crosses the basolateral membrane of the cell through ferroportin [13]. The activity of ferroportin in transporting iron at the basolateral side of enterocytes and macrophages is limited by another protein, the Hpcidin [14, 10]. To get successful transporting mechanism by FPN, it cooperate with ferroxidase hephaestin which is responsible for conversion of Fe<sup>+3</sup> into Fe<sup>+2</sup> and enhance the bounding of iron to the plasma transporter protein [15,16]. Transferring - Fe<sup>+2</sup> transporter is circulated in the blood and has the capacity to deliver Fe to cells by binding to a specific

receptors the transferring receptor, binding of Tf to TfR1 make an active pair dimmers complex. Once it formed, it enclosed in endosome, then iron be released to the cytosol immediately incorporated into a protein [17, 14].

Treatment of IDA is essential to improve our health and performance. Iron may constipation, diarrhea, nausea and gastric pain [19]. A second disadvantage is oral route is that iron absorption restricted by intestinal conditions [20]. Previously reported used iron oxide nanoparticles to treatment iron deficiency anemia [21, 22]. There for the present study aimed to biosynthesis anew IONPS, with small size, low harmful, less expensive and highly effective, to rehomeostasis of iron intracellular and extracellular regulatory mechanisms in iron deficiency anemia

## MATERIALS AND METHODS

### Biosynthesis of Pr-IONPs

The Pr-IONPs synthesized by propolis according to the method described by [23]. The iron concentration measured using flame atomic absorption (Shemadzu, Japan) at the laboratory of Ministry of Science and Technology.

### Experimental animals and diet regimens

Iron deficiency anemia was induced by feeding iron deficient diet provide with 5 – 10 mg / kg diet and phlebotomy. One to 2 ml of blood was taken at each and subsequently at two weeks intervals for iron and Hb analysis for two months.

Fifty weaning age female rats were divided into five groups, the 1<sup>st</sup>, control group, fed on IAD (40 mg /kg diet), the other groups fed on IDD (5-10 mg / kg diet) for two months, further divided in to 4 groups and treated as following, 2<sup>nd</sup> group Iron deficiency anemia group IDA, 3<sup>rd</sup> group, Iron deficient anemia rats treated I/P with two dosages of Pr-IONPs (iron:30mg /kg B.W) and 10 days interval (IDA2X), 4<sup>th</sup> group Iron deficient anemia rats treated I/P with single double dosage of Pr-IONPs (iron: 60mg /kg B.W) magnetic nanoparticles (IDAX<sup>2</sup>), 5<sup>th</sup> group Iron deficient anemia rats treated with multiple I/P injection (IDAD) of Iron Dextran (iron:30mg / kg B.W) each 72 hours.

The prepared particles were sterilized by filtration through 0.2 μm milpore filter. During the experiment (30 days) all the anemic rats stayed with the same feeding regimens with exception of control,

feeding and water supply were ??????. At the end of experiment, blood samples were collected from anesthetized experimental rats.

**Iron status measurements**

**Serum iron** concentration measured by spectrophotometric method using enzymatic assay kit (human, Germany).

**Total iron binding capacity (TIBC)** measured using a colometric method by special kit (human, Germany).

**Unsaturated Iron Binding Capacity (UIBC) µg /dl**

$$\text{UIBC } (\mu\text{g/dl}) = \text{Total iron binding capacity} - \text{totals serum iron}$$

equation 1

**Transferrin saturation / Soluble Transferrin receptor1**

The transferring saturation calculated from the following equation

$$\text{TS\%} = \text{Total serum iron} / \text{Total iron binding capacity} \times 100$$

equation 2

**Quantitative serum rat soluble transferrin receptor 1**

was determined by ELISA kit (My Biosource Diagnostics, USA). Transferrin saturation (TfS) were determination by spectrophotometric method using enzymatic assay kits (human, Germany) and ferritin by (spectrum ,Egyptian ), gene expression of Rattus hepatic hepcidin, and divalent metal transporter protein (Bioneer / Korea).

**Hepcidin synthesis and availability.**

Serum hepcidin determined using serum Rattus hepcidin ELIAS kit (My Biosource Diagnostics, USA).

**Hepcidin (Hamp) gene expression by hepatocytes as mRNA levels were determined by quantitative Real Time PCR**

The RNA were extracted from a piece weighing 50mg of each experimental rat liver tissue was homogenized by micropestlel in 1ml of TRIzol® reagent kit. The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by promega company, USA. There are two quality controls were performed on extracted RNA. First one is to determine the quantity of RNA (ng/µL), the second is the purity of RNA by reading the absorbance in at 260 nm and 280 nm in nanodrop spectrophotometer (Thermo,USA).

Total RNA samples were used in cDNA synthesis step using AccuPower ® RocktScript RT PreMix Kit that provided from Bioneer, Korea. Quantitative Real –Time PCR(qRT-PCR) master mix prepared according to AccuPower™ Green Star Real-Time PCR kit that depend on syber green dye detection of gene amplification in Real-Time PCR system using a housekeeping gene . The relative expression of target genes (Hamp) in rat liver tissue was calculated (2<sup>-ΔΔCT</sup>) [24]. That dependent on normalization of RT-qPCR (CT values) of target genes relatively to housekeeping gene ( GAPDH) as reference gene in control and different treatment groups ,The two primers for Hamp were used the forward: 5'-TGATGCTGAAGCGAAGGAAG-3' and the reverse: 5'-TGTGTTGAGAGGTCAGGACAAG-3' with 135bp amplicon and the NCBI RefSeq = NM\_017008 obtain from margaecrogen (LIGO), Korea,

**Transferrin saturation / Transferrin receptor1**

Transferrin saturation ratio (Tf%) calculated according to the following equation;

**Total Serum Iron / Total Iron Binding Capacity X100 = TS% equation 4.** Quantitative serum rat soluble transferrin receptor 1(**sTfR1**) was determined by ELISA kit (My Biosource Diagnostics, USA).

**Statistical analysis**

The data of the present study was analyzed using SPSS (version 2010) in one ways analysis, Least Significant Difference (LSD) test used to compare between means.

**RESULTS**

**Induction of iron deficiency anemia**

Iron deficiency was gradually induced in IDA groups by iron deficient diet and phlebotomy. After two months serum iron and Hb were reduced significantly in IDA table1.

Role of Pr-IONPs in iron status of normal and IDA groups in table 2. Revealed that iron level was increased to semi normal in treated groups IDA2X (339.95±32.65), IDAX<sup>2</sup> (339.53±61.51) and IDAD (240.94±19.79). The TIBC and UBIC level had significantly ( P<0.05) increased in IDA group (643.54±7.43, 738.29±24.54) and decreased in IDA2X (637.89±40.25, 297.72±27.78), IDAX<sup>2</sup> (625.09±43.51, 293.99±24.38 and IDAD (583.59±27.78; 342.65±26.91) respectively.

**Table 1 : Hemoglobin (g/L) and iron in control and iron deficiency anemia rats during 60 days, mean ±SE.**

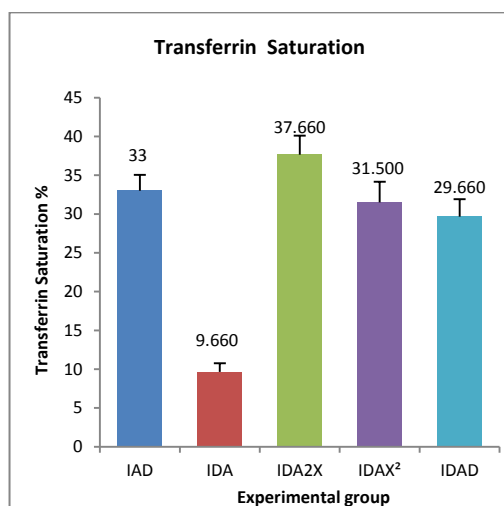
	Experimental diet (No)	T0(0days)	T1(15Days)	T2(30days)	T3(45days)	T4(60days)
Hb (g/l)	IAD (10)	14.80±0.24 <sup>a</sup>	14.65±0.18 <sup>a</sup>	14.61±0.18 <sup>a</sup>	14.5±0.10 <sup>a</sup>	14.64±0.13 <sup>a</sup>
	IDD(60)	15.15±0.25 <sup>a</sup>	14.60±0.17 <sup>a</sup>	11.34±0.32 <sup>b</sup>	9.56±0.33 <sup>c</sup>	9.02±0.46 <sup>c</sup>
Iron ( µg/dl)	IAD(10)	360.52±4.37 <sup>aA</sup>	358.12±5.88 <sup>aA</sup>	355.11±3.20 <sup>aA</sup>	354.52±1.95 <sup>aA</sup>	353.66±2.19 <sup>aA</sup>
	IDD(60)	356.84±2.62 <sup>a</sup>	351.14±5.31 <sup>aA</sup>	286.34±4.82 <sup>b</sup>	199.95±3.75 <sup>c</sup>	147.192±10.73 <sup>d</sup>

**Table 2: Role of Propolis –IONPS on Iron (µg/dl), TBIC (µg/dl), and UBIC (µg/dl) in iron deficiency anemic female rats in compare with Iron dextran, M±SE.**

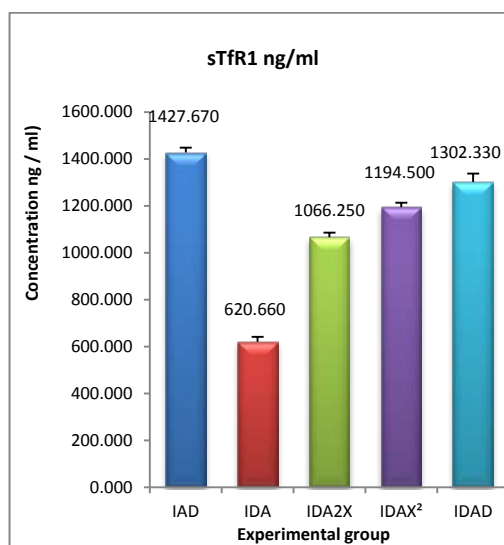
GROUP	Iron µg/dl	TIBC µg/dl	UBIC µg/dl
IAD	302.75±21.74 <sup>a</sup>	643.54±7.43 <sup>b</sup>	340.79±18.38 <sup>b</sup>
IDA	110.74±13.01 <sup>b</sup>	852.04±20.63 <sup>a</sup>	738.29±24.54 <sup>a</sup>
IDA2X	339.95±32.65 <sup>a</sup>	637.89±40.25 <sup>b</sup>	297.72±27.78 <sup>b</sup>
IDAX <sup>2</sup>	339.53±61.51 <sup>a</sup>	625.09±43.51 <sup>b</sup>	293.99±24.38 <sup>b</sup>
IDAD	240.94±19.79 <sup>a</sup>	583.59±27.78 <sup>b</sup>	342.65±26.91 <sup>b</sup>
LSD	99.929	89.933	71.724

**Transferrin saturation / soluble transferrin receptor 1**

Fig.1 represent the levels of transferrin saturation (TfS) statistically ( $P < 0.05$ ) decreased in IDA group ( $9.66 \pm 1.11$ ) and increased ( $P < 0.05$ ) in IDA2X ( $37.66 \pm 2.44$ ), IDAX<sup>2</sup> ( $31.50 \pm 2.66$ ) and IDAD groups ( $29.66 \pm 2.26$ ). The characteristics effect of magnetic iron oxide nanoparticles on soluble transferrin receptor1(stfr1) shown in Fig-2 revealed that the level of serum transferrin receptor 1 (stfr1) has significantly ( $P < 0.05$ ) lowered in IDA ( $620.666 \pm 42.984$  ng/ml) and significant increased ( $P < 0.05$ ) in IDA2X, IDAX<sup>2</sup> and IDAD groups ( $1066.25 \pm 19.539$ ,  $1194.50 \pm 18.912$ , and  $1302.33 \pm 35.35.5$ ) respectively.



(Figure 1.) Efficacy of Propolis –IONPS on rehomeostasis of transferrin saturation In iron deficiency anemic female rats in compare with iron dextran

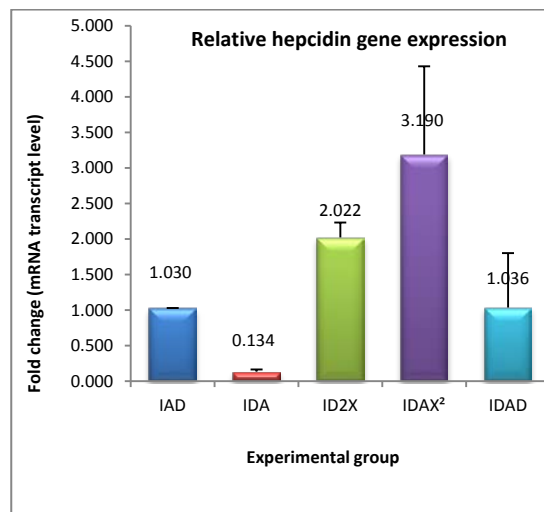


(Figure 2) Efficacy of Propolis –IONPS on rehomeostasis of sTfR1 In iron deficiency anemic female rats in compare with iron dextran

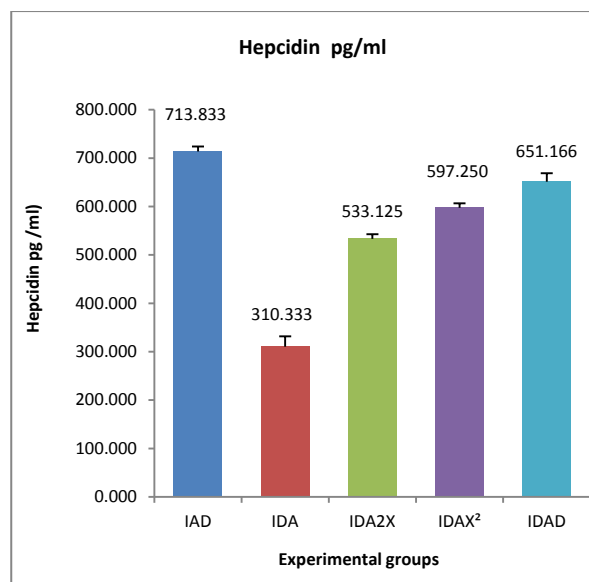
**Hepcidin synthesis and availability**

The present study tends to clarified the role of propolis mediated iron oxide nanoparticles in iron rehomeostasis mechanisms. After 90 days of stating anemia, serum hepcidin level and as gene expression affected by anemia. There was a significant down regulation for the expression of hepcidin in hepatocytes and hepcidin in serum of IDA rats reached statistical significance when compared with control group (1.035). Also results indicated that the Hepcidin transcription was positively regulated by the treatment of anemic rats with Propolis –IONPs, at 30 and 60 mg/kg BW and iron sulfate. The highest fold-induction of

Hepcidin transcription was in IDAX<sup>2</sup> group (3.190) when compared with IDA2X and IDAD 2.022, 1.036, respectively). The standard curve of the Rattus Hepcidin by ELISA, showed the significantly in ( $p < 0.05$ ) lower Hepcidin levels in the IDA groups were  $310.33 \pm 21.492$  pg/ml on another hand. The level of hepcidin significantly increased in ( $P < 0.05$ ) in IDA2X, IDAX<sup>2</sup> and IDAD groups ( $533.125 \pm 9.769$ ,  $597.25 \pm 9.456$  and  $651.166 \pm 17.677$  pg/ml, respectively).



(Figure 3 ) Role of propolis –IONPS on rehomeostasis on hepatic Hepcidin expression in iron deficiency anemic female rats, IAD=Iron adequate diet ,IDA1= Iron deficiency anemia group (60days) , IDA2= Iron deficiency anemia group (90 days) IDA2X= Iron deficient anemia rats+ two-dosage of Pr-IONPs 30mg /kg B.W . , IDAX2= Iron deficient anemia + double dosage of Pr-IONPS 60m /kg B.W., IDAD= Iron deficient anemia+ Iron Dextran 30mg /kg B.W.



(Figure 4) Role of propolis –IONPS on rehomeostasis on Hepcidin concentration pg/ml in iron deficiency anemic female rats, IAD=Iron adequate diet, IDA= Iron deficiency anemia group (60days) , IDA2X= Iron deficient anemia rats+ two-dosage of Pr-IONPs 30mg /kg B.W . , IDAX2= Iron deficient anemia + double dosage of Pr-IONPS 60mg /kg B.W., IDAD= Iron deficient anemia+ Iron Dextran

**DISCUSSION**

The present study designed to study the role of Pr-IONPs in rehomeostasis the systemic iron and the regulatory mechanisms for iron in the body. The procedure for induction of iron deficiency anemia used in the present study was successful.

Anemia can be decided by baseline hemoglobin quintiles reduction. In the present study after 60 days of feeding rats on iron deficient diet and the phlebotomy anemia was induced experimentally. In IDA groups, the systemic iron low level resulted from low diet iron in addition to the continuous phlebotomy affected on iron storage and lead to iron depletion in the body. The intraperitoneal injection of Pr-IONPs is considered a faster route to deliver iron to circulation. There was sharp increase in iron circulated in the blood, this will facilitate iron transportation through cellular membrane of the hepatocytes, macrophages and erythroid cells. During this step the transferrin saturation will be increased, resulting in lower TIBC and UBIC.

### Transferrin saturation / transferrin receptor 1

Plasma transferrin has been known as a central player in iron metabolism, assigned to circulate iron in a soluble, non-toxic form and deliver to the erythron and other tissues, delivering of iron from transferrin to intracellular via receptor mediated endocytosis through transferrin receptor 1 (TfR1) [25]. Transferrin is member in the IRP which are played a roles regulator of cellular iron metabolism. Furthermore, transferrin that captures iron released into the plasma mainly from intestinal enterocytes or macrophage. Normally there will be daily variation in the transferrin saturation [26]. The TfR1 receptor released to the circulation and called sTfR1. Therefore, sTfR is a useful marker of iron-deficient erythropoiesis, due to both absent iron stores and restricted iron supply due to anaemia in chronic disease. The sTfR is the most sensitive serum biochemical marker for the identification of iron-deficient erythropoiesis. The sTfR levels correlate better than BM iron stores with decreased MCV and MCHC. The present results referred to a hyperactivity of bone marrow erythroblast as indicated by the E/M ratio. When there is insufficient iron for the synthesis of hemoglobin, there was an increased expression of sTfR on the erythroblasts in the bone marrow, which also increases the concentrations of sTfR in circulating blood. The sTfR is mainly expressed by erythroid precursors and as such, sTfR concentrations reflected the erythropoietic activity and cellular iron status [27]. Concentrations of sTfR are proportional to the cellular iron demand and thus, reflected early functional iron deficiency [28]. The present results of reduced transferrin saturation demonstrated a depletion in iron stores, since the macrophages iron efflux was not functional. In normal circumstances there must be iron efflux from the stores mainly hepatocytes and macrophages to elevate the levels that saturated the binding capacity of transferrin, which will be taken up into hepatocytes [29]. In the controversy the unbinding transferrin-iron was elevated. Our findings regarding the transferrin saturation and the sTfR1 level in plasma seems unusual in the concept of iron deficiency anemia. But current results suggested that the severe decrease in transferrin saturation 9% much less than the 15% recorded ratio by [30], it is a figure mark for severe iron deficient anemia included the intracellular iron depletion (Cellular iron deficiency). The main source for sTfR1 is from the erythroblast cells, current findings of bone marrow cells showed erythroid hyperplasia. The sequence of erythroid maturation was defect and uncontrolled proliferation of erythroblast results in decreased their number. The present results could be explained by a detectable reduction in the erythroblast and TfR1. The same results were recorded by [31], who found a low sTfR1 which reflected a very low activity of bone marrow erythropoiesis.

The iron hydrolyzed from IONPs into two portion, the bounding with transferrin and the free or labile iron. Entrance of the free iron is pointed clearly by hepatocytes. Presence of free iron in the cytosol will activate the IRP /IRE resulting in over expression of transferrin receptors specifically type1 (sTfR1). The small size of the present Pr-IONPs was a courage factor for bioavailability for

cells. Different researches have been conducted on different iron compounds, and it was observed in all of them that reduction of particle size increases Bioavailability [32, 33]. The sTfR can be used for monitoring the erythropoietic response to various treatments such as iron supplementation treatment. The concentration of serum sTfR is proportional to the amount of TfR in cells and most of the sTfR is derived from the erythroblasts instead of the reticulocytes. Any changes in systemic iron metabolism there will be altered in plasma iron level which could be resulted from intestinal absorption or cell sequestration of iron. Both TfR and DMT1 were used as markers of genes to evaluate whether ABS-derived iron reduced the effect of IDA. They are very responsive to iron deficiency in both *in vitro* and *in vivo*.

### Hepcidin synthesis and availability

The connection between hepcidin and iron metabolism was first recorded by Pigeon [34] through the investigation of hepatic responses to iron overload. The main way that hepcidin interfere with iron regulatory mechanism and homeostasis [14] is through its direct action on the cellular exporter ferroportin. In normal conditions gastro intestinal tract iron absorption consider as a dynamics process, could be suppressed in order to maintain stable extracellular iron level. Studies suggested that hepcidin inhibits iron absorption in the small intestine, the release of recycled iron from macrophages [35]. According to these observations, it has been suggested that hepcidin is a key component of iron homeostasis that acts as a negative regulator of iron metabolism. Systemic iron level is controlled by hepcidin protein (Hamp) that is secreted from the liver [36]. The Hamp leads to ferroportin protein degradation during elevated iron level in human body. The inflammatory cytokines induce hepcidine overproduction which causes the endocytosis and proteolysis of ferroportin (FPN), favoring trapping iron in the absorbing enterocytes resulting in iron deficiency anemia (IDA) [37, 38]. Serum hepcidin level was reduced in iron deficient anemic subject [39]. Several studies have reported increased hepatic hepcidin expression [40], enhanced hepcidin mRNA levels in the substantia nigra [41] and elevated serum hepcidin levels [42]. hepcidin is predominantly expressed in the liver in both mice and humans. Expression is also detectable in the heart and brain, but to a much less extent [34]. Moreover, liver is also important for iron homeostasis in terms of iron storage [43].

In the current study, the increase in hepcidin expression in hepatocytes of iron deficient anemic rats treated with Pr-IONPs indicated a stimulatory effect of the Pr-IONPs on the molecular pathways that responsible for hepcidin transcription in liver cells specifically. As cited by [44] there are three main pathways for modulation of hepcidin transcription. The Janus kinase (JAK) to signal transducer and activator of transcription 3 (STAT3), bone morphogenetic protein (BMP) to mothers against decapentaplegic homologue (SMAD) and haemochromatosis protein HFE/TfR2 signaling pathway. IL6-induced inflammatory stimuli activate the JAK/STAT3 pathway which in turn triggers hepcidin production. Another pathway is triggered by increased hepatic cellular iron which may induces BMP6 expression which then interacts with BMPR and HJV, forming a complex, thereby activating the SMAD pathway. The SMAD pathway involves phosphorylation of SMAD1, 5, and 8 (pSMADs), formation of the pSMADs/SMAD4 complex and subsequent translocation of this complex to the nucleus to activate hepcidin gene expression. Extracellular Tf-Fe<sup>+2</sup> mediates a second iron signal. When transferrin saturation increases, Tf-Fe<sup>+2</sup> displaces HFE from TfR1. HFE then interacts with TfR2 to form the HFE/ TfR2 complex. Consequently, this complex activates hepcidin transcription via the Hemojuvelin HJV/BMP/SMAD and/or ERK/MAPK signaling pathways. The increase in hepcidine and its role in iron sequestration within cells particularly

macrophages, Hepcidin induction during pneumonia to be essential to preventing bacterial dissemination by limiting extracellular iron availability. Hepcidin agonists may represent an effective therapy for Gram-negative infections in patients with impaired hepcidin production or signaling [45]. hepcidin may become a useful tool for diagnosis and management of iron disorders. Furthermore, a number of strategies that target hepcidin, its receptor, and its regulators are under development as novel therapeutic approaches for diseases associated with iron dys-regulation [46]. Therapeutic modulation of hepcidin is a promising method to ameliorate these conditions. Several approaches have been taken to enhance or reduce the effects of hepcidin *in vitro* and *in vivo*. Based on these approaches, hepcidin modulating drugs have been developed and are undergoing clinical evaluation. In this article we review the rationale for development of these drugs, the data concerning their safety and efficacy, their therapeutic uses, and potential future prospects [47]. These data show Hepcidin induction during pneumonia to be essential to preventing bacterial dissemination by limiting extracellular iron availability. Hepcidin agonists may represent an effective therapy for Gram-negative infections in patients with impaired Hepcidin production or signaling [45]. Stefanova in 2017 [48] illustrates how differential levels of Hepcidin expression change the iron concentration in various body compartments with likely consequences for the susceptibility to infection. Hepcidin-induced hypoferrremia in circulating plasma protects against the acute threat of bacterial and yeast sepsis. Prolonged up-regulation of hepcidin leads to the anemia of inflammation or of chronic infection and protect against malaria [49]. During inflammation even low level of liver will secrete hepcidin as acute- phase proteins and there will be blocking for iron absorption from duodenum this will lead to iron deficiency anemia, this required a high hygiene and infection control, such intervention is important in poor population, the Clinical trials are underway for both hepcidin agonists and antagonists and it is likely that these will become commonly used therapeutic agents in the mid-term future [ 50].

The elevation of Hepcidine in the present study demonstrated for the first time that the Pr-IONPs are a good candidate for The findings of the present investigation are in concomitant with other studies demonstrated up regulation of hepcidin expression following acute iron [51, 52]. As hepcidin is one of the acute phase proteins released from liver cells in response to conditions such as inflammation and iron overload [53]. In iron-deficiency anemia, the body seeks to return to iron balance by increasing the absorption of dietary iron. This is achieved by down-regulating the expression of hepcidin, which increases ferroportin expression in enterocytes thereby increasing iron transfer into portal blood. In the present experiment this mechanism did not work because of iron deficient diet even there is activation of ferroportin. The iron-regulatory hormone hepcidin is induced early in infection, causing iron sequestration in macrophages and decreased plasma iron; this is proposed to limit the replication of extracellular microbes, but could also promote infection with macrophage-tropic pathogens. The mechanisms by which hepcidin and hypoferrremia modulate host defense and the spectrum of microbes affected, are poorly understood. Hepcidin analogs may be useful for treatment of siderophilic infections.

In humans, the majority of iron in circulation is derived from the recycling of senescent red blood cells by macrophages and a relatively small amount is obtained daily through the absorption of dietary iron [36]. Since excretion of iron is not modulated, iron storage and release must be tightly regulated in order to prevent iron deficiency, iron toxicity and to prevent excess iron availability for pathogens. Hepcidin, a 25 amino acid peptide hormone produced in the liver, is a major regulator of iron homeostasis. Hepcidin regulates systemic iron levels as well as

the redistribution of tissue iron by binding to the iron exporter ferroportin, causing it to be degraded [36]. Ferroportin is highly expressed on macrophages and enterocytes [54]. Hepcidin decreases serum iron concentrations by redistributing iron into macrophages and impairing dietary iron absorption. As a result, excess hepcidin can cause anaemia since iron availability for erythropoiesis is decreased. On the other hand, low hepcidin leads to excessively high systemic iron levels as a result of increased dietary iron uptake and iron release from erythrophagocytic macrophages.

#### **Expression of divalent metal transporter protein (dMTP) gene in hepatocyte.**

The dMTP1 could be named according to its role in iron metabolism, natural resistance associated macrophage protein 2, because it play pivotal role in iron uptake from both Tf and non Tf bound [50]. Transcription of this protein by DMT1 gene, this gene used as marker genes to evaluate whether drugs such as ABS-derived iron reduced effect of IDA. DMT1 are very responsive to iron deficiency *in vitro* and *in vivo*. The liver senses changes in systemic iron requirements and can regulate iron concentrations in a robust and rapid manner. After endocytosis of TfR1-Tf, initiates the release of iron from Tf via acidification of endosomes. Ferric iron is reduced to ferrous iron by an endosomal ferric reductase [55]. Ferrous iron is then transported to the cytosol via DMT1. In the present result where there were decrease in Tf/ TfR1 iron influx to cells to hepatocytes. Liver DMT1 gene was up regulated for expression of DMT1 protein in hepatocytes in iron overload and down regulate in iron depletion [56]. Several mechanisms have been shown to contribute to non-transferrin bound iron (NTBI) uptake including membrane bound DMT1 facilitating direct uptake of iron into the hepatocytes [57] but in our study the iron was sever depleted as indicated by low total iron.. In the present study in Pr-IONPs treated rats when plasma iron was reregulated to normal or higher level, the TfR1 also was higher and did not reduced. This increase of iron influx in to the cytosol.

#### **CONCLUSION**

The parameters studied strongly suggest recovery of iron deficiency anemia. The single or the double dosage of the Pr-IONPs could be considered as the gold goal for the treatment of iron deficiency anemia.

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