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Chelating effects of siderophore in reducing organ dysfunction caused by iron overload in ICR mice

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RESEARCH ARTICLE

Abstract

Background and Objectives: Iron is an essential element that plays a vital role in a wide variety of cellular processes but when present in excess concentration in organs it may increase the risk for liver disease, heart failure, and diabetes. Recently, siderophores which are iron-chelating agents produced by microorganisms have attracted tremendous attention because of its strong binding and high selectivity to the ferric form of iron. Thus, the use of siderophore in sequestering excess iron in the body as a form of therapy is very attractive. This study determined the effects of commercially available siderophore in sequestering excess iron in organs such as liver, heart, and pancreas under excess iron conditions.

Methodology: First, iron-overload was induced by injecting iron dextran (20 mg) into male ICR mice for three consecutive days. The effects of iron to the liver, heart, and pancreas and the possible sequestration by siderophore were determined by scoring histological sections. The liver iron concentration was also assessed by atomic absorption spectroscopy (AAS).

Results and Conclusion: The study showed that iron-overloaded mice exhibited skin hyperpigmentation and hemosiderosis in liver, heart, and pancreas. Significant changes in the liver include hepatomegaly and development of tumor. Iron-overloaded mice had 2,935% increase in liver iron content compared to the saline-treated mice. However, when iron-overloaded mice were treated with either 100 µg or 200 µg siderophore, there was a 77% and 84% decrease in liver iron content, respectively. Moreover, the treatment of iron-overloaded mice with siderophore prevented the development of hemosiderosis, tumor, and structural changes in the tissues studied. The results showed that siderophore can effectively reduce excess iron and organ damage in iron-overloaded mice and can be potentially employed in chelation therapy of iron-overload diseases. Further studies on the possible mechanisms of siderophore aside from decreasing iron excess and lowering organ dysfunction are recommended.

Keywords: *siderophore, iron overload, iron chelating agents, hemosiderosis, hepatomagaly, hepatoprotection*

Introduction

Iron (Fe) is vital to a wide variety of cellular processes, including cellular respiration, erythropoiesis, oxygen transport, DNA replication, and protection against oxidative stress [1,2,3,4]. The capability of iron to donate and accept electrons readily makes it physiologically essential as a useful component of cytochromes and oxygen-binding molecules [3]. In humans, it exists in the form of hemoglobin circulating in red blood cells; iron-containing proteins such as myoglobin, cytochromes, and catalase; iron bound to transferrin in plasma; and in storage iron in the liver [1]. However, iron is also biochemically dangerous in excessive quantities because it can damage tissues by catalyzing the conversion of hydrogen peroxide to free radical ions that attack cellular membranes, protein, and DNA [5,3]. Moreover, as the iron content of the body increases, the saturation of circulating transferrin with iron increases, resulting in the production of increased amounts of non-

transferrin-bound iron, and the off-loading of iron, especially to cells with high levels of transferrin receptors. The excess iron in these cells may act as a Fenton agent, catalyzing the Haber-Weiss reactions. The reactive oxygen species (ROS) produced by these reactions presumably oxidizes lipids, proteins, and perhaps RNA and DNA, thereby causing tissue damage and subsequently fibrosis [1].

Since humans have no physiological mechanism by which excess iron is eliminated, the body's iron content is regulated primarily by absorption which may lead to progressive and pathological iron overload. Excess iron in the body is the result of prolonged intake of iron-containing supplements, multiple blood transfusions, hereditary disorders of iron metabolism, and chronic ineffective erythropoiesis [5].

Excessive iron intake from iron-containing supplements can be highly toxic. This toxicity involves many organs, leading to complications such as hepatic cirrhosis symptoms, myocardial traits, pancreas function disorders, endocrine glands function disorder, degenerative diseases of connective tissue, hyperpigmentation of skin, and degenerative changes in central nervous system [6].Overwhelming evidence supports the observation that excessive storage of iron in body tissues over time among transfusion-dependent patients results in increased morbidity and greater mortality, especially due to cardiac dysfunction. Symptoms related to organ dysfunction due to iron accumulation are often apparent only after significant irreversible organ damage has already occurred. The accumulation of iron therefore in the body could be fatal in the absence of therapy [7].

In the Philippines, no studies have been conducted on the effects of iron overload to different organs of the body and the possible role of siderophores on iron chelation therapy. Some of the local studies conducted are mostly focused on assessment of iron status particularly among Filipino pregnant women or determining the blood iron concentration among Filipino pregnant women particularly those taking oral supplements to treat iron deficiency conditions such as anemia [8,9].

Siderophores are low molecular weight (500-1000 Da), high affinity ferric-iron-chelating compound secreted by microorganisms in response to insoluble nature of iron in the environments. They are common products of aerobic, facultative anaerobic bacteria, and of fungi [10,11]. Today, siderophores are of interest because of their exceptionally strong binding and high selectivity for ferric ion [12]. In addition to this, it was previously been reported that a number of siderophores have good antiplasmodial activity and low toxicity towards mammalian cells [13].

The present study focuses only on the use and efficiency of deferoxamine mesylate, a trihydroxamic acid produced from a bacterial siderophore, Streptomyces pilosus [7,14]. In this regard, the concern of this study is to provide scientific basis of the *in vivo* chelating therapeutic effect of siderophore in iron-overloaded mice. Specifically, to assess the iron-unloading effect of deferoxamine mesylate on key organs including the liver, heart, and pancreas, to correlate the degree of organ damage and the total iron concentration absorbed by the liver, and to determine the influence of iron administrations in different organs. Significantly, this study presents an animal model which helps understand the cardiac, hepatic, and pancreatic function changes in patients with secondary iron overload due to excessive iron intake. The results of the study may help characterize the pathophysiologic features of cardiac, hepatic, and pancreatic failure in patients with iron overload. This study is the first to report about the hepatic-, cardio-, and pancreatic-protective effect of deferoxamine mesylate in a murine model of iron overload in the Philippines.

Methodology

Approval from the Ethics Committee of Miriam College, Quezon City was first obtained prior to the conduct of the study. Twenty (n=20), eight-week old ICR inbred male mice with an average weight of 23.9 grams were obtained from Bureau of Animal Industry (BAI) in Visayas Ave., Quezon City, Metro Manila. The mice were acclimated for a period of seven days before various treatments. The mice were housed in plastic cages (five mice per cage) in a laboratory modified as an animal house maintained at room temperature with 12/12 dark-light cycle. The mice given access to commercially available food pellets and supplied with tap water *ad libitum*. The commercially available siderophore deferoxamine mesylate (Desferal®) was obtained from Merck Pharmaceuticals (New Jersey, USA) and 10µg/µl solution was prepared by dissolving 1 mg of desferal in 100 µl sterile phosphate buffered solution (PBS). On the other hand, iron dextran (Iron-D®) was procured from Progressive Poultry Supply Corporation (Quezon City) and 100 mg/ml Iron-D® was prepared for elemental iron injection. The pH of the iron solution was maintained at 5.2 to 6.5 and was stored at 15-30ºC.

For the experimental set-up, the mice were divided into four groups namely; the control group (C), iron overloaded

group (T1), iron overloaded and supplemented with 100 µg siderophore (T2) and iron overloaded and supplemented with 200 µg siderophore (T3). Each set-up contains five experimental animals each. For the control group, mice received intraperitoneal (i.p.) injections of 0.1 ml of 0.9% NaCl solution per mouse/day for three consecutive days. Meanwhile, the iron-overloaded (T1) mice received 20 mg iron dextran i.p. per mouse/day for three consecutive days. Similarly, the iron-overloaded with siderophore treatment groups (T2 and T3) mice also received 20 mg i.p. iron dextran per mouse/day for 3 days. After iron overload, the T2 treatment group received 100 µg siderophore while the T3 treatment group received 200 µg siderophore administered three times a day for additional one week. The summary of the treatment dosage and the number of animals used in each group is shown in Table 1.

Table 1. *Summary of the treatment dosage and number of mice used in each experimental group.*

Group	Treatment	Number of animals
Control (C)	0.9 % i.p. NaCl	5
Treatment 1 (T1)	20 mg i.p. Iron dextran	5
Treatment 2 (T2)	20 mg i.p. Iron dextran + 100 µg siderophore	5
Treatment 3 (T3)	20 mg i.p. Iron dextran + 200 µg siderophore	5

Effects of iron overload to the external and internal organs of mice

To determine the effect of iron overload in mice, morphological changes in the external and internal organs were observed and compared with the control group. Mice were weighed before and after injections to determine the effect of iron overload in the body and liver weights of the mice. The mortality rates were also noted during the experimental period.

Histological analysis of the liver, heart, and pancreas

After a total of 17 days of experimental period, mice were sacrificed by cervical dislocation and then weighed. Whole heart, pancreas, and liver were removed, weighed and subsequently cleaned and rinsed in sterile 0.9% NaCl solution. Organs were cut into small pieces and immersed in 10% formaldehyde. Samples were then sent to the Philippine Kidney Dialysis Foundation, Medical Laboratory, Quezon City for histological processing. The presence of iron in the liver, heart and pancreas was scored by obtaining the percentage of the sections of the organs with iron deposits. Scoring was done by randomly selecting areas in the tissue samples and evaluating whether the area has iron deposits. The degree of inflammation was obtained by the percentage of the neutrophil aggregations caused by accumulation of iron deposits. Since presence of neutrophil is a hallmark of inflammation, it was used as marker in this study. Other structural and architectural changes were also evaluated using a light microscopy.

Liver Iron Content (LIC)

The elemental iron concentration was determined in liver tissues since it is the major storage organ of iron. The determination for total iron concentration was performed at the Philippine Nuclear Research Institute (PNRI) in Quezon City, Metro Manila by Atomic Absorption Spectroscopy (AAS). One gram of the liver per mouse was removed, rinsed in 0.9% NaCl solution and stored in the freezer until analysis. Briefly, liver weight was determined and representative samples were dried at 60°C for 48 h, dry weight was determined, after which they were ashed in a furnace at 550°C. The ash was dissolved in 10 ml concentrated HCl, diluted up to 20 ml distilled water and its total Fe content determined by Varian AA240 atomic absorption spectrometer at 386.0 nm and AA240 statistical software [15]. To ensure accuracy and precision, calibration standards were employed.

Statistical Analysis

Results were presented descriptively as means ± standard deviation (SD) in each group of five mice using SPSS (Statistical Program for the Social Sciences) Statistical Software. The differences in the liver iron content among the control, iron overloaded, iron-overload supplemented with 100 µg siderophore, and iron-overloaded supplemented with 200 µg siderophore treatments were evaluated by one-way analysis of variance and Kruskal Wallis (H) test. Data that were significant were further analyzed by using Tukey's Comparison of Means to determine if there are real differences among treatments. Differences with p-values less than 0.05 were considered significant.

Results and Discussion

Effects of iron overload in external and internal organs in mice

The effects of iron overload to the external and internal organs in mouse models were observed. There were no

changes in skin color of the saline-treated (control) group was observed however, Treatments 1, 2 and 3 (T1, T2, T3) exhibited skin hyperpigmentation characterized by redness of their skin evident on their ears (Fig. 1B-D). This might be because the deposition of iron causes skin inflammation and enhances melanin production causing darker and discoloration [16]. Moreover, the liver of control mice appear as normal reddish coloration (Fig. 2A) while those of T1, T2 and T3 showed a bronze coloration (Fig. 2B-D) which may indicate severe inflammation caused by excess iron in the body. The results are consistent with studies that also described changes of skin coloration, inflammation and bronze coloration in major organs such as the liver in iron-overloaded animals [17,18,4,16].

Development of tumor was observed in T1 which was characterized by yellowish nodules (Fig. 2B), this was not seen in the control group (Fig. 2A) or T2 and T3 (Fig. 2C-D). The presence of tumor in the liver of iron-overloaded animals was also observed in previous studies and was diagnosed as hepatocellular carcinoma [19]. It was also previously reported that the mechanisms whereby iron may act in carcinogenesis were due to the overproduction of free radicals, induction of oxidative stress, facilitation of tumor growth, and modification of the immune system that is capable for chronic cell toxicity and DNA damage [19, 18,4]. This supports the carcinogenic role of iron suggested by numerous experimental and epidemiological studies [3]. Meanwhile, it was observed that the excised heart and pancreas in T1 appeared normal as no difference was observed in comparison to control mice. This result suggests that iron may have not accumulated and have no substantial effects in these organs.

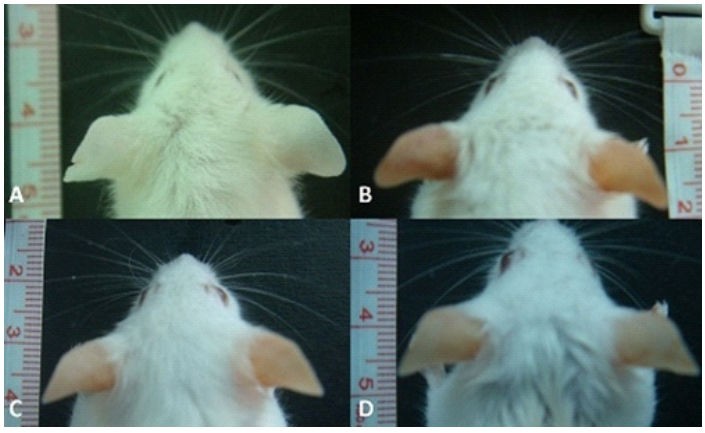

Figure 1. *External appearance of control mice (A), iron-overloaded (B), iron overloaded supplemented with 100 µg siderophore (C), and iron overloaded supplemented with 200 µg siderophore (D). Hyperpigmentation due to possible internal inflammation evident in the ears in Fig. B, C, and D (arrow) which is indicative of iron overload.*

Effect of iron-overload to the body and liver weights of mice

The effect of iron overload and treatment of siderophore on the body weight is presented in Table 2. The body weight of control mice was increased by 9.2% while by 7.9% for the iron overloaded mice (T1). The increase in body weights were also observed in a previous study wherein body weights of saline-treated and iron-overloaded gerbils had an approximately 1-fold increase [20].

On the other hand, the body weight of mice treated with 100 µg siderophore (T2) was increased only by 4.9% which was lower than those of iron overloaded mice. This result suggests that siderophore treatment at 100 µg concentration (T2) might have sequestered iron from the different organs, thus, preventing the accumulation of iron deposits. Moreover, when the concentration of siderophore was increased at 200 µg concentration (T3), a decrease in body weight by 7.9% was observed. It is therefore plausible that that 200 µg concentration of siderophore was more effective in the sequestering iron accumulated from different organs.

The effect of iron overload in mice and treatment with siderophore on the liver weight and liver/body weight ratio is presented in Table 3. Significantly, the liver weights as well as the liver/body weight ratio of the T1 were increased by 2.5 ± 1.112 g than the normal weight of the liver. On the other hand, the liver weights in T2 was decreased by 2.3 ± 0.385 g while in T3 it decreased more efficiently by 1.8 ± 0.038 g as compared

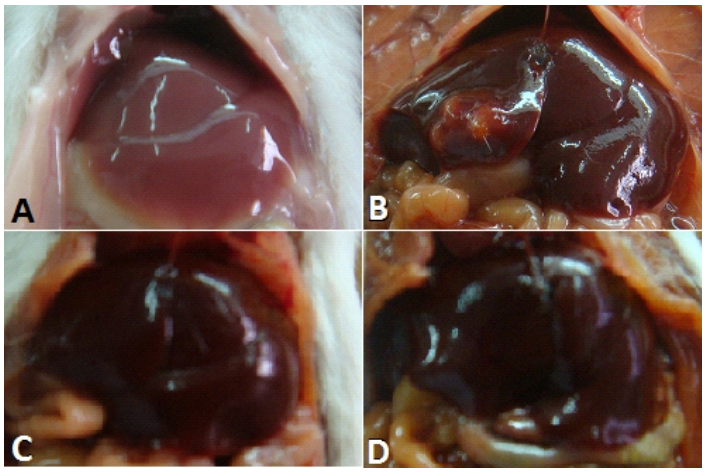

Figure 2. *Internal features of the liver in control mice (A), iron-overloaded with the presence of yellowish nodules (white arrow) (B), iron-overloaded supplemented with 100 µg siderophore (C), and iron-overloaded supplemented with 200 µg siderophore (D). The bronze appearance of the excised liver was evident in Fig. B-D probably due to accumulation of iron.*

Table 2. *Body weights of mice in control and experimental groups.*

Group	Control	Treatment 1	Treatment 2	Treatment 3
Initial body weight (g) Final body weight (g) Increase body weight Loss in body weight	22.8 ± 2.1* 25.1 ± 3.0 (9.2)** -	23.1 ± 2.5 25.1 ± 1.7 (7.9) -	25.1 ± 1.4 26.4 ± 1.2 (4.9) -	22.6 ± 2.9 20.8 ± 3.0 - (7.9)

**Means and standard deviations of 5 mice in each group.*
***Values within parentheses express data in percentage.*

Table 3. *Liver Weights of Mice in Control and Experimental Groups.*

Group	Control	Treatment 1	Treatment 2	Treatment 3
Liver weight (g) Liver/body ratio x 100 (%)	1.3 ± 0.212* 0.054 ± 0.008	2.5 ± 1.112 0.101 ± 0.005	2.3 ± 0.385 0.09 ± 0.015	1.8 ± 0.038 0.078 ± 0.022

**Means and standard deviations of 5 mice in each group.*

Table 4. *Effect of siderophore in decreasing iron deposits and inflammation of the liver, heart, and pancreas.*

Group	Control	Treatment 1	Treatment 2	Treatment 3
Liver Iron deposits Neutrophil aggregation	n=58 0/58 (0%) 0/58 (0%)	n=79 79/79 (100%) 79/79 (100%)	n=115 115/115 (100%) 64/115 (55.7%)	n=73 73/73 (100%) 35/73 (47.9%)
Heart Iron deposits Neutrophil aggregation	n=20 0/20 (0%) 0/20 (0%)	n=20 14/20 (70.0%) 11/20 (55.0%)	n=16 10/16 (62.5%) 2/16 (6.25%)	n=18 11/18 (61.1%) 0/18 (0%)
Pancreas Iron deposits Neutrophil aggregation	n=10 0/10 (0%) 0/10 (0%)	n=12 12/12 (100%) 10/12 (83.3%)	n=4 4/4 (100%) 3/4 (75.0%)	n=13 13/13 (100%) 6/13 (46.0%)

to the liver weight of T1. This showed that iron overload can result to hepatomegaly. This manifestation of a metabolic defect can be caused by the accumulation of heavy metals such as iron stored in the liver causing it to become enlarged [17]. In addition to this, the results also suggested that siderophore treatment can decrease the liver weight of iron overloaded mice, thus preventing hepatomegaly condition.

Mortality rate in iron-overloaded mice

After the third day of iron-overloading, 1 out of 5 (20%) mice from T1 died while no deaths were observed in control, T2 and T3. Similar report had been done whereby inducing iron overload causes 20-28% mortality rates [17,20]. Since animals have no mechanism for excreting iron, iron gradually accumulates in various tissues, causing morbidity and mortality [21]. This result suggested that accumulation of iron in the body can lead to death in the absence of therapy.

Iron deposits and neutrophil aggregation in the liver, heart, and pancreas

The percentage of iron deposits and iron deposits with neutrophil aggregations in the liver, heart, and pancreas is presented Table 4. It is commonly believed that the presence of ferritin particles in cell cytoplasm is a manifestation of iron overload [22].

In the liver (Table 4), control mice had no 0/58 (0%) iron deposits and 0/58 (0%) iron deposits with neutrophil aggregates which showed the normal histology. On the other hand, after iron administration, there were heavy iron depositions in all of the hepatocytes of T1, T2 and T3 mice. T1 mice had the most number of iron deposits of 79/79 (100%) with neutrophil aggregations of 79/79 (100%). Meanwhile, treatment of siderophore appeared to reduce iron load in terms of deposition of iron with neutrophil aggregations. In T2 and T3, the liver sections revealed a decreased amount of iron deposits with neutrophil aggregations of 64/115 (55.7%) and 35/73 (47.9%), respectively. Sections of the T1, T2 and T3 had demonstrated accumulation of hemosiderin iron granules in cytoplasm of hepatocytes which is an indicative of hemosiderosis and inflammation [22]. However, inflammation was decreased when siderophore treatment was administered with T2 being more effective than T3. This result might also be because of the difference in the n values of the number of sections used between the two treatments.

In the heart (Table 4), control showed no visible iron depositions of 0/20 (0%) and neutrophil aggregations of 0/20 (0%). T1 mice showed that 14/20 (70.0%) of the heart sections contained iron deposits whereby 11/20 (55.0%) were surrounded with clusters of neutrophils that entailed inflammation. In T2 mice, iron deposits were decreased by 10/16 (62.5%) and inflammation caused by iron deposits with neutrophil aggregations were decreased by 2/16 (6.25%). However, in T3 mice, there was a decrease of iron deposits by 11/18 (61.1%) but no effect on iron deposits with neutrophil aggregations by 0/18 (0%). This showed that siderophore treatment can also decrease iron burden and inflammation in the heart with T2 being more effective than T3.

In the pancreas (Table 4), control mice showed no visible iron deposits of 0/10 (0%) and iron deposits with neutrophil aggregations of 0/10 (0%). In T1 mice, the presence of iron deposits appeared in all samples 12/12 (100%) and iron deposits with neutrophil aggregations in 10/12 (83.3%). In T2 mice, iron deposits was seen in 4/4 (100%) sections, however, iron deposits with neutrophil aggregations were decreased by 3/4 (75.0%). On the other hand, T3 mice appeared to have all iron deposits of 13/13 (100%), however, iron deposits with neutrophil aggregations were decreased by 6/13 (46.0%). These results suggest that siderophore treatment allowed the reduction of inflammation in tissues despite the presence of the presence of iron deposits in the sections with T2 being more effective than T3.

Histological analysis of the liver, heart, and pancreas

In the liver, control mice showed normal lobular architecture and histology without stainable iron deposits (Fig. 3A). On the other hand, T1 mice showed a heavy iron depositions characterized by the reddish granules that were uniformly distributed in the cytoplasm of the hepatocytes (Fig. 3B). Moreover, the development of tumor reported earlier might be due to the accumulation of iron in the liver because the tumor cells which were characterized with small, actively dividing cells were surrounded by the iron deposits.

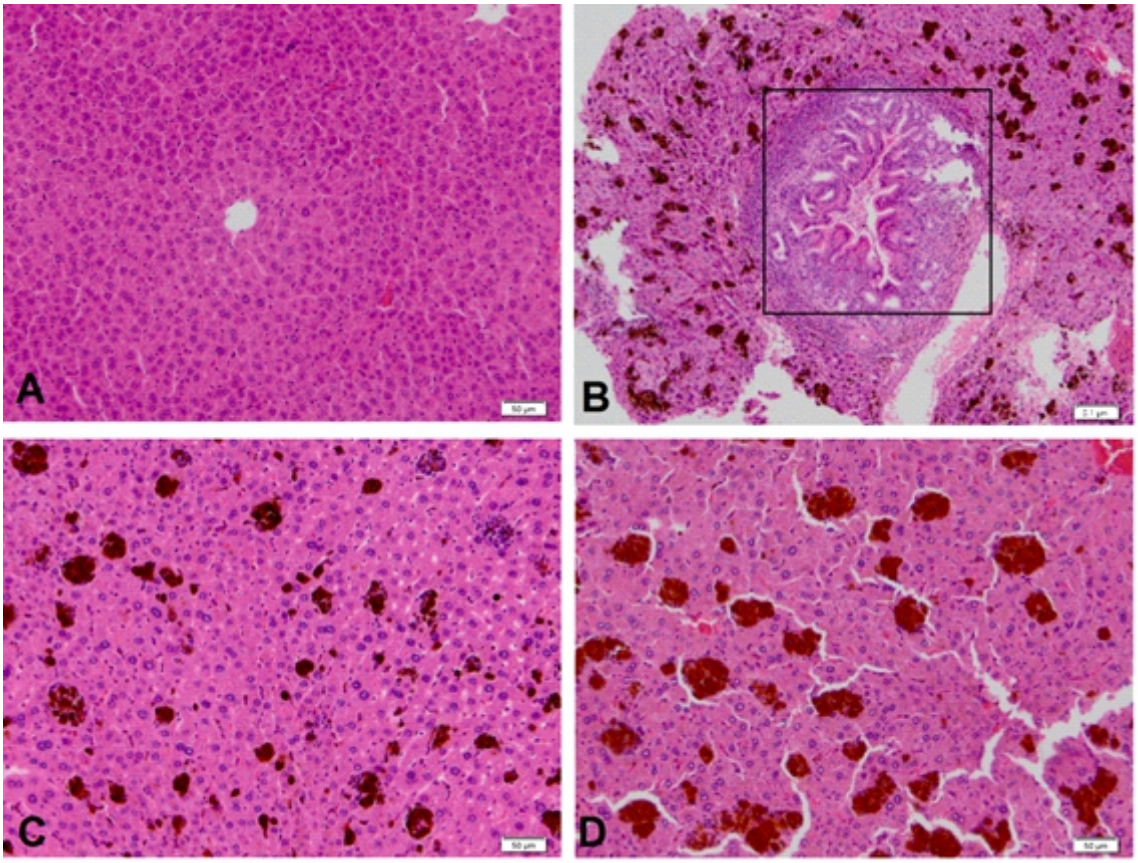

Figure 3. *Histological section of liver parenchyma in control mice (A, 20x) without stainable iron, iron-overloaded mice (B, 10x) showing iron deposits surrounding tumor cells (boxed area), iron-overloaded supplemented with 100 µg siderophore mice (C, 20x), and iron-overloaded supplemented with 200 µg siderophore (D, 20x). Treatment groups (B-D) showed iron depositions in the cell cytoplasm after iron overload (arrow). H&E stain was used..*

On the other hand, though T2 and T3 mice also showed uniform iron depositions, no significant structural changes was found in this group since it remains to have similar structure with the control saline-treated mice despite the iron depositions (Fig. 3C-D). This proves that the siderophore might be effective in terms of preventing or minimizing iron overload complications such as inflammation and tumor development in the liver. Similar to a previous study, iron depositions were also abundant in all hepatocytes because it is capable of storing excess iron and can cause hemosiderosis in liver tissues [22].

In the heart, there were no morphological changes found in control mice, hence, appeared normal histologically (Fig. 4A). Iron accumulations in T1 mice were seen in the cardiomyocetes, ventricles, and less in atria. Sections of the ventricle from iron-overloaded mice showed areas of iron-induced destruction of cardiac muscle due to the presence of iron (Fig. 4B). Other studies showed that cardiomyopathy, characterized by small foci of degenerative and necrotic myofibers, was inevitably diagnosed with patients who do not undergo iron chelation therapy [17]. In this study, however, cardiomyopathy and necrosis were not observed as previously reported by the group of Whittaker [17]. T2 mice appeared to be normal in structure with only few iron deposits (Fig. 4C). On the other hand, T3 mice iron deposits were localized in the veins of the heart (Fig. 4D). Apart from iron deposits, there were no morphological changes observed in the heart of T2 and T3 mice. Moreover, iron deposits in the heart were much lesser in comparison to the heavy iron deposits in the liver. This suggested that heart had limited access to iron unlike the liver which is the major organ for iron storage.

In the pancreas, control mice appeared to have normal pancreatic cells (Fig. 5A). After iron administration, the mice had accumulated iron in the islets of Langerhans but a higher

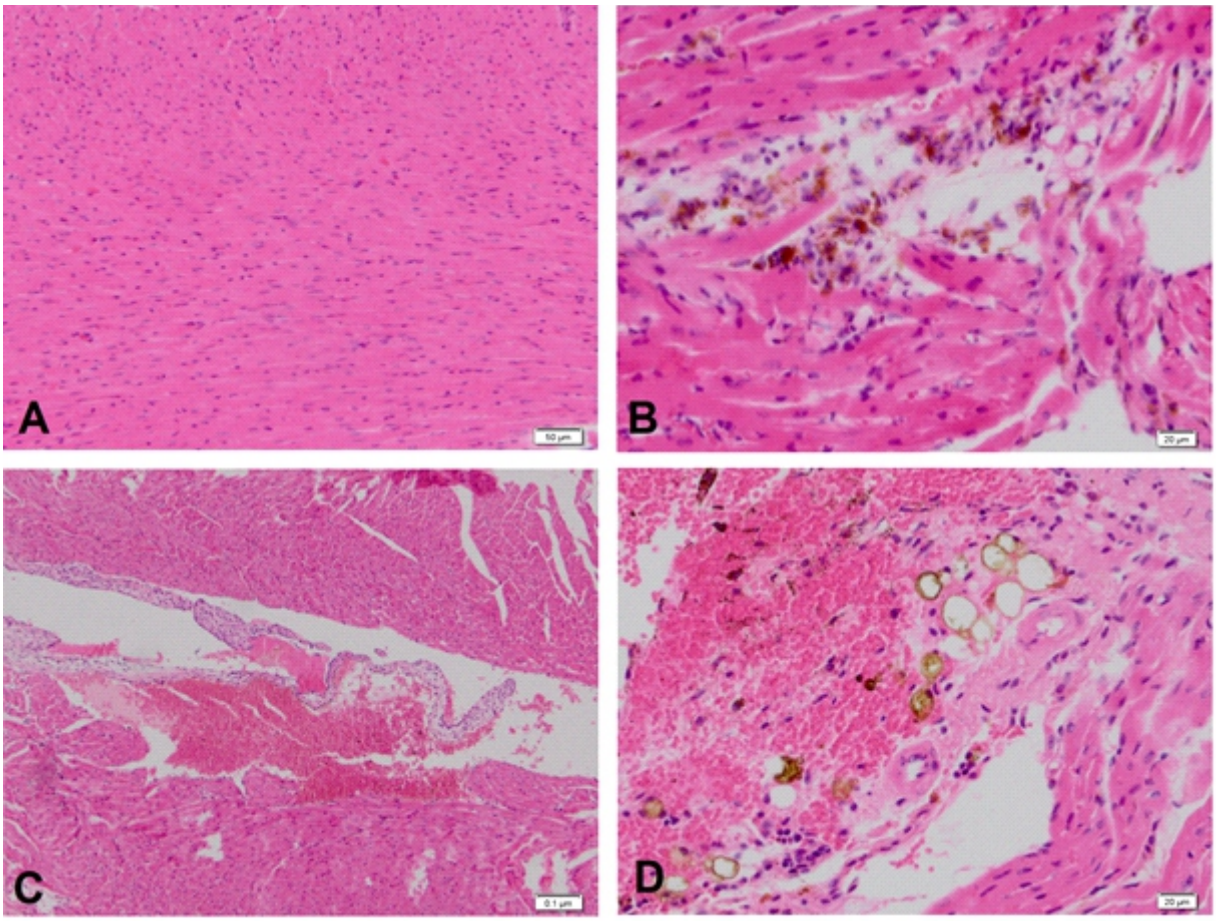

Figure 4. *Histological section of cardiomyocytes in control mice (A, 20x) without stainable iron, iron-overloaded mice (B, 40x), iron-overloaded supplemented with 100 µg siderophore (C, 40x), and iron-overloaded supplemented with 200 µg siderophore (D, 40x).Treatments groups (B and D) showed iron depositions in the cell cytoplasm after iron overload (arrow). H&E stain was used.*

degree of deposition was also observed in the pancreatic acini or acinar cells similar to what was observed by [15,17] (Fig. 5B). As expected, T1 had more iron deposits in the pancreatic cells compared to T2 and T3. Siderophore treatment appeared to reduce the presence of iron granules in the cells (Fig. 5C-D). Moreover, pancreas like the heart also had lesser iron deposits as compared to the liver. This finding is consistent with a previous study by the group of Nick [23], whereby iron loading in mice plateaued later in the heart and pancreas than in the liver, indicating delayed iron deposition based from histological evaluation which is consistent with the liver being the primary storage organ for iron.

Liver Iron Concentration (LIC) by atomic absorption spectroscopy (AAS)

The liver is considered to be the major organ for iron storage as also evident in this study, thus, the total concentration of accumulated iron was measured by atomic absorption spectroscopy (AAS) in the liver. The iron concentrations in the liver in different treatments are shown in Table 5.

Results showed that there was a significant difference in the concentration of iron among control and the treatment groups. T1 had a significant increase of iron concentration to

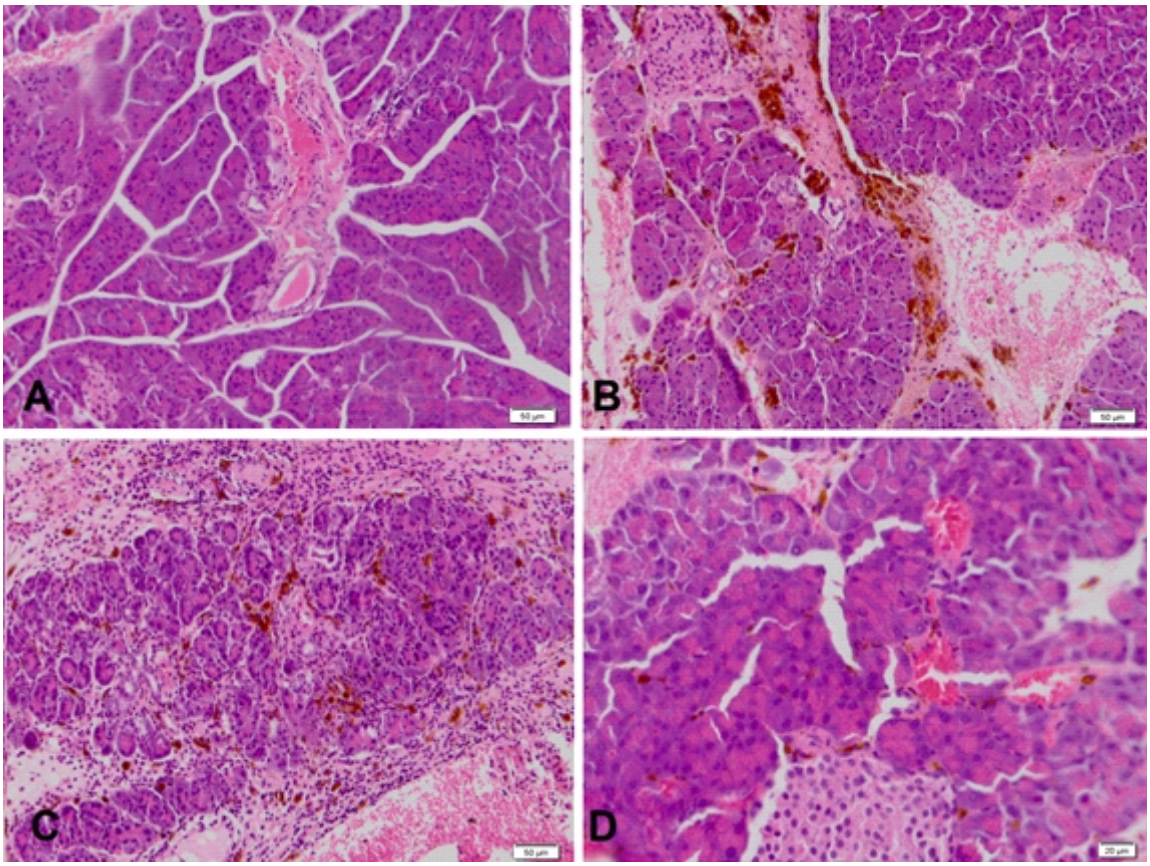

Figure 5. *Histological section of pancreatic cells in control group (A, 20x) without stainable iron, iron-overloaded mice (B, 10x), iron-overloaded supplemented with 100 µg siderophore-treated mice (C, 20x), and iron-overloaded supplemented with 200 µg siderophore (D, 40x). Treatments groups (B-D) showed iron depositions in the cell cytoplasm after iron overload (arrow). H&E stain was used.*

Table 5. *Average post-treatment hepatic iron concentrations determined by Atomic Absorption Spectroscopy (AAS).*

Treatment	Concentration of Iron (ppm)
0.9% saline solution (Control)	3015.16 ± 2624.03
20 mg iron dextran (T1)	91539.44 ± 94194.93
20 mg iron dextran + 100 µg siderophore (T2)	20914.33 ± 6017.08
20 mg iron dextran + 200 µg siderophore (T3)	14706.76 ± 5643.81

91539.44 ± 94194.93 ppm (p=0.041) compared to the control group. This result suggested a 2,935.97% increase in iron content as compared to the normal iron content of 3015.16 ± 2624.03 in control mice. However, iron-overloaded mice treated with siderophore (T2 and T3) showed significant reduction to 20914.33 ± 6017.08 ppm and 14706.76 ± 5643.81 ppm, respectively. In addition to this, there was a statistically significant difference in the concentration of iron among T1, T2, and T3. These results also suggested that siderophore treatment was able to reduce excess iron by 77.15% and 83.93%, respectively. However, there were no statistically significant differences between T2 and T3 (Fig. 6). This result indicates that the efficacy of siderophore in this study may not be dose-dependent. It might be possible that a sufficient amount of siderophore was already maximized to chelate iron in the liver or the siderophore concentration interval was too low to exhibit significant difference. A similar effect of siderophore was reported, wherein oral administration of multiple doses of FBS0701 novel iron chelator also showed no apparent dose-dependency in terms of iron excretion in urine among transfusionally iron-overloaded patients [24]. However, a previous study showed that lipophilic siderophores, infused into the root of aorta, were capable of reducing cardiac reperfusion injury in a dose-dependent manner [25]. Other synthetic iron chelators like ICL670 and 2-pyridylcarboxaldehyde thiophenecarboxyl hydraxone (PCTH) showed dose-dependent increase in iron excretion when orally administered to mice [14].

This study showed a good correlation between iron concentration by AAS and degree of organ damage by histological assessment. It was found out that the higher the total iron concentration in the liver, the more severe the iron overload if not treated with iron chelation therapy through siderophore. It was also shown in this study that iron is generally localized in the liver because iron deposits were lesser in the heart and pancreas. Moreover, iron deposits in these tissues were decreased by siderophore treatment. A clear trend toward decreasing iron burden and organ damage was also found as the concentration of siderophore increases.

Siderophore treatment was able to reduce iron concentration in the liver because it complexes with iron and reoxidizes it to non-toxic form. Consequently, the siderophore prevented oxidative stress and the generation of reactive oxygen species through the Fenton reaction in iron-

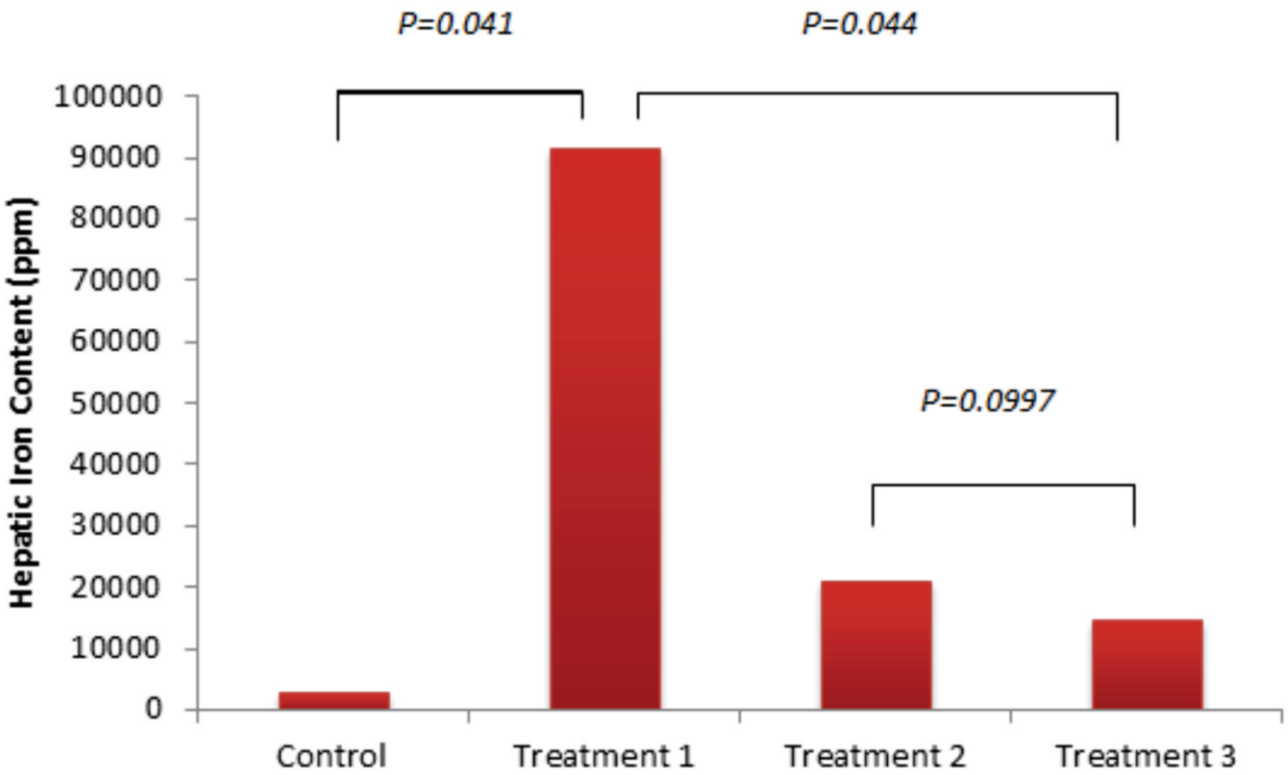

Figure 6. *Hepatic iron content of mice in control and experimental groups by AAS analysis. The iron content of liver of saline treated (control, n=5), iron overloaded (T1, n=5), 100 µg siderophore-teated (T2, n=5), and 200 µg siderophore-treated (T3, n=5).*

overloaded cells, thus, protecting the tissues against cellular damage and restoring their normal cellular activity [20].

Currently, the only way to prevent iron overload in patients requiring regular red blood cell transfusions is by long-term iron chelation therapy [5]. Recently, deferasirox, an orally active and long-acting iron chelator, has been approved for use in transfusional iron overload. In support of this knowledge, the effects of deferasirox has been assessed by the reduction of liver and heart iron content in murine model of juvenile hemochromatosis [23]. Similar to the present study, the results suggest that deferoxamine provided significant hepatic, cardiac, and pancreatic iron removal and the prevention or reduction of structural abnormalities in the murine model of secondary iron overload established in this study.

Previous studies showed that although deferoxamine has been established as the current clinical chelator of choice for treatment of iron overload, there had been reports on the role of siderophore in the pathogenesis and virulence of microbial infections [26] or could decrease iron acquisition and growth of pathogens as previously reported by the group of Olakanmi [27].

Conclusion and Recommendations

This study revealed that excess iron uptake could produce morphological changes in mouse models in relatively short period of time and this model can be used for further parenteral iron overload investigations. In addition to this, siderophore was able to mobilize excess iron from the liver and other organs, at the same time, provide structural protection in tissues by decreasing the excess iron, preventing further toxicity and damage. This study contributes to the assurance of the clinical safety and therapeutic efficacy of siderophore in its iron-removing role. Furthermore, it hopes that iron chelation by siderophore will become an important strategy in the future to lower the incidence of liver, cardiac, pancreatic failure, and most importantly, human cancer. The effects of iron overload to other organs such as the skin, kidney and spleen are recommended. Imaging studies that may help identify and quantify iron overload in various organs may also be done. The use of different concentrations of siderophore is also recommended to determine if the effect is dose dependent.

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References

1. Angelucci E, Cogoni C, Pettinau M, Pilo F, Targhetta C, Zacchedu F. (2008) Organ damage and iron overload. Hematology Meeting Reports 2(5):3-5.
2. Garner DK. (2003) Siderophores: New understanding of strong metal chelation. Journal of Biochemistry 25-27.
3. Gurzau ES, Neagu C, Gurzau AE. (2003) Essential metals—case study on iron. Ecotoxicology and Environmental Safety56:190-200.
4. Kohgo Y, Katsuy I, Takaaki O, Yoshihiro T, Junji K. (2008) Body iron metabolism and pathophysiology of iron overload. International Journal of Hematology 88:7-15.
5. Shander A, Sweeney JD. (2009) Overview of current treatment regimens in iron chelation therapy. Journal of US Hematology 59-62.
6. Szatko F, Paradowska-stankiewicz I, Grzybowski A. (1999) Iron overload in the organism as a health problem. Polish Journal of Environmental Studies 8(1):1-5.
7. Merson L, Olivieri N. (2002) Orally active chelators. Blood Reviews, Elsevier Science Ltd. 16:127-134.
8. Kuizon MD, Tajaon RT, Madriaga JR, Perlas LA, Desnacido JA. (1989) Assessment of iron status of Filipino pregnant women. Southeast Asian Journal of Tropical Medicine and Public Health 20(3):461-70.
9. Timoteo VJA, Dalmacio LMM, Nacis, JS, Marcos JM, Agarrado REL, Capanzana, MV. (2019) Determining Significant predictors of blood iron concentration and status in Pregnant Filipino Women using linear models. Philippine Journal of Science, 148(1):167-177.
10. Gokarn K, Pal RB. (2010) Siderophores and pathogenicity of microorganisms. Biosciences Technology 1(3):127-134.
11. Neilands JB. (1995) Siderophores: Structure and function of microbial iron transport compounds. The Journal of Biological Chemistry 270(45):26723-26726.
12. Allard KA, Dao J, Sanjeevaiah P, *et al.* (2009) Purification of legiobactin and importance of this siderophore in lung infection by Legionella pneumophila. Infection and Immunity 22(7):2887-2895.
13. Rotheneder A, Gernot F, Lothar H, *et al.* (2002) Effects of synthetic siderophores on proliferation of Plasmodium falciparum in injected human erythrocytes. Antimicrobial Agents and Chemotherapy 46(6):2010-2013.

14. Kalinowski DS, Richardson DR. (2005) The evolution of iron chelators for the treatment of iron overload disease and cancer. The American Society for Pharmacology and Experimental Therapeutics Reviews 57(4):547-583.
15. Tsay J, Zheiwei Y, Patrick R, *et al.* (2010) Bone loss due to iron overload in a murine model: importance of oxidative stress. Blood: The American Society of Hematology 116(14):2582-9.
16. Crowe SL. (2001) Cardioprotective effects of dihydropyridine antagonists in a murine model of chronic iron overload. Masteral Dissertation. Queen's University, Kingston, Ontario, Canada.
17. Whittaker P, Hines FA, Robi MG, Dunkel VC. (1996) Histopathological evaluation of liver, pancreas, spleen, and heart from iron-overloaded Sprague-Dawley rats. Toxicology and Pathology 24(5):558-563.
18. Deugnier Y, Margues S, Brissot P, Deugnier MB, Bourel M, Ferrand B. (1982) Comparative study between biochemical and histological methods and image analysis in liver iron overload. Journal of Clinical Pathology 35:45-51.
19. Blanc JF, Balabaud C. (2000) Iron overload and cancer. Bulletin of Academy of Natural Medicine 184(2):355-363.
20. Walker EM, Epling CP Parris C. (2007) Acetaminophen protects against iron-induced cardiac damage in gerbils. Annals of Clinical and Laboratory Science 37(1):22-33.
21. Hershko C. (2007) Iron loading and its clinical implications.American Journal of Hematology 82(12):1147-1148.
22. Özgüner M, Sayın N. (2002) Histological changes in rat liver after chronic iron-sorbitol overload. Journal of Ankara Medical School 24(2):49-54.
23. Nick H, Allegrini PR, Fozard L. *et al.* (2009) Deferasirox reduces iron overload in a murine model of juvenile homechromatosis. Institute of Zoology, University of Zurich, Switzerland. Exp Biol Med. 234:492-503.
24. Rienhoff HY, Viprakasit V, Jay L, *et al.* (2001) A phase 1 dose-escalation study: safety, tolerability, and pharmacokinetics of FBS0701, a novel iron chelator for the treatment of transfusional iron overload. Haematology 96(4);521-525.
25. Horwitz LD, Sherman NA, Kong Y, *et al.* (1998) Lipophilic siderophore of Mycobacterium tuberculosis prevent cardiac reperfusion injury. Proceedings of the National Academy of Science 95:5263-5268.
26. Collins HL, Kaufmann SHE, Schaible UE. (2002) Iron chelation via deferoxamine exacerbates experimental salmonellosis via inhibition of the nicotinamide adenine dinucleotide phosphate oxidase-dependent respiratory burst. Journal of Immunology 168:3458-3463.
27. Olakanmi O, Schlesinger LS, Britigan BE. (2006) Hereditary hemochromatosis results in decreased iron acquisition and growth by Mycobacterium tuberculosis within human macrophages. Journal of Leukocyte Biology, 81:1-10.