Effects of of α– tocopherol and L.NAME on experimental chronic hepatic iron overload in adult male albino rats

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Abstract :Background: Although an optimum level of iron is always maintained by the hepatic cells to balance between essentiality and toxicity, in some situations this is disrupted, resulting in iron overload induced oxidative stress with the release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) e.g. Nitric oxide (NO). **Objective:** The current study aimed at evaluating the *in vivo* antioxidant effects of both vitamin E (α – tocopherol) and NG – nitro – L – arginine methyl ester (L.NAME) which is a specific non- selective nitric oxide synthase (NOS) inhibitor on chronic hepatic iron overload oxidative stress in a adult male albino rats. **Design:** 40 adult male albino rats divided into four equal groups were included. Group (1): served as a control group. Group(2): Injected with intra peritoneal(IP). Iron dextran at a dose of 1000mg /kg/day, three alternate days/week for 4 weeks, to induce a chronic iron overload state. Group (3): Iron dextran co-administrated with α – tocopherol (100 mg / kg i.p) three alternate days/week for 4 weeks Group(4): iron dextran co-administrated with L.NAME(100 mg/kg subcutaneously), three alternate days/week for 4 weeks. Blood and tissue samples were collected to estimate serum iron levels, liver enzymes, bilirubin, albumin hepatic iron deposition and histopathologic changes in all groups. **Results:** The administration of iron dextran has significantly increased serum levels and hepatic content of iron when compared with the control group, which was proved by increased iron deposition by perl's stain and was associated with pathological changes represented by significant decreases in liver albumin level together with significant increases in liver enzymes and bilirubin as well as injurious cellular changes observed in haematoxylin and eosin stained sections. In addition, there was a significant improvement of serum hepatic biomarkers induced by co-administration of the antioxidant α – tocopherol with iron dextran when compared with their elevated levels in the iron-dextran group alone and associated with marked reduction in hepatic cellular injury observed in haematoxylin and eosin stained sections. In chronic iron overload.However, the present study revealed a significant worsening in the hepatic biomarkers by co-administration of L-NAME with iron-dextran versus their increased levels in iron-dextran group. The liver sections obtained from this group showed histopathological worsening denoting enhanced hepatotoxicity. **Conclusion:** The present study revealed that the antioxidant α – tocopherol has a partial protective effect against iron-overload-induced hepatic toxicity. This is evidenced by biochemical and histopathological improvement suggesting its beneficial use as a promising hepatoprotective agent in hepatic iron overload states. However, the treatment by the antioxidant L-NAME was harmful in such condition.

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Key words: Chronic iron overload; Hepatotoxicity; Antioxidants; L-NAME; Reactive oxygen species.

1.Introduction

Iron is an essential element for the normal body health, being found in functional form in many molecules as hemoglobin, myoglobin, cytochromes, and enzymes harboring iron sulphur complexes among other iron-dependent enzymes **(1).**

Cells maintain free iron concentration to the minimum required level to avoid toxic effects of excess iron. However, in some conditions as genetic hemochromatosis, hereditary sideroblastic anemia, severe alpha and beta thalassemia, myelodysplastic syndrome (MDS) and transfusional siderosis, iron balance becomes disrupted resulting in iron overload **(2).**

Free iron is a potent promoter of hydroxyl radical formation which causes increased lipid peroxidation and depletion of chain-breaking antioxidants (oxidative stress), resulting in several health problems including diabetes, heart failure, gallbladder disorders, infertility, and cancer **(3).**

Hepatotoxicity is the most commonly met with manifestation in iron overloading patients as liver is the physiologic main site of iron storage in our bodies**(4).**

It was suggested that hepatic oxidative stress induced by iron overload could be through production of both ROS as well as RNS or otherwise decreased activity of antioxidants **(5).**

ROS and RNS are formed in normal hepatocytes. They are considered critical for normal physiological processes including oxidative respiration, growth, regeneration, apoptosis, and microsomal defenses **(6).**

When the capacity of normal antioxidant systems in the hepatocytes is overwhelmed by increased levels of oxidation products, oxidative stress occurs.

In This type of stress, ROS and RNS can directly attack cellular membrane polyunsaturated fatty acids (PUFAs), and induce damage to all liver cells, including hepatocytes, Kupffer cells, stellate cells, and endothelial cells, through initiation of inflammation, ischemia, fibrosis, necrosis, apoptosis, or even through malignant transformation by damaging lipids, proteins, and/or DNA**(7).**

Iron chelation therapy is an effective and life-saving strategy in different iron overload induced diseases. The currently available iron-chelating agents used clinically are known to have several serious adverse effects as cardiac dysrhythmias, bone marrow suppression, and renal failure, **(8).**

The therapeutic trials using radical trap antioxidant compounds are so limited which mandates further workup as it could be an effective, safe, and beneficial agents in the therapeutic handling of iron overload states.

The antioxidant vitamins are suggested by many authors to have the capacity of controlling oxidative stress related functional and histopathological changes **(9, 10)**

Alpha-tocopherol, is the most abundant form of vitamin E in nature. It possesses the highest biological activity and is considered as the main lipid-soluble antioxidant in the body. It acts on cell membranes where it prevents the propagation of free radical reactions, scavenges peroxyl & alkoxyl radicals, prevents lipid peroxidation and promotes the production of antioxidant enzymes **(11).**

In addition, vitamin E was found to protect the body's biological systems in some heavy metals induced poisonings. It was suggested to be a potentially useful therapeutic agent in the treatment of several disorders associated with oxidative damage induced by heavy metals **(12, 13).**

NO is a reactive nitrogen species which has a critical role in the reduction/oxidation (redox) biological processes of hepatocytes. It is produced by nitric oxide synthase (NOS) which is present in three forms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) **(14).**

iNOS is known to be expressed in different hepatic cells as hepatocytes, Kupffer cells, vascular endothelial cells, and stellate/Ito cells. The iNOS is proved to have a role in the development and propagation of inflammation in the liver **(15).**

The current study was designed to evaluate the in vivo antioxidant effects of both vitamin E (α – tocopherol) and L-NAME which is a specific non-selective nitric oxide synthase inhibitor on a model of experimentally induced chronic hepatic iron overload oxidative stress

2.Materials and methods

Animals:

Forty (40) adult male albino rats weighing 220– 250g, supplied from faculty of Veterinary Medicine, Zagazig University, were utilized in the present study. Rats were housed in stainless steel rodent cages under environmentally controlled conditions and allowed one week for acclimatization at room temperature (23 ± 2°C) and mean relative humidity of (50 ± 5%), with a 12 hours dark / light cycle before begging the experimental work. During the acclimatization period and throughout the study period, rats were kept in the animal unit of the faculty of Medicine, Zagazig University. They were fed the standard rat diet (commercial rodent chow) and allowed water ad libitum [i.e. free access to water).

All investigations were conducted in accordance with the guiding principles for the care and use of research animals and were approved by the Institutional Research Board.

Experimental design:

One, week after acclimatization, rats were randomly divided into four (4) equal groups (n = 10, for each). Three (3) groups received 12 doses [3 doses per week in three alternative days] of iron dextran-saline [1000mg/kg/dose, i.p]. The other group received i.p. injection of an equivalent volume of saline-dextran as a blank or control. Thus, the groups of the study were:

1- First group: This group consisted of 10 rats which were i.p injected with saline-dextran and served as a control group.

2- Second group: This group consisted of 10 rats which were i.p injected with iron dextran prepared in saline solution at a dose of 1000mg /kg/dose/day, three alternate days/week, for 4 weeks to induce chronic iron overload. **(16, 17).**

3- Third group: This group consisted of 10 rats in which chronic iron overload was induced as previously mentioned in the second group but with co-administration of α–tocopherol (100 mg/kg i.p.) **(18)**.

4- Fourth group: This group consisted of 10 rats in which chronic iron overload was induced as previously mentioned in the second group but with co-administration L.NAME (100 mg/kg subcutaneously) **(19).**

Drugs and Chemicals:

Iron dextran, α – tocopherol and L-NAME were purchased from Sigma Chemicals Co. [Aldrich, St. Louis, Mo]. L-NAME was dissolved in distilled water (**19**).

Blood and tissue sampling:

At the end of the experiment, animals were fasted overnight; blood was collected via retro-orbital bleeding in dry clean centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. Serum was collected, divided into aliquots and stored at – 20°C until used. Then, rats were killed by decapitation, livers were immediately excised, rinsed with ice-cold normal saline (4°C) to exclude the blood cells, blotted and dried with filter paper, then divided into two portions. The first portion was quickly "snap frozen" in liquid nitrogen (-70°C) and stored at – 20°C for further determination of other biochemical parameters such as total iron. The other tissue portions from liver were kept in 10% buffered formalin – saline at 4°C for at least one week (1ry fixation), then the specimens were dehydrated with a series of ascending grade of ethanol from 75 to 100%. Tissues were placed thereafter in xylol and embedded in paraffin wax.

1- Haematoxylin and eosin (H & E) stain **(20)** to study general microscopic characters of the liver.
2- Perl's Prussian blue stain to record iron deposition (haemosidrin) in liver [21]

Methods

I) Determination of serum iron: Serum iron was determined according to the method described by **Burits and Ashwood** [22]using commercial kit provided by Spinreact, Co., Spain.

II) Determination of total iron in rat hepatic tissues: Total iron concentration was determined in tissues by flame atomic absorption Spectrophotometer according to the method described by **Basset *et al.*** [23]**.**

III) Determination of serum alanine aminotransferase (ALT): Serum ALT was determined according to the method described by **Rec (1970)(24)** [24]using kits supplied by ELITECH: Division de SEPPIM S.A zone industrielle 61500 SEES France.

IV) Determination of serum aspirate aminotransferase (AST): Serum AST was determined according to the method described by **Rec** [24] using kits supplied by ELITECH: Division de SEPPIM S.A zone industrielle 61500 SEES France.

V) Determination of alkaline phosphatase (ALP): Serum ALP was quantitatively determined according to the method described by **Abicht** [25] using Roche/ Hitachi Cobas c system kits.

VI) Determination of serum bilirubin: Serum total bilirubin was quantitatively determined according to the method described by **Doumas *et al.*** [26] using Roche/ Hitachi Cobas c systems kits.

VII) Determination of albumin:

Serum albumin was quantitatively determined according to the method described by **Junge *et al.*,** [41] using Roche/ Hitachi cobas c systems kits.

Statistical analysis:

Statistical methods used in this study for analysis of data was according to the statistical analysis SPSS released 10.0 program for Windows (SPSS Inc. Chicago, IL, USA). All data were expressed as mean ± SD (Standard deviation), the intergroup differences were compared by unpaired student's t test. The acceptable level of significance was p <0.05 **(27).**

3. Results

Serum and liver iron

Tables (1&2) show a significant increase in serum levels and hepatic contents of iron after induction of chronic iron overload by i.p injection with iron dextran at a dose of 1000 mg /kg/day, three alternate days/week for 4 weeks where mean values ± standard deviations ($\overline{x}$ ± SD) of (2001.58±331.83 µg / dL), and (3469.6 ± 320.36µg/g) respectively compared to controls (185.28 ± 44.64 µg / dL), and (106.7±16.50 µg/g) respectively & (P < 0.001 for both).

In addition, Co-administration of iron dextran with the antioxidant, α –tocopherol, had no significant changes in both serum levels and hepatic total iron contents (1817.11±292.67 µg/dL), and (3278.5±301.24 µg/g) in comparison with the above serum and tissue iron contents induced by iron dextran alone (P>0.05 for both). Moreover, co-administration of iron dextran with the specific non-selective nitric oxide synthase (NOS) inhibitor, L-NAME, had no significant effects of both serum iron level and hepatic total iron content (1909.48±313.38µg/dL), and (3374.05±310.58µg/g) versus the above mentioned serum and tissue iron contents induced by iron dextran(P>0.05 for both).

The previous results indicated that both α – tocopherol and L- NAME are not iron cleating agents.

Serum biochemical parameters

Tables (3, 4, 5,6 &7) illustrated the effects of chronic iron overload, on the serum levels of ALT, AST, ALP. bilirubin and albumin (used as sensitive indicators of liver damage). there was a significant increase in all pervious biomarkers except albumin which showed a significant decrease the mean values ± SD were 265.2±44.25 IU/L, 313.3±32.02 IU/L, 379.6±46.05 U/L, 1.68 ± 0.11 mg/dL and 2.47± 0.20 g/dL respectively compared to means ± standard

deviations of their controls (44±12.05 IU/L), (67.8±12.22 IU/L), (88.2±19.19 U/L), (0.34±0.41 mg/dL) and 3.74± 0.41 g/dL respectively & (P < 0.001for all parameters).

Also,the above tables illustrated the effects of antioxidant, α – tocopherol, on the elevated serum enzymes & bilirubin and the decreased serum albumin in chronic iron overload induced by iron-dextran, demonstrating a significant reduction of these markers except albumin which showed a significant elevation.The mean values ± SD were 157.4±22.29 IU/L, 183±18.02 IU/L, 173.7±15.48 U/L, 1.27 ± 0.06 mg/dL and 3.25 ± 0.28 g/dL respectively in comparison with their values induced by iron-dextran alone (P <0.001 for all parameters).

These results indicated the improvement of liver pathology created by the iron overload after treatment with α – tocopherol. But on contrary, these reductions in liver enzymes and bilirubin together with elevation of albumin by α – tocopherol co-administration were still significantly higher than their controls levels (P <0.001 for all).

As regard, the effects of co-administrated L-NAME with iron-dextran on the liver biochemical markers.The present study showed a significant elevation in the serum levels of ALT,AST, ALP, bilirubin together with significant decreased in serum albuimin by co-administration of iron-dextran with L-NAME ($\overline{X}$ ± SD) of (314.66 ± 45.66 IU/L), (361.88 ± 35.22 IU/L), (429.02±48.44 U/L), (2.17 ± 0.15 mg/dL) and (1.79±0.14 g/dL) respectively versus their levels in iron-dextran group mentioned above and (P <0.05), (P <0.01), (P <0.05) (P <0.001) and (P <0.001) respectively, indicating worsening of the liver pathological condition induced by iron overload after treatment with L-NAME.

Histopathological Study.

Histological observations were performed along with the level of various biochemical parameters in circulation to mark the extent of hepatic damage.

1- Detection of -iron deposition (haemosidrin) in liver tissue by Perl's Prussian blue stain: Figure (2) and (3) showed extensive aggregation of iron granules in nearly all studied sections in iron dextran treated group which was not reversed to normal Photomicrographic pattern after treatment by either α – tocopherol or L-NAME, indicating that both of them are not iron chelating agents.

2- Figures (4 - 9) represent study of general microscopic characters of the liver by Haematoxylin and eosin; in which liver sections of normal rats showed normal cell morphology with well-preserved esinophilic cytoplasm, prominent central nucleus, and well-brought-out central vein (fig.4).

Iron overload group showed various degrees of pathological changes including moderate fatty change of the hepatocytes, loss of cellular boundaries and congested central vein (fig.5).

Moreover, the liver sections taken from α – tocopherol treated rats showed lessening of the pathology and revealed marked reduction in hepatic injuries induced by chronic iron overload represented by mild fatty changes of the hepatocytes without central vein congestion and exhibited the improved histology of liver sections taken from α – tocopherol treated group (fig.6,7). These observations indicate the in situ hepatoprotective effect of α – tocopherol treatment on Liver Iron overload. However, liver sections taken from L-NAME treated group revealed sever hepatotoxicity evidenced by marked fatty changes; sever congestion of the central vein and hepatic sinsoides with necrotic hepatocytes (fig. 8, 9). These observations indicate the in situ hepatoinjurious effect of L-NAME treatment on Liver Iron overload.

Table (1):Serum iron levels (μg/dL) in studied groups.

<table>
<tr><th></th><th>Control</th><th>Fe-overload</th><th>α-tocopherol treated</th><th>L.NAME</th></tr>
<tr><td>$\overline{x}$ ±SD</td><td>185.28±44.64</td><td>2001.58±331.83</td><td>1817.11±292.67</td><td>1909.48±313.38</td></tr>
<tr><td rowspan="4">Unpaired t test</td><td rowspan="4"></td><td>17.15
P< 0.001
vs control</td><td>17.43
P < 0.001
vs control</td><td>17.20
P < 0.001
vs control</td></tr>
<tr><td colspan="2">1.32
NS(non significance)
α-tocopherol vs Fe-overload</td><td></td></tr>
<tr><td></td><td colspan="2">0.68 NS
α-tocopherol vs L.NAME</td></tr>
<tr><td colspan="3">0.64 NS
L.NAME vs Fe-overload</td></tr>
</table>

Table (2):Hepatic total iron content [µg/g tissues] in studied groups.

	Control	Fe-overload	α-tocopherol	L.NAME
$\bar{X}$ ±SD	106.7±16.50	3469.6±320.36	3278.5±301.24	3374.05±310.58
Unpaired t test		33.15 ($P < 0.001$)	33.247 ($P < 0.001$)	33.225 ($P < 0.001$)
		1.374 (NS)		
			0.698 (NS)	
		0.677 (NS)		

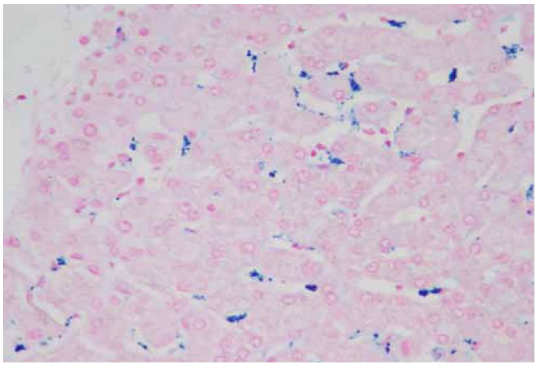

Fig. (1): Photomicrograph of control liver tissue with normal amount of bluish iron granule in the cytoplasm [Perl's Prussian blue stain x 400.

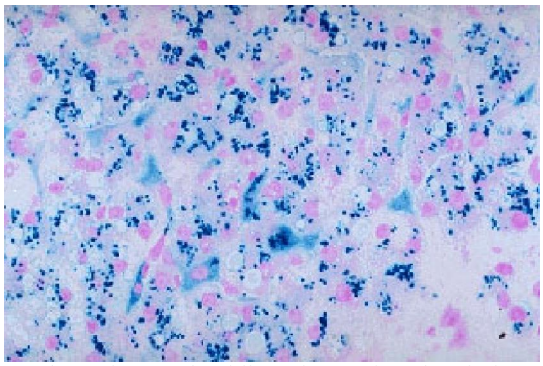

Fig. (2): Photomicrograph of liver tissue after chronic iron overload by iron dextran showing sever aggregation of iron granules in nearly all hepatocyte [Perl's Prussian blue stain x 400].

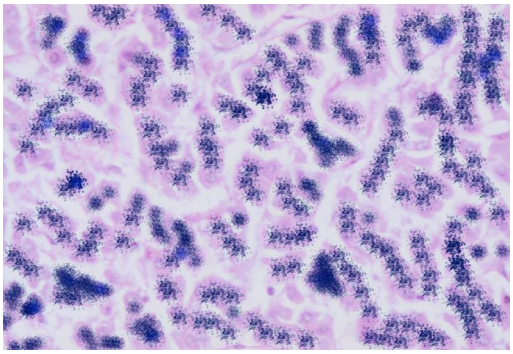

Fig. (3): Photomicrograph of liver tissue after treatment by L-NAME showing marked aggregation of iron granules in nearly all hepatocyte similar to the Photomicrograph of iron dextran group [Perl's Prussian blue stainx 400].

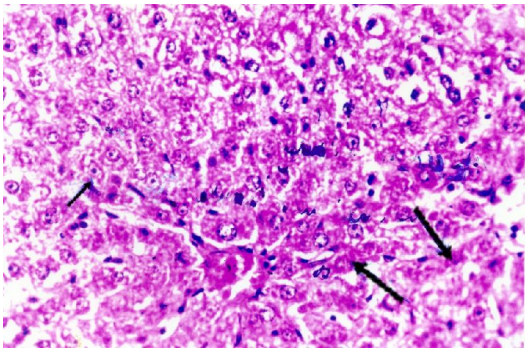

Fig. (4): Photomicrograph of normal liver(control group) formed of cords of polygonal hepatocytes with central nuclei and to esinophilic cytoplasm (↑) [Hematoxylin& Eosin x 400]

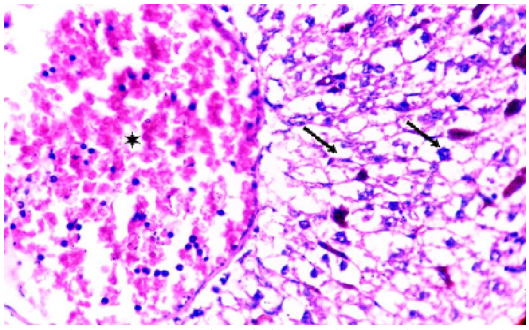

Fig. (5): Photomicrograph of liver tissue (iron overload group) showing moderate fatty change of the hepatocytes (↑) and congested central vein (✶) [Hematoxylin& Eosin x 400]

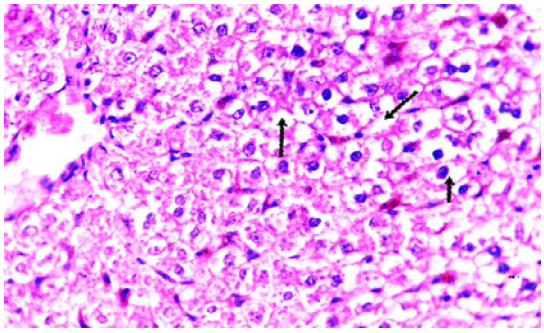

Fig. (6): Photomicrograph of liver tissue (iron overload + vitamin E group) showing mild fatty change of the hepatocytes (↑) [Hematoxylin& Eosin x 400]

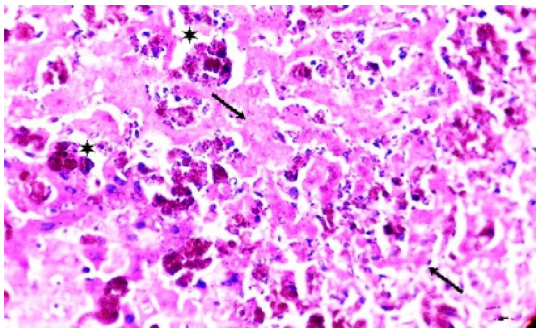

Fig. (7): Photomicrograph of liver tissue (iron overload + vitamin E group) with necrotic hepatocytes (↑) and congested blood sinusoides (✶) [Hematoxylin& Eosin x 400]

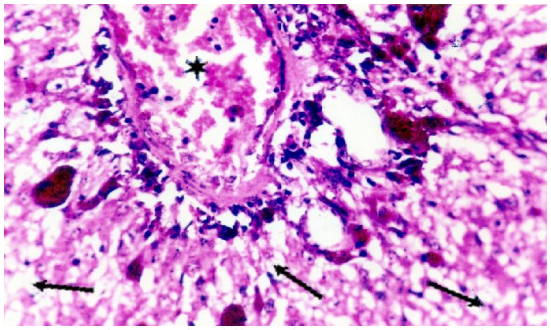

Fig. (8): Photomicrograph of liver tissue (iron overload + L-NAME group) with marked fatty change (↑) and sever congestion of the central vein and blood sinusoides (✶) [Hematoxylin & Eosin x 400]

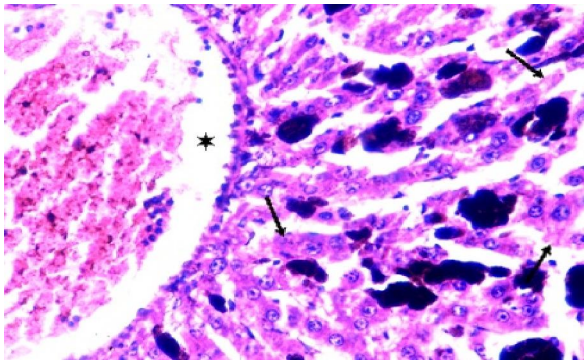

Fig. (9): Photomicrograph of liver tissue (iron overload + L-NAME group) with necrotic hepatocytes (↑) and markedly congested blood sinusoides and central vein (✶) [Hematoxylin & Eosin x 400]

Table (3):Serum alanine aminotransferase (ALT = SGPT, IU/L) levels in studied groups.

	Control	**Fe-overload**	**α-tocopherol**	**L.NAME**
$\bar{x}$ ±SD	44±12.05	265.2±44.25	157.4±22.29	314.66±45.66
Unpaired t test		15.26 ($P < 0.001$)	14.16 ($P < 0.001$)	2.46 ($P < 0.001$)
		6.88 ($P < 0.001$)		
			9.81 ($P < 0.001$)	
		2.46 ($P < 0.05$)		

Table (4): Serum aspartate arninotransferase (AST = SGOT, IU/L) levels in studied groups.

	Control	**Fe-overload**	**α-tocopherol**	**L.NAME**
$\bar{X}$ ±SD	67.8±12.22	**313.3±32.02**	**183±18.02**	**361.88±35.22**
Unpaired t test		**22. 65 ($P < 0.001$)**	**16.74 ($P < 0.001$)**	**24.95 ($P < 0.001$)**
		11.21 ($P < 0.001$)		
			14.29 ($P < 0.001$)	
		3.22 ($P < 0.01$)		

Table (5): Serum alkaline phosphatase (ALP, U/L) levels in studied groups.

	Control	**Fe-overload**	**α-tocopherol**	**L.NAME**
$\bar{X}$ ±SD	88.2±19.19	379.6±46.05	173.7±15.48	429.02±48.44
Unpaired t test		18. 48 ($P < 0.001$)	10.98 ($P < 0.001$)	20.68 ($P < 0.001$)
		13.40 ($P < 0.001$)		
			15.88 ($P < 0.001$)	
		2.34 ($P < 0.05$)		

Table (6): Serum total bilirubin levels (mg/ dL) in studied groups.

	Control	**Fe-overload**	**α-tocopherol**	**L.NAME**
$\bar{X}$ ±SD	0.34±0.41	1.68± 0.11	1.27 ± 0.06	2.17 ± 0.15
Unpaired t test		28.23 ($P < 0.001$)	19.27 ($P < 0.001$)	26.52 ($P < 0.001$)
		10.58 ($P < 0.001$)		
			18. 04 ($P < 0.001$)	
		7.98 ($P < 0.001$)		

Table (7): Serum albumin levels (g/dL) in studied groups.

	Control	**Fe-overload**	**α-tocopherol**	**L.NAME**
$\bar{X}$ ±SD	3.74± 0.41	2.47±0.20	3.25±0.28	1.789±0.136
Unpaired t test		9.07 ($p < 0.001$)	3.10 ($p < 0.01$)	14.34 ($p < 0.001$)
		7.09 ($p < 0.001$)		
			14.90 ($p < 0.001$)	
		9.72 ($p < 0.001$)		

4. Discussion

Iron is an essential nutritional element with a marked bioavailability. However its excess may induce harmful effects to cells and tissues, therefore iron homeostasis has to be tightly regulated **(28).**

This is clearly documented in iron overload diseases, where excessive iron accumulation induces tissue damage, organs failure and cancer development **(3, 4).** Iron toxicity is mostly referred to its capacity to catalyze the generation of free radicals, which attack and damage cellular macromolecules promoting cell death and tissue injury **(5).**

In the current study, we induced chronic iron overload by i.p injection of iron dextran in a rat model and confirmed the condition by the significant increase in serum levels of iron as well as by detection of extensive iron deposition in the liver sections stained by Perl's Prussian blue.

The serum enzymes are very important adjuncts to clinical diagnosis of diseases and tissue injury.(29). In the present study, a significant elevation in the enzymes (ALT, AST & ALP) as well as bilirubin together with a significant reduction in albumin were noted.

This indicated that Hepatic injury has occurred by excessive iron deposition manifested by the leakage of cellular enzymes into the bloodstream. Moreover, the histopathological changes seen in the hepatic sections stained by hematoxylin and eosin have confirmed the presence of the hepatic insult.

These findings are in agreement with **Zhao *et al.* (30)** who reported that, in cases of iron overload, the natural storage and transport proteins such as ferritin and transferrin become saturated and overwhelmed, and then the iron spills over into other tissues and organs. At the same time, oxidative stress arises because of the catalytic activity of the metal iron which produces high reactive oxygen radicals leading to tissue injury.

In addition, it was found that the hepatic iron overload was associated with a decrease in the total antioxidant status. This was achieved through changing in the activities of some antioxidant enzymes including catalase. It enhances biliary & sinusoidal efflux of glutathion disulfide, and lactate dehydrogenase (antioxidant enzymes), and increases hepatic myeloperoxidase activity (oxidative enzyme), consequently leading to an increase in the lipid peroxidation process in the liver **(7).**

Moreover, many researchers detected histological changes similar to ours in iron hepatic overload states. It was in the form of lymphatic and neutrophil infiltration of the portal spaces, peri-portal fibrosis, hyperplasia and hypertrophy of kupffer cells, hepatocellular necrosis, and finally established cirrhosis. They concluded that excess hepatic iron could cause chronic necroinflammatory hepatic disease **(29, 31,32).**

In addition, in the present study we demonstrated that, Co-administration of iron dextran and the antioxidant, α –tocopherol, did not change either serum levels or total hepatic iron contents.

The above results indicate that α –tocopherol, has no iron chelating activity.

However, there was a significant increase in the reduced albumin associating with significant decreases in the elevated liver enzymes as well as bilirubin induced by chronic iron overload after co-administration of iron dextran and α –tocopherol.

In concordance with these findings **Balakrishnan *et al.* (12)** stated that α–tocopherol exhibited protective effects against chromium-induced oxidative stress to the liver and kidney in

rats. They suggested that the antioxidant effects were through inhibition of lipid peroxidation.

In addition, many searches conducted on humans and animals have demonstrated the protective antioxidant effect of vitamin E on hepatic oxidative stress induced by exposures to different common environmental metals e.g. Lead, Arsenic, Fluoride, 4,4.dibromodiphenyl ether (BDE-15), 4,4.dihydroxydiphenyl ether(HODE-15), and 4,4.dicholorodiphenyl ether(SDE-15)(13,33).

Also, many investigators have established that the Plasma α-tocopherol level was decreased in patients with thalassemia and sickle cell disease (SCD) after severe iron overload induced by life-saving repeated blood transfusions.It was suggested that the observed changes in α-tocopherol levels were not because of variation in dietary intake but was probably related to its consumption as a scavenger of oxidants in the hepatic as well as other tissues. **(34).**

In contrary, to our findings, **Asare *et al.* (35)** found that the oxidative liver damage induced by chronic oral administration of iron for 12 month as determined by serum AST and ALT levels, was not attenuated by α –tocopherol co-administration in male albino rats.

The discrepancy between the results of our study and that of the previous study could be explained by the differences in the duration and the rout of administration of iron together with the dose and type of tocopherol used.

Finally the present results indicate that improvement of liver function tests and the near normalization of hepatic tissue architecture is created by α – tocopherol reducing oxidative stress and through inhibition of lipid peroxidation counteracting the harmful effects of iron overload.

The co-administration of iron dextran with the specific non-selective nitric oxide synthase (NOS) inhibitor, L- NAME, in our study had no significant effects on both serum iron level and total hepatic iron content compered to serum and tissue iron contents induced by iron dextran alone indicating that L-NAME possess no iron chelating activity

In the present study, we found significant elevations in the serum levels of ALT,AST, ALP and bilirubin and significant reduction in serum level of albumin induced by co-administration of iron-dextran and L-NAME versus their levels in the iron-dextran group, indicating worsening of the liver pathological condition which was further confirmed by the sever histopathological changes denoting enhanced hepatotoxicity.

The role of iNOS in liver injury is complex as the amount and duration of iNOS expression determines the amount of NO released and thus, the level of reactive nitrogen species created **(7)**.

The effects of iNOS are also dependent on the other proinflammatory cascades active in the cell at the time of NO production. For example, iNOS activation is protective in both preventing reactive nitrogen species formation and by inhibiting apoptosis **(36)** on the other hand, it is associated with deleterious effects in both ischemia reperfusion injury **(37)** and hemorrhagic shock **(6)** due to oxidative damage and activation of inflammatory cascades **(38)**.

When iNOS was activated by TNF-α and N-galactosamine or by carbon tetra chloride (CCl4), it was found to assume a protective role through inhibition of apoptosis and reduction of oxidative stress **(39)**.

Moreover, in cultured hepatocytes, when iNOS is activated by TNF-α and Fas antibody, NO is released and performed a protective role in these cells as it inhibited caspases and thus suppressed apoptosis**(40)**.

Similarly, hepatocytes stimulated by hydrogen peroxide were found to be protected by NO induced heme oxygenase-1 upregulation. (6).

These examples of iNOS activation and subsequent downstream regulation of protein/gene expression is just a sample of the complexity inherent in redox signaling in hepatocytes. The amount of NO and the previous redox conditions of the cell seem to be important in determining the role of NO as an inducer or otherwise an inhibitor of apoptosis

From the previous studies and current results we suggest that NO released in iron overload states has a protective role. The co-administration of L-NAME (the NO and NOS antagonist) was associated with worsening of the in situ hepato-injurious effect induced by iron overload.

Conclusion

From the present study, we concluded that antioxidant α – tocopherol has a protective effect against iron-overload-induced hepatic toxicity. This is evidenced by biochemical and histopathological improvement suggesting its beneficial use as a promising hepatoprotective agent in hepatic iron overload However, the treatment by antioxidant L-NAME is harmful in such condition.

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