Efficiency of PIH as an iron chelator on the alteration of haematological aspects in rats suffering from iron overload

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ABSTRACT

Iron is a vital element that participates in many metabolic activities of the cell. However, excess iron is a major reason for iron-induced oxidative stress and several human diseases. Pyridoxal isonicotinoyl hydrazone (PIH) is well-known antioxidants, and could be efficient treatment agents. Therefore, the current study was undertaken to evaluate the Efficiency of PIH as an iron chelator on the alteration of haematological aspects in rats suffering from iron overload (IOL). 85 adult male albino rats were employed in the current study. To induce IOL, rats were given 50 mg iron – sorbitol – citric acid complex/100 g BW subcutaneously on four successive days. PIH were prepared prior to the injection 50 mg PIH/kg BW (low dose) and 100 mg PIH/Kg BW (high dose). The current work included two experiments; the first one was carried out to follow up the changes that could occur in the haematological parameters as a result of IOL. In the second experiment, five comparisons were made between normal control rats, positive control rats (animal which treated with both low dose and high dose) for 4, 8 and 12 weeks and iron overload rats (groups treated with the same previous doses of PIH for the same intervals time). At the end of the 1st and the 2nd experiment (4, 8 and 12 weeks) post iron overload induction. Rats were overnight fasted, anaesthetized under diethyl ether and venous blood of all the groups was collected from the venous plexus of the eye by standard venipuncture with glass capillary tubes where a part of the blood was collected in dry clean tubes containing ethylene diethyl tetra acetic acid (EDTA) for the determination of haemoglobin. Other blood samples were collected in dry clean test tube and left to clot. Sera were separated and divided into small aliquots to avoid the effects of repeated thawing and freezing. All sera were stored at -20°C until analysis. Blood hemoglobin, serum iron profile [iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), transferrin (Tf) and unsaturated transferrin %] and ferritin were estimated. The obtained results discovered that IOL caused in significant rise in blood hemoglobin, serum iron, TIBC, UIBC, Tf, and ferritin levels. Meanwhile, it decreased serum unsaturated transferrin %, when compared with the control rats. PIH administration to IOL-rats resulted in significant decrease in blood hemoglobin, serum iron, TIBC, UIBC, Tf and ferritin levels and increased serum unsaturated transferrin %, compared with the IOL-rats. This study provides in vivo evidence that PIH administration can increase the antioxidant protection systems against IOL-induced haematological oxidative stress in rats. This effect may be due to both the antioxidant and metal chelation activities.

Keyword: - *Iron overload, Iron chelator, PIH, haematological aspects, and male albino rats.*

1. Introduction

Iron (Fe) is an important and essential nutrient that is required by almost all cells [1]. Fe is indispensable for life since it is involved in oxygen transport, electron transfer, nitrogen fixation, and DNA synthesis [2,3]. Within the mitochondria, Fe is incorporated into protoporphyrin IX and forms heme. As a part of the hemoglobin molecule, iron is a component of red blood cells which bind oxygen and delivers it to all the tissues in the body. In solution, iron exists in two oxidation states, ferrous Fe (II) and ferric Fe (III) that respectively donate or accept electron relatively easily. These different redox states are important for biochemical functions of iron; however, they may become dangerous for the organism. At physiological pH and oxygen tension, Fe (II) is readily oxidized to Fe (III), which is prone to hydrolysis and forms insoluble ferric hydroxide and oxohydroxide polymers [4-6]. Moreover, iron, due to its catalytic action in one-electron redox responses, plays a key role in the formation of harmful oxygen radicals which ultimately cause peroxidative damage to vital tissues and organs. Hence organisms have developed specific iron sequestering proteins to maintain iron in soluble form [7,8]. During evolution, sophisticated mechanisms have emerged to manage transport and storage of iron. In vertebrates, the plasma glycoprotein transferrin is responsible for shuttling iron among sites of absorption, storing, and utilization. On the other hand ferritin is an intracellular protein involved in iron storage [7,8].

Transferrin is a glycoprotein consisting of a single polypeptide chain. The concentration of transferrin in humans is about 240 mg/kg, and in normal adults only about 30% of Tf is saturated with iron. Tf contains two specific high-affinity Fe (III) binding sites and di-ferric transferrin is recognized by specific membrane receptors which are responsible for cellular iron acquisition [9]. The affinity of iron to Tf is pH dependent such that in plasma (pH 7.4) Fe affinity to Tf is extremely high but with decreasing pH the affinity decreases. Erythroid cells expressing Tf receptors, easily strip iron from Tf by a process referred to as transferrin-receptor-mediated endocytosis [10,11].

Ferritin is a ubiquitous protein that has only one clearly defined function: to sequester and store Fe. This protein has been identified in almost all animal and plant tissues, and also in fungi and bacteria [11]. In humans, ferritin consists of a protein shell composed of 24 subunits with a molecular weight of 450 000 Daltons. This relatively large protein can bind up to approximately 4500 Fe atoms. As it was previously mentioned, free unbound Fe is relatively insoluble and can catalyze formation of harmful oxygen radicals. The ferritin molecule consists of a para-crystalline iron core surrounded by a protein shell, which allows the complex to remain soluble, also helps prevent the Fe from mediating oxidative damage to cell constituents [12].

Under normal conditions, the body can regulate iron absorption to meet the general needs of the organism for this important metal. There is about 1 mg of dietary iron absorbed in 24 h, and net organismal iron balance is maintained by excretion of 1 mg iron. Fe acceptance happens predominately in the proximal small intestine, with the duodenum being the site of maximal absorption [13]. There is a series of overlapping steps involved in Fe absorption, which include iron binding to the brush border, uptake of iron into the interior of the cell, intracellular handling of the iron, transcellular transport. Finally, transport of the metal from the basolateral surface of the mucosal cell into the portal circulation where it binds to transferrin [13].

Iron represents normally about 40 and 50 mg/kg body weight in adult female and male, respectively [14]. The major portion of iron (60 to 70%) is found in erythrocytes in the form of hemoglobin dedicated to oxygen transport and delivery. 20 to 30% of the total body iron is deposited in ferritin as storage iron held in reserve by hepatocytes and macrophages in the liver, bone marrow and many other tissues. The remaining iron (less than 10%) is found as myoglobin iron in muscle, cytochromes, and as iron-containing and iron-dependent enzymes throughout the cells of the body. Although the plasma iron pool is very small (transferrin bound iron represents less than 0.1% of organismal iron) this is dynamically the most important iron pool with the highest rate of turnover [15]. Within 24 hours, plasma Fe can turn over 10 times in humans, total daily turnover of transferrin Fe being 30 mg. Generally, about 24 mg of this iron is moved to the bone marrow for hemoglobin synthesis in developing erythroid cells. In humans, mature erythrocytes circulate in the blood for about 120 days. Senescent erythrocytes are phagocytized by macrophages (reticuloendothelial system) and the heme moiety is split from hemoglobin. Heme is catabolized by oxygenase enzyme to form biliverdin, carbon monoxide by products, and the liberated iron is released back to plasma Tf at a rate which normally matches the rate of iron transport for erythropoiesis [16]. The remaining 5 mg of

plasma iron turnover is exchanged with nonerythroid tissues, mainly the liver. Daily intestinal absorption of dietary Fe is about 1 mg and this gain is usually balanced by small iron losses in the bile and urine and via cells shed from skin and gut mucosa [16].

Humans lack an effective mechanism for the excretion of excess iron. Even when iron stores are markedly increased [17], the body is unable to eliminate more than 1 mg Fe in 24 h. Under physiological conditions, the total iron in the body can be as high as 100 g due to the progressive iron loading caused by excessive dietary iron absorption and/or by blood transfusion. This excess iron is retained in the body and accumulates in hepatocytes, cardiac muscle, pancreas and endocrine glands. Such patients are likely to die because the accumulation of iron eventually leads to cirrhosis of the liver, congestive heart failure or diabetes mellitus [18]. Treatments of iron overload differ from patient to patient according to medical circumstances. In patients with hereditary hemochromatosis (HHC), the phlebotomy (removal of excess iron) should be initiated as quickly as possible in order to avoid hazardous complications such as diabetes mellitus, hepatic and cardiac failure. Phlebotomy therapy cannot be used in patients suffering from secondary iron overload because of their requirement for blood transfusion. Since humans are unable to eliminate the excess Fe that has accumulated in the body due to numerous transfusions, attempts to interrupt the cycle of iron overload and iron-induced tissue damage have centered on the use of iron chelating drugs [19].

Initially the search for effective iron chelators was primarily driven by the need to treat Fe overload diseases such as β-thalassemia. However, it has become clear that Fe chelators may be useful for the treatment of a wide variety of disease conditions, including cancer, malaria, and free radical-mediated tissue damage. Despite the synthesis and biologic assessment of a varied range of ligands, only little compounds have ever been effective and safe enough to reach clinical trials [20]. At present, the most common Fe chelator in widespread clinical use is the desferrioxamine (DFO) [21-23]. Even though DFO is an extremely effective drug with little side effects, it suffers from a number of serious problems, including its high cost and poor intestinal absorption. This latter disadvantage coupled with the fact that DFO has a very short plasma half-life necessitates that the drug must be infused subcutaneously [24-25]. Most patients treated with DFO require 12 to 24 hours of subcutaneous infusion 5 to 6 times / week to achieve Fe balance [26]. These difficulties with DFO have directed to the search for another Fe chelators that are economical, orally effective, and highly efficient. One group of compounds that satisfies all of these conditions is that PIH [27].

The iron chelator PIH is a potent antioxidant against OH formation induced by Fe (III)-EDTA, ascorbate and O_2 or by Fenton reagents Fe (II) and H_2O_2 [28]. Furthermore, Hermes-Lima et al. [29] demonstrated that iron-mediated lipid peroxidation, 2- deoxyribose oxidative dilapidation, plasmid DNA strand breaks and 5,5- dimethyl-1-pyrroline-N-oxide (DMPO) hydroxylation is inhibited by PIH. The antioxidant activity of PIH was explained by its ability to form a complex with iron that does not participate in Haber-Weiss reactions.

The current work aimed to study the effect of iron overload on some physiological and biochemical aspects and the role of PIH as an iron chelator which has a potent antioxidant against OH- formation. The long duration in this study was chosen as a critical period to focus the attention on early and lately signs of unwarranted changes in physiological and biochemical status of the treated animals.

2. Material and Methods

The current study was carried out on male albino rats Rattus rattus as an animal model for induction of iron overload. 85 adult male albino rats were employed in the current study. Their average weight was of 100±10 g representing 9±1 weeks of age. Animals were allowable 10 days pre-experiment period to acclimatize to laboratory conditions in order to avoid any complications along the period of the experiment. Rats were housed in metallic cages at 28±2oC and 50% relative humidity and received food and water ad-libitum with new supplies offered daily.

To induce IOL, rats were given 50 mg iron – sorbitol – citric acid complex (Jectofer–Auenntis Pharm. Co.) /100g BW subcutaneously on four successive days [30]. Pyridoxal hydrochloride, pyridoxal-5- phosphate and pyridoxine hydrochloride were obtained from Sigma Chemical Co., USA. PIH were prepared by the method of Johnson et al. [31] and were recrystallized from methanol / petroleum ether to yield crystalline PIH. Solution of PIH was prepared

using 1M sodium hydroxide in normal saline solution (0.9% sodium chloride) and adjusted to pH 10 with 1M hydrochloric acid prior to the injection 50 mg PIH / kg BW (low dose) and 100 mg PIH / Kg BW (high dose).

This work included two experiments; the first one was carried out to follow up the changes that could occur in the haematological parameters as a result of iron overload. To achieve this purpose, a comparison was done between two groups of animals, the first group (normal control, n = 5 rats) received daily injection subcutaneous of normal saline (0.9 % Na Cl) for four successive days. The second animal group (n = 5 rats) injected subcutaneously with 50 mg iron - sorbitol - citric acid complex for the same previous period to induce iron overload.

In the second experiment, 75 rats were employed in this experimental. Five comparisons were made between normal control rats, positive control rats [animal which treated with both low dose (50 mg PIH / kg BW) and high dose (100 mg PIH / kg BW) for 4, 8 and 12 weeks] and iron overload rats [groups treated with the same previous doses of PIH for the same intervals time]. At the end of the 1st and the 2nd experiment (4, 8 and 12 weeks) post iron overload induction, rats were overnight fasted, anaesthetized under diethyl ether (Sigma Co. USA) and Venous blood of all the groups were collected from the venous plexus of the eye by standard venipuncture with glass capillary tubes. A part of the blood was collected in dry clean tubes containing EDTA for the determination of haemoglobin. Other blood samples were collected in dry clean tubes and left to clot. Sera were separated and divided into small aliquots to avoid effects of repeated melting and icing. All sera were kept at -20°C until analysis.

Haemoglobin was estimated using the commercial kit purchases from Randox, U.K. according to the method of Dacie & Lewis [32]. Serum iron was determined by the method of Seidel et al., [33]. The determination of total iron binding capacity was determined according to the method of Ceriotti and Ceriotti [34]. To calculate the unsaturated iron binding capacity (UIBC), substrate the serum iron concentration from the total iron binding capacity. UIBC = TIBC – Serum iron concentration. The levels of serum ferritin were determined in stored sera using radioimmunoassay (RIA) procedures. The RIA methodology was undertaken according to the working manual of specialized kit. The employed kit was purchased from ICN pharmaceuticals, New York, U.S.A. The method for serum transferrin involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. By measuring the ring diameters produced by a number of samples of known concentration, a calibrator curve may be created. The concentration of the antigen in unknown samples may then be determined by evaluating the ring diameter created by that sample and reading off the calibration curve [35]. Serum unsaturated transferrin (%) was calculated by dividing serum iron concentration by total iron binding capacity and multiplying by 100 according to Tietz et al. [36].

Statistical Analysis

All documented data were expressed as mean ± standard error and analyzed by applying the following mathematical principles, two-way analysis of variance (ANOVA) test followed by Duncan's multiply range test [37,38]. The Statistical Package for the Social Sciences (SPSS) version 15 at a statistical significance level of $P<0.05$ and 95% confidence interval were used.

3. Results

The current study was conducted to elucidate the therapeutic role of PIH as an iron chelator in terms of reducing and correction the toxic effects of iron overload on haematological parameters, which induced in male albino rats. To induce iron overload, rats were given 50 mg iron – sorbitol – citric acid complex / 100 g BW subcutaneously on four successive days. From the inspection of the data presented in table (1), a significant ($p<0.05$) increase in the blood haemoglobin and serum iron were noted in iron overload rat group. The percentage of these increases reached to 13.58 for haemoglobin and 10.98 for serum iron as compared to the normal control rats.

On the other hand, the data showed a significant ($p<0.05$) elevation in TIBC, UIBC, ferritin and transferrin in iron overload rat group. The mean values recorded 68.13 ± 0.619 for TIBC, 48.12± 0.387 for UIBC, 110.37 ± 0.961 for ferritin, and 2.96± 0.027 for Transferrin regarding to 55.07± 0.523, 37.19± 0.318, 91.67± 0.867 and 2.22± 0.015 respectively in normal control rats. In relation to the control rats, a significant ($p<0.05$) decrease in unsaturated transferrin was reported in iron overload rats. The percent of these decreases reached to 11.01 regarding to control.

Table - 1: The Mean and standard error value of blood Hb content as well as serum Fe, TIBC, UIBC, Ferritin, Transferrin and Unsaturated Transferrin in control and iron overload groups.

Parameters \ Groups		Control	Iron overload	%
Hb (g / dl)	Mean ± SE	15.02± 0.339	17.06± 0.412*	13.58
Fe (µmol / l)	Mean ± SE	18.03± 0.168	20.01 ± 0.191*	10.98
TIBC (µmol / l)	Mean ± SE	55.07± 0.523	68.13 ± 0.619*	23.72
UIBC (µmol / l)	Mean ± SE	37.19± 0.318	48.12± 0.387*	29.39
Ferritin (ng /ml)	Mean ± SE	91.67± 0.867	110.37 ± 0.961*	20.40
Transferrin (ng /ml)	Mean ± SE	2.22± 0.015	2.96± 0.027*	33.33
Unsaturated Transferrin (%)	Mean ± SE	32.71 ± 0.287	29.11± 0.271*	- 11.01

On detecting the blood haemoglobin content, TIBC, UIBC, Ferritin and Transferrin the data graphically represented in figures (1),(2),(3). The data showed that the mean values of blood haemoglobin content, TIBC, UIBC, Ferritin and Transferrin were significant ($p<0.05$) decreased with the progress of time in positive control rats animal (which treated with both low dose (50 mg PIH / kg BW) and high dose (100 mg PIH / kg BW) for 4, 8 and 12 weeks). In addition to iron overload rats group (treated with the same previous doses of PIH for the same intervals time) compared to their corresponding normal control rats.

While, the treatment of different groups with PIH does not show any significant ($p<0.05$) changes to iron either at 4 week or 8 weeks for positive control rats animal and elucidate a significant ($p<0.05$) decreased at week 12. In contrary PIH showed a significant ($p<0.05$) decreased of iron at week 4 and week 8 backing into normal value at week 12 compared to their corresponding normal control rats.

On the other hand, the data showed a significant ($p<0.05$) elevation of unsaturated transferrin in positive control rat groups and iron overload groups at all-time intervals but didn't reach the normal value even in week 12 according to normal control rats.

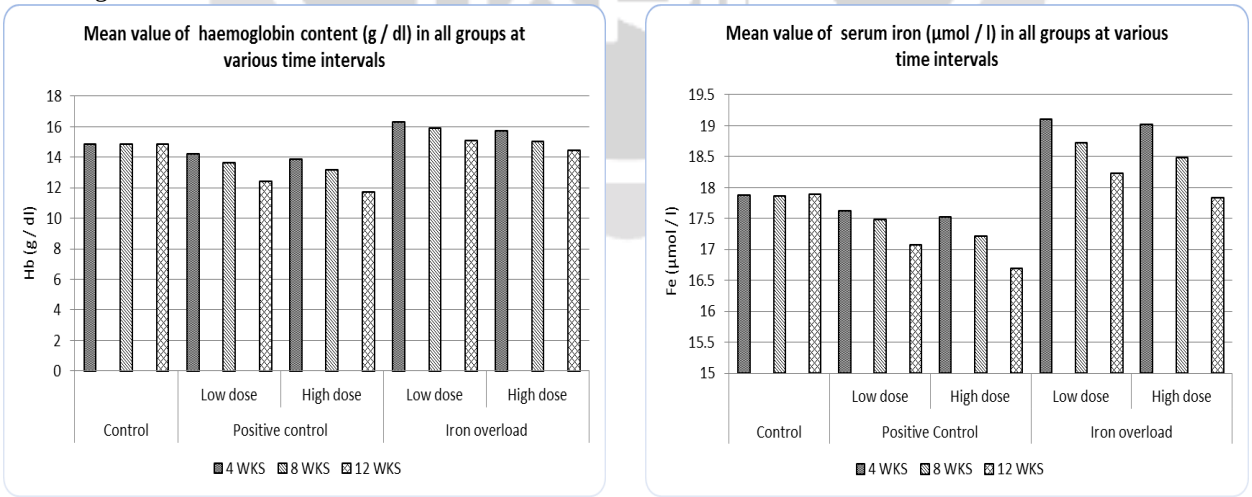
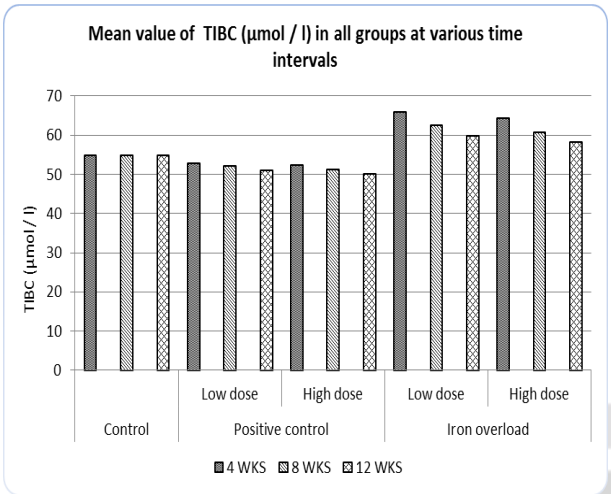


Figure-1: The mean values of blood haemoglobin and serum iron in normal control, positive control (low & high dose) and iron overload (low & high dose) rat groups treated with PIH at various time intervals.



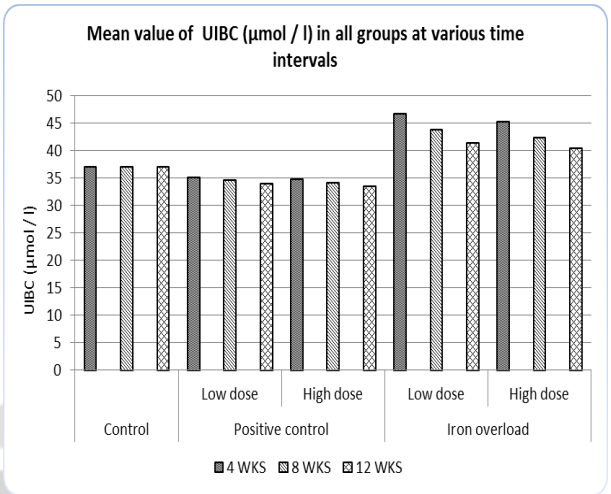
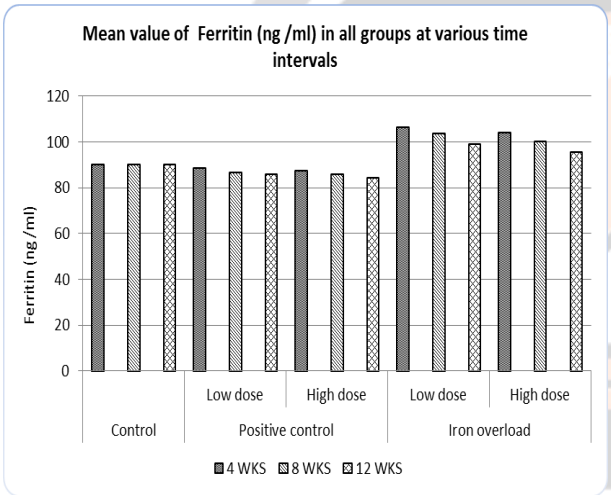
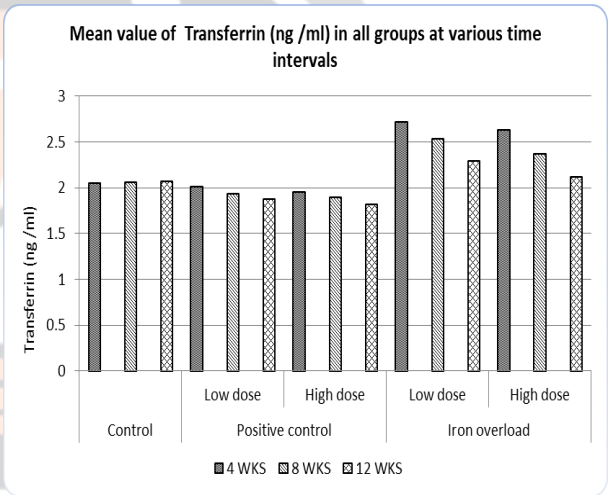


Figure-2: The mean values of serum TIBC and UIBC in normal control, positive control (low & high dose) and iron overload (low & high dose) rat groups treated with PIH at various time intervals.





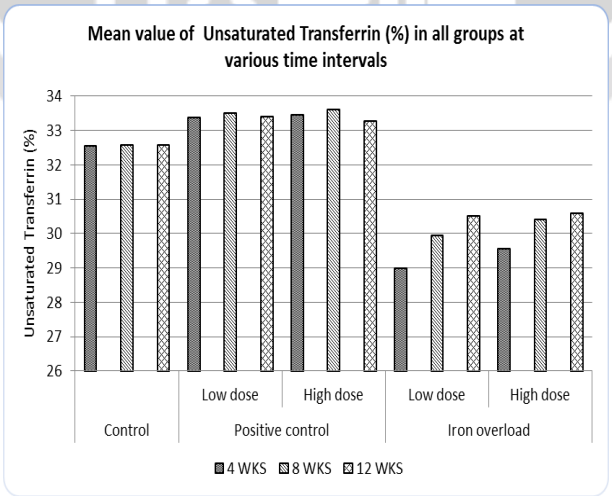


Figure-3: The mean values of serum ferritin, transferrin and unsaturated transferrin (%) in normal control, positive control (low & high dose) and iron overload (low & high dose) rat groups treated with PIH at various time intervals.

4. Discussion

Iron overload is a serious clinical condition that develops in-patient with either hereditary hemochromatosis or stubborn anemia's such as thalassemia or other anemia's requiring regular blood transfusions. Because of the limited ability of the organism to eliminate excess iron, excessive amounts of iron derived from the diet or contained in transfused red cells. It is deposited in the liver, heart, pancreas and other organs, resulting in cirrhosis, heart disease, diabetes and other disorders. Fe in excess produces cellular injury by promoting the formation of oxygen-derived free radicals, which damage to cellular components such as lipids, proteins and DNA. Untreated patients are likely to die because iron accumulation leads to progressive fibrotic changes and subsequent organ failure [39].

Patients suffering from HHC are haematologically normal and can be treated by phlebotomy that effectively reduces the total body iron stores. Obviously, phlebotomy is not an option for anemic patients requiring regular blood transfusion and such patients are treated with iron chelating agents that are capable of removing excess iron from afflicted cells and reversing iron-inflicted tissue damage. The most common clinical iron-chelating agent that is currently used is DFO. Although DFO therapy is the most effective and safe form of treatment for patients with transfusional iron overload, the major drawbacks with the use of this drug are that DFO requires long subcutaneous infusion (12-24 h/day, 5-6 times/week), It is weakly absorbed from the gut and it is very costly [40]. Although iron chelators have been utilized since the 1960s and there has been much development in available treatment, there is still requirement for new medicine candidates due to limited long-term infusion and drug weak absorption [41].

The task of developing an ideal Fe chelator is a challenge. Three different parameters should be considered in an attempt to design effective compounds for Fe chelation. These are (1) bioavailability, (2) selectivity & affinity for iron, and (3) disposition and toxicity. Bioavailability is simply defined as the percentage of absorbed drug, which eventually reaches the systemic blood circulation. Obviously, it would be fruitless to create a compound that has high first pass metabolism by hepatocytes, resulting in low bioavailability. With orally active iron chelators, a high bioavailability is ideal in order to achieve general systemic delivery of the compound to sites or tissues where iron levels are elevated. It is important that the chelator must be selective for Fe(III) since Fe(II) binding ligands possess appreciable affinities for other physiologically important elements such as zinc(II), copper(II), manganese (II) and cobalt (II). Another key property for an ideal oral iron chelator is its ability to cross biological membranes, not only enabling it to be absorbed from the intestinal tract, but also to allow access into cells from tissues such as liver and heart. Effective drugs enter cells by simple diffusion via the hydrophobic region of the cell membrane, Since uncharged molecules permeate more rapidly than charged molecules [42], neutral compounds are more likely to cross epithelial cells of the intestine and be more orally active than charged molecules. Finally, an orally effective chelator must be designed in such a way so as to resist enzymatic cleavage and the acidity of the stomach. Any ideal Fe-chelating drug should be nontoxic as the free chelator and iron-chelate complex, and should not inhibit DNA synthesis or incorporation of iron into heme. Furthermore, the iron-chelate should not increase or promote infection by supplying microorganisms with iron. Obviously, the drug should be effective when given orally and inexpensive [43,44].

Pyridoxal isonicotinoyl hydrazone is an orally effective Fe chelator [45]. This chelator is inexpensive and can efficiently cross the plasma membrane and reach intracellular iron pools where it binds Fe (III), forming a 2:1 Fe-PIH, complex. PIH has been shown to mobilize iron in vitro [46-48] and in vivo [49-51] to a greater degree when compared to DFO. Previous studies have demonstrated that PIH was able to enter into reticulocytes fairly rapidly and via an energy independent pathway (simple diffusion). However, the release of the Fe-PIH2 complex from Fe59-reticulocytes was energy dependent and cellular energy depletion [52]. Hence the exit of Fe-PIH2 complex seemed to be dependent on an energy supply and an intact microtubule network. It was thought that the cell considers the Fe chelate as a foreign entity and sequesters and shuttles the complex out of the cell along a microtubule scaffold [53].

The purpose of the current research was to explore the protective effects of PIH on iron overload in rats. It has been established for their protecting result against iron-induced toxicity. In the present study, the accumulation of iron in blood was effectively reduced by PIH, which revealed that PIH chelate the iron. Moreover, the PIH active metabolites might bind with iron and improved the elimination of iron, which in consequence decrease accumulation

and reduce the toxic effects of iron. It is quite well known that PIH act as antioxidant molecule [51,52] which can scavenge the excess iron in biological system. Fe is the most common cofactor within the oxygen management biological machinery and precisely lipid peroxidation of living membranes is the main pathogenic mechanism of iron overload prompted tissue damage [54]. The mitochondrion is a mark for iron toxicity, with oxidative mitochondrial injury and poisoning of enzymes of the tri-carboxylic acid cycle and energy metabolism known as possible targets [55]. Iron is also an essential component whose redox properties and synchronization chemistry suits it for a number of catalytic and transport functions in living cells [56]. However, these same properties render Fe toxic, to a great extent due to its ability to generate reactive oxygen species (ROS) [57,58]. Fe is a well-known inducer of ROS. Its ability to accelerate lipid peroxidation is well established [59,60]. Harmful effects of extreme iron deposition in liver are possible through iron overload, which has been linked with the beginning and proliferation of ROS induced oxidative damage to all bio-macromolecules (proteins, lipids, carbohydrates and DNA) that can lead to a serious failure of biological functions and ultimately cell death [61]. Earlier studies have demonstrated the critical role of iron in the formation of ROS that eventually cause peroxidative injury to vital cell constructions [62]. An effective beneficial method of PIH can show a dual role in reducing the degree of oxidation - first by sequestering and chelating cellular iron stocks and other as radical trap (i.e., antioxidant activity) since PIH has shown antioxidant and free radical scavenging activity [63,64]. When iron accumulates in the body and exceeds the antioxidant defense, the cells undergo oxidative stress [65] that causes cell damage [66]. Commonly the results support a useful effect of antioxidants that are substances with both chelating and free radical scavenging properties [67]. PIH possibly be a very useful medicine as a chelating cellular iron stocks and improvement of the antioxidant defense system against iron overload in rats.

Blood haemoglobin, serum iron, TIBC, UIBC, ferritin and Tf levels were significantly elevated in the iron loaded rats while unsaturated transferrin was significantly decreased (Table-1). When rats were administered with PIH, blood haemoglobin content, TIBC, UIBC, Ferritin and Tf were significantly decreased with the progress of time in positive control and iron overload rat groups compared to their corresponding normal control rats. While, the treatment of different groups with PIH does not show any significant changes to iron either at 4 week or 8 weeks for positive control rats animal and elucidate a significant decreased at week 12. In contrary PIH showed a significant decreased of iron at week 4 and week 8 backing into normal value at week 12 compared to their corresponding normal control rats. On the other hand, the data showed a significant elevation of unsaturated transferrin in positive control rat groups and iron overload groups at all-time intervals but didn't reach the normal value even in week 12 according to normal control rat's figure (1),(2),(3).

These results came in accordance with the recorded data of Papanikolaou and Pantopoulos [66] who reported that when plasma Fe content exceeds the Iron Binding Capability (IBC) of transferrin, iron gathers in the body and causes cell injury. Likewise, Crisponi and Remelli [65] reported that, serum iron was 75% higher in rats getting diet without cholesterol together with iron dextran treatment compared with those of the untreated control group and also presented higher transferrin.

Additionally, Zhang et al. [68] noted that, hepatic Fe in mice was significantly increased after administration of 500 mg kg-1 iron-dextran for forty five days. Furthermore, Silva et al. [69] detected that, liver Fe was significantly prejudiced by iron-dextran administration, as treated rats showed 6-times levels higher than that of the control group. Moreover, Torti and Torti [70] recorded that, treatment with iron increased basal levels of ferritin. Also Zhang et al. [71] indicated that, when mouse under iron overload, serum ferritin level was significantly increased. Cornejo et al. [72] observed that, chronic IOL leads to a substantial rise in liver Nitric oxide synthase activity in rats, a feature that is accompanied by ferritin induction.

Excess iron induced rise in hepcidin mRNA level that was not adequate to prevent increased intestinal iron absorption and onset of iron overload. This record is compatible with the observation that serum iron was very high in that condition and transferrin saturation was extra than 100%. The most definitely results in the incidence of Non-transferrin-bound serum iron (NTBI), although they were not able to assess this directly [73]. When the iron load increases, the iron binding capacity (IBC) of serum transferrin is exceeded and a NTBI fraction of plasma iron appears which generates free hydroxyl radicals and induces dangerous tissue damage [65], suggesting a novel mechanistic link between dopaminergic glutathione (GSH) depletion and increased Fe levels based on amplified

translational guideline of transferrin receptor 1(TfR1) [74]. Thus, the increase in hepatic iron amount due to GSH reduction mechanism show a robust relation between alterations of cellular redox condition/increase in ROS generation due to GSH depletion with altered iron homeostasis in hepatic cell that led to iron deposition, but in conditions when GSH depletion sustained for long, iron-storage capacity of ferritin might be a limiting factor to sequester continuously generating free Fe [75]. However, Fe in excess regulates iron storage protein ferritin production via translational mechanism due to synchronized release of bound iron regulatory proteins from iron responsive elements of ferritin [76].

PIH administration to iron overloaded rats protect against iron-induced lipid peroxidation by forming a complex with Fe (III) [64]. Similar results were reported by Huang, et. al. [52] who noted that, the marked capacity of PIH for iron binding, suggested that the mechanism of action of PIH at the cellular level using an in vitro Fe59-reticulocyte model involves three steps: (1) passive diffusion of PIH Into cells, (2) chelation of Fe (III) within the mitochondria, and (3) the active extrusion of Fe-PIH2 complexes via an energy dependent pathway requiring an intact micro-tubular network. Such an observation indicated that the effectiveness of PIH as an iron chelator depended mainly on the capacity of the cell to release the Fe-PIH complex rather than on the transport of PIH into the cells. The obtained results indicate that, iron overload encouraged oxidative stress that shown in alteration of the blood biochemical parameters in rats and PIH treatment can increase antioxidant status, reducing iron content in the blood of iron overloaded rats.

4. CONCLUSIONS

It can be concluded that the using of PIH may prevent or delay primary and secondary effects associated with iron overload-related diseases. The therapeutic effect of PIH may be due to iron chelation activities and antioxidant status which reducing iron content in the blood. We recommend that using PIH with a further study as it may be a source of medicine for treatment iron overload diseases. Therefore these compounds deserve further vigorous evaluation for iron chelation therapy.

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