Evaluating the Effect of PIH on Rats-Liver Overloaded with Iron

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Abstract: *The accumulation of iron (Fe) in the liver is a serious complication of thalassemia and other iron-loading anemias requiring long-term blood transfusions. Such patients are directed to suffer death unless they are treated with Fe chelating agents. Desferrioxamine (DFO), deferiprone (L1) and desferasirox (ICL-670) are clinically approved iron chelators used to treat secondary iron overload, but there is still need for new drug candidates due to limited long-term efficacy and drug toxicity. Pyridoxalisonicotinoylhydrazone (PIH) is tridentating iron chelator effective at scavenging and mobilizing iron. Therefore, the present study was undertaken to evaluate the role of PIH to improve rat antioxidant systems against iron overload (IOL) induced hepatic oxidative stress. To produce IOL, rats were intraperitoneal(IP) injected by 50 mg iron–sorbitol–citric acid complex/100g b.w. subcutaneously on four successive days.AST and ALT activities, moreover, MDA, TBARS and NO levels were significantly (p<0.05) increased. The toxic effect of iron was also indicated by significant (p<0.05) decrease in the levels of liver GSH, Gpx, CAT, SOD and Ceruloplasmin when compared with the control rats. Administration of PIH at different doses 50 mg PIH/kgb.w.(low dose) and 100 mg PIH/Kg b.w. (high dose), significantly (p<0.05) reversed the levels of AST, ALT, MDA, TBARS and NO levels and restored the levels of GSH, Gpx, CAT, SOD and Ceruloplasmin to the normal levels. This study provides in vivo evidence that PIH administration can improve the antioxidant defense systems against IOL-induced hepatic oxidative stress in rats. This therapeutic effect of PIH in iron overloaded rats liver may be due to both the antioxidant and metal chelation activities.*
Keywords: *Antioxidant, Iron overload, Lipid peroxidation, Liver, Oxidative stress, PIH.*

I. Introduction

Fe is a foremost minute element of the body, being found in operation form in hemoglobin, myoglobin, and cytochrome enzymes with iron sulphur complexes [1]. During iron overload, red blood cells are very susceptible to iron-mediated cell injury but, they do not bear the assault of reactive oxygen species (ROS) alone. Damage to cells in other organs accumulates gradually and becomes clinically significant. Hepatocytes, the primary component cells of the liver, are the major site for body iron. With IOL, these cells are unremittinglybarraged by ROS and eventually die. Also, the hepatocytes are replaced by fibroblast cells and collagen laid down by fibroblasts produces hepatocellular carcinoma. Liver is one of the main organs in the body and the highest site for intense metabolism and excretion [2],[3]. Hepatotoxicity is the furthermostshared finding in patients with IOL as liver is mainly the active loading site of Fe in the body [4].Although an optimum level of iron is always maintained by the cells to balance between essentiality and toxicity, in some situations it is disrupted, resulting in IOL which is related to the oxidative stress madeillnesses including anemia, heart failure, hepatocellular necrosis and cirrhosis [5].

In IOLpromptedsicknesses, Feelimination by iron chelation therapy is an actual life-saving treatment. IOLraises the creation of ROS which implicatesin the start of lipid peroxidation, protein oxidation and liver fibrosis. However, excess iron is stored as Fe^{3+} in ferritin and IOL sustains for long period and released depends on the efficiency of iron chelating drugs [6]. The currently available iron-chelating agents used clinically are deferoxamine, 1,2-dimethyl3-hydroxypyrid-4-one (deferiprone, L1), and deferasirox. The body lacks to excrete unnecessaryFe and therefore the attention has been focused to develop Fe chelators that are economical, orally effective, and highly competentpotent chelating agent capable of complexing with Fe and helpingin its excretion [7],[8].

One group of compounds that satisfies all of these criteria is that of the Pyridoxalisonicotinoylhydrazone (PIH) class.PIH is tridentate iron chelator produced by the Schiff base condensation of pyridoxal and isonicotinic acid hydrazide (INH), this method is simple and economical because it is a one-step procedure. In addition, both pyridoxal and INH are readily available. Hence the cost of preparing PIH is very low, which is of major importance, since the thalassemia syndromes occur at highest frequency in developing countries [9]where the population cannot pay forcostly drug routines. PIH is tridntate ligands that bind Fe through their phenolic oxygen, carbonyl oxygen, and aldimine nitrogen groups[10], [11].PIH active at scavenging and mobilizing iron. The possible utility of this type of chelator, as well as its ability as anti-proliferative agents, preventing free-radical mediated damage.PIH has been shown to chelate both forms of Fe,

at pH 7.4 these chelators exist as a mixture of the neutral molecule. In fact, at physiologic pH, PIHs are predominantly in the form of the neutral molecule(the neutral charge of PIH ensures oral absorption and allows access to the cytosol, where labile Fe can be chelated), allowing access across cell membranes to intracellular Fe pools and absorption from the gut. The mainly uncharged state of pyridoxalisonicotinoylhydrazone at pH 7.4, its small sizeand its relatively high lipophilicity probably account for its rapid uptake by cells to chelate intracellular Fe pools [12],[13],[14].PIH displayedabilityforeliminatingFe from rat reticulocytes which enclosed labile non-hemeFe and mobilizing Fe from Chang cells. From the speciation schemes of these ligands it was revealed that the neutral species was maximal at pH 6.3 Hence the greatest absorption of these compounds may occur in the small intestine, where the pH has been reported to be in this range [15]. PIH is a strong antioxidant against OH^-creationmade by Fe^{+3}-EDTA, ascorbate and O_2 or by fenton reagents Fe^{+2} and H_2O_2. The antioxidant activity of PIH was explained by its ability to form a complex with iron that does not participate in Haber-Weiss reactions [16], [17], [18], [19].

Hence the present study aimed to investigate effect of iron overload on liver and the role of PIH as an iron chelatoragainst iron-induced liver injury.

II.   Material And Methods

The current study was carried out on male albino rats Rattusrattus as an animal model for induction of iron overload. Eighty fivemature male albino rats were used 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 ± 2^oC and 50% relative humidity and received food and water ad-libitum with new supplies offered daily.

To induce iron overload, rats were given 50 mg iron–sorbitol–citric acid complex (Jectofer–Auenntis Pharm. Co.) /100g BW subcutaneously on four successive days [20]. 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. [21] 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 1Mhydrochloric acid prior to the injection 50 mg PIH/kg b.w. (low dose) and 100 mg PIH/Kg b.w. (high dose).

This work included two experiments; the first one was carried out to follow up the changes that could occur in the liver 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 dosehypodermic of normal saline (0.9 % NaCl) 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 b.w.) and high dose (100 mg PIH/kg b.w.) 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 random blood samples and liver tissue specimens were collected from all animals groups.

Venous bloods of all the groups were collected from the venous plexus of the eye by standard venipuncture with glass capillary tubes in dry, clean and screw capped 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 used for subsequent biochemical analysis.

Liver tissues; incompletion of each investigational period, rats were forgone by cervical decapitation. The liver specimen was rapidly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter study and stored at -20°C. Liver tissues were cut and milled into minorbits, make uniform with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 rpm for fifteen minutes at 4°C then the supernatant was used for the subsequent biochemical analysis.

Biochemical analysis: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT),glutathione (GSH), catalase (CAT), superoxide dismutase (SOD),ThioBarbituric Acid Reactive Substances (TBARS),malondialdehyde (MDA), Nitric Oxide (NO),glutathione peroxidase (GPx), andceruloplasmin, were determined according to the methods described by [22], [23], [24], [25],[26], [27],[28], [29], and[30] respectively.

2.1 Statistical Analysis

All documented data were expressed as mean±standard error and analyzed by applying the next mathematical principles, 2-way analysis of variance (ANOVA) test followed by Duncan's multiply range test [31],[32]. 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.

III. Results

The present study was directed to interpret the beneficial role of PIH as an iron chelator in relations of decreasing and correction the noxious effects of iron overload on liver functions, which induced in male albino rats. To induce iron overload, rats were given 50 mg iron–sorbitol–citric acid complex/100g b.w. subcutaneously on 4continuous days. From the inspection of the data presented in Table (1), a significant (p<0.05) increase in the AST and ALT were noted in iron overload rat group. The percentage of these increases reached to 95.35 for AST and 70.58 for ALT as compared to the normal control rats.

Also, the data revealed a significant (p<0.05) elevation in MDA, TBARS and NO in iron overload rat group. The mean values recorded 187.60 ± 18.38 for MDA, 161.76 ± 1.79 for TBARS, and 0.201 ± 0.005 for NOregarding to 129.81 ± 5.07, 110.91 ± 1.59, and 0.164 ± 0.003 respectively in normal control rats.
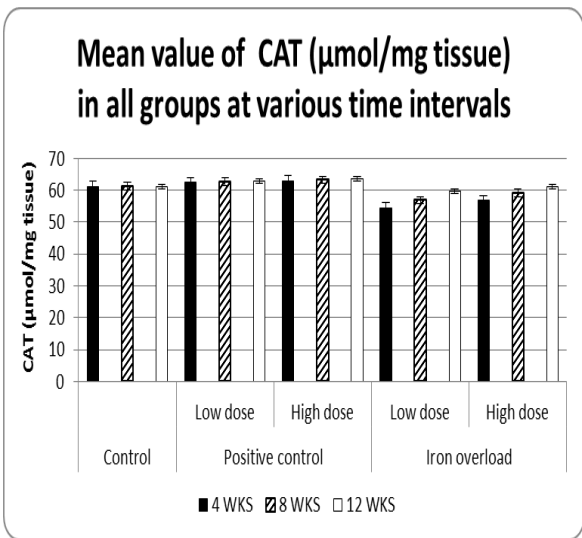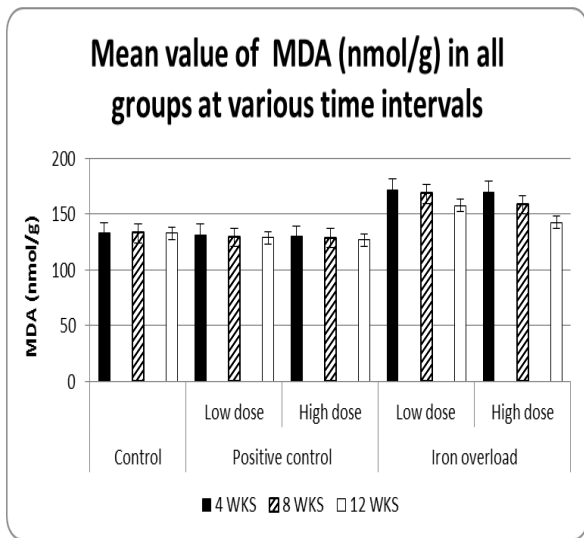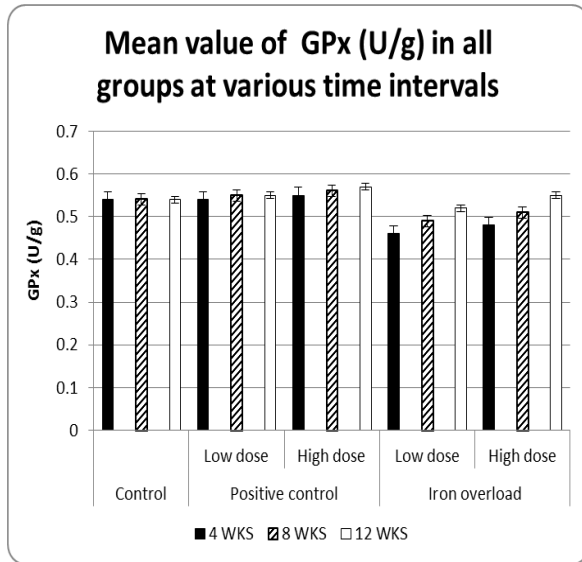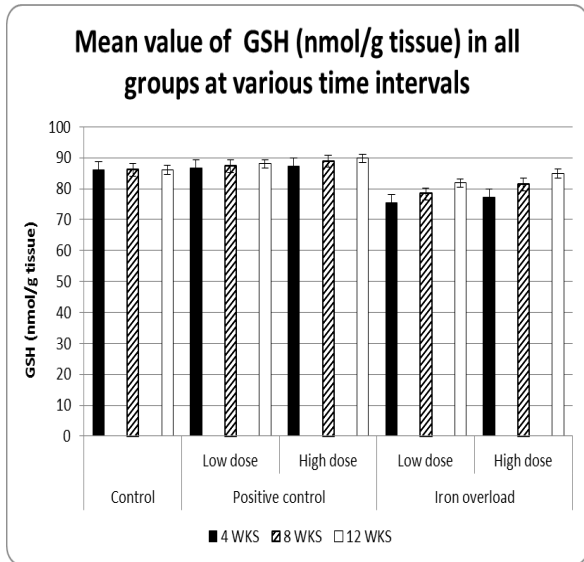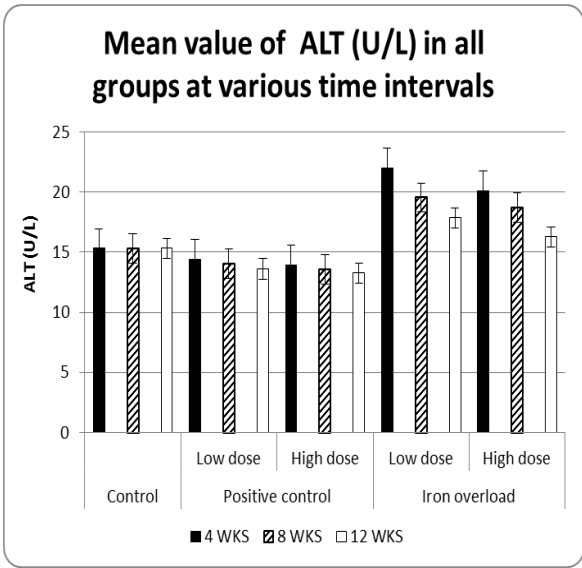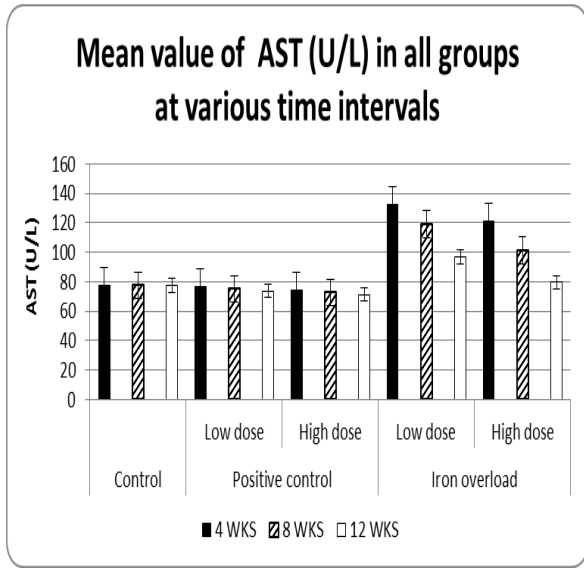
In relation to the control rats, a significant (p<0.05) reduction in GSH, GPx, CAT, SOD and Ceruloplasmin weredescribed in iron overload rats. The percent of these decreases reached to -15.07043, -18.51851, -14.1107, -22.7387 and -44.33962 respectively.

Table - 1: The Mean and standard error value of AST, ALT, GSH, GPx, MDA, CAT, SOD, TBARS, NO, and Ceruloplasmin in control and iron overload groups.

Parameters	Groups	Control	Iron overload	%
AST (U/L)	Mean±SE	77.50 ± 5.87	151.41 ± 5.56*	95.35484
ALT (U/L)	Mean±SE	15.32 ± 1.32	26.13 ±1.08*	70.58824
GSH (nmol/g tissue)	Mean±SE	86.12 ± 0.46	73.14 ± 0.47*	-15.07043
GPx (U/g)	Mean±SE	0.54 ± 0.03	0.44 ± 0.02*	-18.51851
MDA (nmol/g)	Mean±SE	129.81 ± 5.07	187.60 ± 18.38*	44.53005
CAT (µmol/mg tissue/ 60 min)	Mean±SE	61.23 ± 1.74	52.59 ± 1.59*	-14.1107
SOD (Nu/mg tissue/30min)	Mean±SE	7.96 ± 0.035	6.15 ± 0.051*	-22.7387
TBARS (nmol/g tissue)	Mean±SE	110.91 ± 1.59	161.76 ± 1.79*	45.84798
NO (µmol/g)	Mean±SE	0.164 ± 0.003	0.201 ± 0.005*	22.56098
Ceruloplasmin (U/g tissue)	Mean±SE	1.06 ± 0.004	0.59 ± 0.002*	-44.33962

On detecting the biochemical analysis for AST, ALT, GSH, GPx, CAT, SOD, TBARS, MDA, NO, and ceruloplasmin, the data graphically represented in Chart (1). The data showed that the mean values of AST, ALT, TBARS, MDA, and NO, 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 b.w.) and high dose (100 mg PIH / kg b.w.) for 4, 8 and 12 weeks) and iron overload rats group (treated with the same previous doses of PIH for the same intervals time) compared to their corresponding normal control rats.

In contrary PIH showed a significant (p<0.05) increased of GSH, CAT,GPx and ceruloplasmin, 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 SOD in positive control rat groups and iron overload groups at all-time intervals but it didn't reach the normal value even in week 12 according to normal control rats.

Mean value of AST (U/L) in all groups at various time intervals

Mean value of ALT (U/L) in all groups at various time intervals

Mean value of GSH (nmol/g tissue) in all groups at various time intervals

Mean value of GPx (U/g) in all groups at various time intervals

Mean value of MDA (nmol/g) in all groups at various time intervals

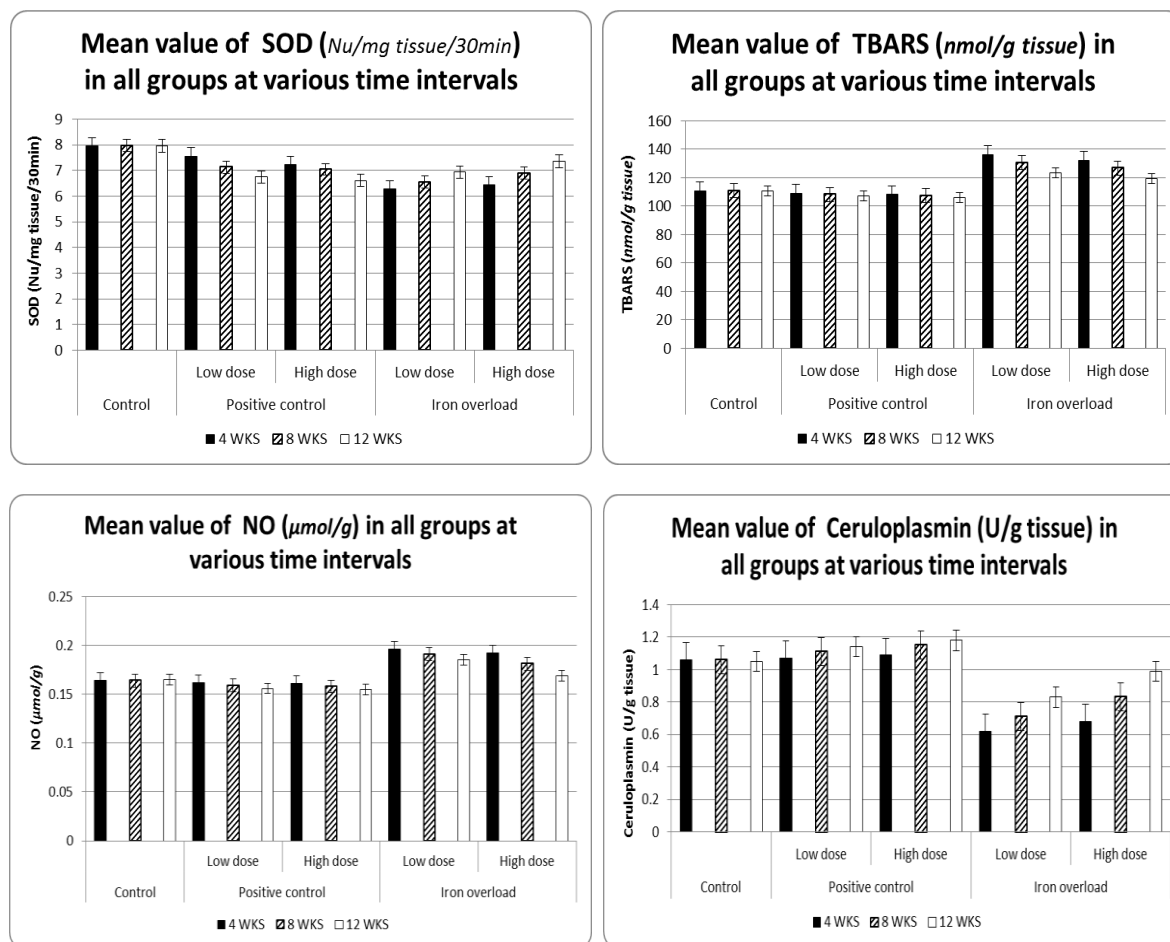Mean value of CAT (µmol/mg tissue) in all groups at various time intervals

Figure -1: The mean value of AST, ALT, GSH, GPx, MDA, CAT, SOD, TBARS, NO, and Ceruloplasmin in all rat groups

IV. Discussion

Iron overload in rats is an outstanding model to investigate the *in vivo*lipid peroxidation (LPO) in which extraFe induced oxidative stress by growing lipid peroxide levels in liver and in serum[33]. Subsequently, malondialdehydewasformedsignificantly contributed to liver injury that is evaluated by AST and ALT levels in the iron-supplemented rats [34]. Excess hepatic iron may thus cause peroxidation of membrane lipids and oxidative liver damage[35] in which IOL increases liver injury and accelerates the process of fibrosis [36]. This tissue injury can be relieved by the treatment of an suitable chelating agent which can association with the Fe and rise its rate of excretion [37], as PIH that are substances with both chelating and free radical scavenging propertiesthus, it may be a very useful medicine for treatment [38].

Iron overload resulted in significant increase in serum AST and ALT activities compared with the normal control rats. The obtained results are nearly parallel to data reported by [39], [40] that presented, in the iron-supplemented rats all of the indices of LPO, including AST and ALT, were enlarged significantly (~5-fold) compared with the control. As AST and ALT were used as sensitive indicators of liver damage [41], the serum enzymes amplifiedactions in Fe-loaded rats can be attributed to the generation of ROS and oxidative destruction by excess hepatic Fe that may outcome in chronic necro-inflammatory hepatic illness, which in turn creates more ROS and causes more oxidative injury[40], [41], [42]. PIH administration in iron-loaded rats reduced serum transaminases activities. Comparable results were reported by the data of [43] thatnoted, PIH administration to iron overload rats reduced serum ALT and AST activities compared to untreated controls. Also, [44]detected that, PIH administration significantly decreased the levels of AST and ALT activities in overloaded rats, suggesting protection by stabilizing the structural integrity of the hepatocellular membrane. PIH also scavenged free radicals [45] and deterring LPO procedure[46], in which the Fe complexes of PIH studied not only reserved the antioxidant properties of PIH, but in many cases displayedimproved free radical-scavenging activity [47].

The obtained datashown that, iron overload resulted in significant increase in MDA concentrations in the iron-loaded rats as compared with control group. These results are nearly related to those of [48] who observed that, the injection of FeCl or FeSO$_4$ (3 mg Fe^{2+}/100 g b.w.) significantly amplified LPO products in the

rat liver. Also, [49] reported that, IOL in rats was accompanied by improvement in LPO as presented by a significant rise in all tissue MDA concentration in iron complemented group and added that, in the liver there was a perfect relationship between MDA level and tissue iron content, suggesting creation of oxidative stress. LPO produced by ROS was measured in terms of MDA[50], which is a secondary end product of the oxidation of polyunsaturated fatty acids [51]. Focusing on the liver organ, the cytotoxic degradation products as MDA[52] can form covalent adducts with proteins, phospholipids and DNA[53], in which the creation of liver microsomal malondialdehyde protein adducts, through IOL in mice and the microsomal function injury may modify protein function and might lead to cellular damage and Fe-associated hepatotoxicity [54]. When rats were administered with PIH hepatic MDA concentrations were significantly reduced than that of iron-loaded control group. The obtained results approve with the data of [55] that described, PIH administration in IOL-rats sharply diminished microsomal LPO in the liver and spontaneous oxygen radical creation by peritoneal macrophages. Also, [56]stated that, LPO level in the liver of the PIH group was significantly reduced in comparison to the control group, so PIH can be both antioxidants and iron chelators; it means that PIH will be good applicants for curing IOL related sicknesses, as they can play a dual role in dropping the rate of oxidation, one act as iron chelator and the other act as radical trap [56]. Also [57] found that, PIH administration suddenly suppressed free radicalconstruction in liver microsomes by phagocytes in iron overload rats. This antioxidant activity due to its Fe(III) complexingthat may be a good antioxidant and may be more effective free radical scavengers matched to other antioxidant. The severe decrease in MDA in IOL-rats may be also attributed to the free radical scavenging property of PIH, in which IOL in rats induces the oxidative stress that was characterized by oxygen radical over-creation in liver microsomes, peritoneal macrophages and blood neutronphils[19].

The current results revealed that, IOL resulted in significant decrease in liver GSH levels when compared with the control group. The obtained data are nearly parallel to those reported by [58] that observed increase in protein carbonylation and decrease in GSH content as well as in the GSH/GSSG ratio of the liver were observed after six weeks of treatment maybe induced by iron-generated free radical activity [59]. Also, [60]stated that, introduction of Fe into mouse liver mitochondrial fraction caused a time dependent reduction in GSHtwice lower than control at 30 min post usage. GSH is a main non-enzymatic tri-peptide multifunctional intracellular antioxidant [61] that was frequently used as an estimation of the redox environment of the cell [62]. Iron deposition and related damages in liver show a strong relation between modifications of cellular redox condition which increase in ROS, e.g., Fe-induced free radical [59] due to GSHreduction, suggesting a novel mechanistic link between dopaminergic GSHreduction and increased iron levels based on enlarged translational regulation of transferrin receptor 1, in which iron deposition and related injuries in liver indicate a strong relation between alterations of cellular redox condtion/increase in ROS generation due to GSHreduction with changed iron homeostasis in hepatic cell that directed to iron deposition [63],[64].At the same time, oxidative stress arises because of the catalytic activity of the metal ion creating high reactive oxygen radicals and lastly leads to tissue damage[37].
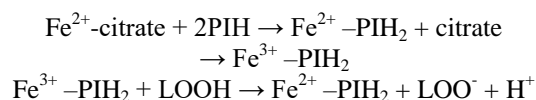
PIH administration to iron-overloaded rats resulted in significant increase in liver GSH level compared with the IOL-group. Likewise,[65] observed that, PIH treatment significantly increase the GSH level in the iron overload rats. Furthermore, [66] reported that, PIH treatment showed significant enhancement in GSH concentration, telling its role in scavenging the free radicals produced by iron-overloaded. The antioxidant imbalance was compensated by the prophylactic treatment of PIH as excessive LPO can cause increased GSH consumption [57], PIH inhibiting *in vitro* lipid peroxidation, oxidative DNAinjury, ascorbate oxidation, and 2-deoxyribosedeprivation through the creation of an inactive complex of PIH with Fe^{3+} that does not catalyze oxyradicalconstruction [67].

The obtained results revealed that, iron overload resulted in significant decrease in liver Gpx, CAT and SOD activities when compared with the control group. Iron overload can altered the balance between pro-oxidants and antioxidants, leading to severe damage of total antioxidant status level. This phenomenon can be seen in most iron overload animal models [68]although these animal cells are equipped with a collection of endogenous antioxidant defense machinery, which includes such enzymes (Gpx, CAT and SOD)[69]. In excess, iron is a major cause of oxidative stress and lowers the levels of these enzymes involved in body's antioxidant defense system. The present results suggest that PIH arrested the iron-induced depletion of these enzymes and thus reduced the iron-induced damage.

Catalase is an iron-containing antioxidant enzyme, it was described that under iron overload, there was a significant reduction of catalase activity in rat liver [70]. Similarly, [37] who displayed that, Fe-dextran injection in mouse caused a significant reduction in hepatic CAT activity. Furthermore, [71]informed that, GPx significantly competes with CAT for H_2O_2substrate and it is the major source of protection against low levels of oxidative stress-induced cancer. These results can be explained as the ROS generated throughusual cellular processes are directly detoxified by endogenous antioxidants similarto GSH, CAT, GPx, glutathione reductase, glutathione-S-transferase, etc. [72].

SOD work in conjunction with CAT and GPX [73], preventing its interaction with Fe and therefore creation of the highly toxic ^-OH, in which SOD and GPx are supportive enzyme system of the first line cellular defense against oxidative injury [74]. GPx decomposes peroxides, that start a chain of free radical creation to H_2O [60] while simultaneously oxidizing GSH; Significantly, GPx competes with CAT for H_2O_2 substrate, as it is the maincause of protection against low levels of oxidative stress [71] and the increase of SOD/GPx ratio in Fe treated cells compared to control cells specified that Fe-induced oxidative damageseemed, that might be indicative of ROS rise due to unefficient scavenging by enzymes [75].

PIH is able to inhibit lipid peroxidation in bio-membranes by forming a complex with Fe^{3+}. The resulting complex is not redox active and thus cannot receive electrons from LOOH. By functionally arresting LOO^- formation, PIH may stop the propagation of the peroxidation process and lead to a reduced formation of by-products of lipid peroxidation, such as aldehydes [76].

$$Fe^{2+}\text{-citrate} + 2PIH \rightarrow Fe^{2+} –PIH_2 + citrate$$
$$\rightarrow Fe^{3+} –PIH_2$$
$$Fe^{3+} –PIH_2 + LOOH \rightarrow Fe^{2+} –PIH_2 + LOO^- + H^+$$

PIH administration to iron-loaded rats resulted in significant increases in liver Gpx, CAT and SOD activities compared with the IOL group. As antioxidants, PIH may increase total antioxidant status level in living body, and could raise hepatic total antioxidant status level in rats and mice [76]. This effect may come from the chelation of free Fe ion with preventing iron-catalyzed oxidative reaction and the direct aggregate antioxidant status that performance as robust antioxidant. Thus, PIHpresented a novel source of antioxidant and could partiallyimpede peroxidation-induced hemedamage and then providing a defense on catalase [77]. The significant rises in liver antioxidant enzymes in PIHcured iron-loaded rats are in conformity with the resultsstated by [78] that noted, PIH has a significant influence on SOD and GPx in rats. As associatedrise in CAT and/or GPx activity is vital if a useful effect from the high SOD be expected. Chronic iron administration induced adaptive responses involving stimulation of the antioxidant defenses. PIH significantly inhibited LPO in IOL-microsomes that may be due to thefree radical scavenging property [79]. The three enzymes can prevent injury by detoxifying ROS. The significant increasing of SOD activity also proposes that its free-radical scavenging action is only actualonce it is complemented by a rise in activity of CAT and/or GPx, because SOD produces H_2O_2 as a metabolite [80], which is more noxious than oxygen radicals in cells and require to be scavenged by CAT or GPx[81].

Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism, it was reported that under iron overload, there was a significant decrease of ceruloplasmin in rat liver. Likewise, [82],[83] who displayed that, ceruloplasmintransmits more than 95% of the total copper in the plasma of healthy human. Ceruloplasmindisplays a copper-dependent oxidase activity, which is connected with possible oxidation of Fe^{2+} into Fe^{3+}, consequentlysupport in its carriage in the plasma in connotation with transferrin, which can transmit iron only in the ferric state. The declining of the level of ceruloplasminrevealed a substantial rise in TBARS levels when compared with the control group. This rise was due to amplified lipid peroxidation, presumably due to altered systemic iron homeostasis, so ceruloplasmin is vital for normal iron metabolism and storage inside cells [84].

PIH administration to iron-overloaded rats resulted in significant increase in ceruloplasmin activity and reduced liver TBARS levels compared with the IOL group. These data may be attributed to the pharmaokinetic properties and antioxidant capabilities of PIH, which acts as free radical scavengers, reducing the manufacture of oxygen and/or nitric free radicals, inhibition of oxidation in mitochondrial matrix and cell membrane stabilizers. Also, PIH acts to neutralize the free radicals, declines the lipid peroxidation and increases the immune system defiance. These data are in agreement with those obtained by [56], [85],[86].

Iron overload caused in significant rise in liver NO levels when matched with the control group. Comparable results were recorded by[87] that found, amplified NO generation was evidenced in the liver under conditions of acute IOL. Furthermore, [88]discovered that, increased manufacture of NO by nitric oxide synthase-2 was confirmed in patients with hepatic cell carcinoma complicating genetichaemochromatosis. That can be clarified as NO is an inorganic reactive nitrogen species created in liver by inducible nitric oxide synthase (iNOS) found in hepatocytes, Kupffer cells and endothelial cells [89], whose appearance is controlled by the redox-sensitive transcription factor, nuclear factor-kappa B [90] and the complex interrelationships between Fe and NO [91] can result in modifications*in vivo* NO production [92]. Also the rise in rat liver NOS activity due to chronic iron overload [93] is related to up regulation of iNOS expression [94].

PIH administration to iron-overloaded rats resulted in significant decreased liver NO levels compared with the IOL group. The data obtained are in synchronization with [95] that displayed, PIH inhibit lipopolysaccharide-induced NO production. NO was proposed to act as a pro-oxidant at high concentration, that is suppressed by PIH by direct scavenging [96],[97].

V.   Conclusion

Altogether, the present results confirmed that, PIH is inhibit the adverse effect of ferric ion induced oxidative stress, lipid peroxidation, and reducing the toxic level of iron in IOL rats, hence protect liver from oxidative stress and fibrosis. PIH treatment improved the cytoprotective enzymatic and non-enzymatic antioxidants as revealed by elevated the GSH concentration, GPx, CAT, ceruloplasmin, and SOD activities and thus, might protect the cellular environments from iron-induced free radical damage. The results indicate that, PIH may have potential effects in inhibiting the iron-induced oxidative stress in human. The effective role of PIH on livers of iron-overloaded rats may be due to its high antioxidant activity, including both its radical scavenging and iron chelation activities. Conclusively, PIH may be used for the development of potential iron chelating drug in the treatment of iron overload-induced liver toxicity.

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