Efficacy of Intravenous N-acetylcysteine as an Adjuvant Antioxidant Therapy in Acute Iron Toxicity in Rats

Aliaa Abd Elhakam Hodeib[1*], Lamees Mohamed Dawood[2] and Manar Maher Ali Fayed[3]

KEYWORDS
Acute Iron Toxicity;
Intravenous N-acetylcysteine;
Antioxidant;
Deferoxamine.

ABSTRACT

Iron is a one of the heavy metals that is necessary for cell function. Acute and chronic exposure to high dose leads to oxidative damage. In the liver, iron overload affect hepatic mitochondrial respiration. Deferoxamine is well established antidote for iron toxicity which acts as a chelator. N-acetylcysteine (NAC) is a safe over the counter mucolytic which is antioxidant and glutathione substitute properties. Its use as an antidote for some toxins is well established nowadays. The aim of this work is to study the efficacy of intravenous N-acetylcysteine as an adjuvant therapy with deferoxamine in acute iron intoxication in rats. This study was carried out on twenty male albino rats, weighted 200-250 gm and divided into group I: 10 rats received 400 mg/kg elemental iron orally followed by 25 mg/kg subcutaneous deferoxamine and group II: 10 rats received 400 mg/kg elemental iron followed by 150 mg/kg IV NAC and 25 mg/kg subcutaneous deferoxamine. The results revealed that N-acetylcysteine use lowered both oxidative stress markers malondialdehyde MDA and cyclic adenosine monophosphate cAMP. On the other hand, the reduction of serum and hepatic iron levels and the elevation of Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) were statistically insignificant. It was concluded that intravenous N-acetylcysteine helps in reduction of oxidative stress caused by acute iron toxicity.

Introduction

Iron is a trace element necessary for normal cell metabolism; however it is cytotoxic in high amounts as there is no physiological mechanism to get rid of excess iron. Iron absorption is regulated to avoid accumulation. Iron overload leads to an increase of serum iron concentration. Acute toxicity presented with abdominal pain, vomiting, diarrhea and gastrointestinal bleeding in the first 6 hours after exposure. In the stage of stabilization (12 hours after ingestion) symptoms improve, as absorbed iron is removed from circulation by cellular uptake. After this mitochondrial function is affected and signs of shock, acidosis, coagulopathy, hyperglycemia or hypoglycemia and acute tubular necrosis start to develop. As a consequence, acute hepatic failure may develop within 2 days. After 2-4 weeks, delayed complications e.g. gastrointestinal scarring can occur (Skoczynska et al., 2007 and Yassin et al., 2017).

Acute iron toxicity can be diagnosed clinically together with elevated serum iron level (about 2-9 hours after exposure). Abdominal X-ray could reveal radiopaque shadows if iron tablets were ingested (Fleming

(1) Lecturer of Clinical Toxicology, Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University.
(2) Assistant Professor of Medical Biochemistry, Medical Biochemistry Department, Faculty of Medicine, Tanta University.
(3) Lecturer of Forensic Medicine and Clinical Toxicology, Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Tanta University.
** Corresponding author: aliaa.hodeib@gmail.com*

et al., 2012). Iron toxicity is common among children due to wide availability of iron containing supplements for children or their mothers. It is rated as the most common toxicity in children less than 6 years old. Also, acute iron toxicity in adults occurs either due to suicidal attempts or iron overdose during pregnancy (Wessling - Resnick, 2017).

Acute iron intoxication leads to depletion of reduced glutathione in liver (Abu-Kishk et al., 2010). The reactive oxygen species (ROS) production and the resulting oxidative stress is a possible mechanism for tissue damage resulting from acute iron intoxication (Jaishankar et al., 2014). N-acetylcysteine (NAC) is a widely available, cheap, over-the-counter drug with antioxidant activity (Breitbart et al., 2011).

Standard treatment of iron toxicity depends on the use of deferoxamine as a chelator forming iron-deferoxamine complex which is a harmless compound excreted in urine (Umemura et al., 2017). Deferoxamine use may be associated with local or systemic side effects reactions e.g. shock, gastrointestinal disturbances, elevated liver enzymes, dizziness, paresthesia, seizures and renal failure. Hypersensitivity reactions also may occur. Moreover, Deferoxamine is pregnancy category C. Hence there is a need for discovering a safer antidote or adjunct for iron toxicity management to replace or reduce the dose of deferoxamine used (Howland, 1996 and Clajus et al., 2007).

Abu-Kishk et al. (2010) suggested that, the administration of oral NAC increases the absorption of iron through the gastrointestinal tract, causing higher serum iron levels. This explained elevated liver enzymes and hepatic destruction reported during his study. On the other hand, Breitbart et al. (2011) reported that intraperitoneal administration of NAC may decrease serum iron levels and mortality rate.

So, in this work we studied the effect of administration of intravenous NAC with IV deferoxamine on serum, hepatic tissue iron levels, and mortality rate. This work was designed to study the efficacy of intravenous NAC as adjuvant antioxidant therapy with deferoxamine in acute iron intoxication in rats.

Material and Methods

Study design and animals:

This study was carried out on 20 developing male albino rats, their weight ranged from 200-250 gram. They were housed in clean cages under standardized laboratory conditions; good lighting (12 hours' light/dark cycles) and good aeration. They were fed a standard laboratory diet and tap water. One week after accommodation, rats were randomly divided into two groups:

Group I: Ten rats received 400 mg/kg elemental iron orally (Abu-Kishk et al., 2010), followed by 25 mg/kg subcutaneous deferoxamine (Wongjaikam et al., 2016).

Group II: Ten rats received 400 mg/kg elemental iron (Abu-Kishk et al., 2010), followed by (150 mg/kg IV NAC (Breitbart et al., 2011) and 25 mg/kg subcutaneous deferoxamine) (Wongjaikam et al., 2016).

The study was carried out following approval of the medical research ethical committee of Tanta Faculty of Medicine- Tanta University (code: 33332/09/19).

Biochemical analysis:

Collection of blood samples:

At the end of the research, blood samples were collected by cardiac puncture using diethyl ether for light anesthesia (Beeton et.al, 2007). Blood was collected in sterile plain tubes then centrifuged at 3000 rpm for 15

minutes to separate serum. Serum was saved in aliquots at –80°C.

Serum was used to measure aspartate aminotransferase (AST) enzyme activity and alanine aminotransferase (ALT) enzyme activity, and iron levels colorimetrically using commercial kits (BioSystems Company, Spain kits number 11567, 11568 and 12509 respectively).

Tissue sampling:

Closely after blood sampling, animals were sacrificed by cervical dislocation under deep ether anesthesia. Liver tissues were taken, rinsed with saline to get rid of contaminating blood then dried by blotting with filter paper. They were weighed and frozen immediately at –80°C for biochemical analysis of tissue homogenate.

Preparation of tissue homogenates:

The liver tissue was homogenized using Glas-Col homogenizer. A 20% w/v homogenate was prepared in ice-cold phosphate buffer (0.01M, pH 7.4). The homogenate was centrifuged for 20 minutes at 3000 rpm for separation of the supernatant to avoid sample thawing and refreezing then kept at −80°C.

The aliquot was used for measuring MDA levels which were estimated by the double heating method to assess the lipid peroxidation (Draper et.al, 2001). This method depends on the use of spectrophotometry to measure the generated color by the reaction of thiobarbituric acid (TBA) with MDA at 532 nm. Then the absorbance coefficient of the MDA–TBA complex (absorbance coefficient of 1.56 × 105cm−1 M−1) was used to calculate the concentration of MDA and the results were expressed as mmol/mg tissue.

The CAMP level in Liver homogenates was assayed using specific Rat ELISA kit (R&D Systems, USA; Catalogs Number: KGE012B).

Iron level in liver homogenate samples was analyzed on a spectrophotometer (Mindray BA-88A) using a commercial kit (BioSystems Company, Spain kits number 12509) at 562 nm.

Statistical analysis:

Continuous variables were reported as mean ± standard deviation and compared with analysis of variance (Student's t test). Categorical variables were expressed as frequencies and compared with $\chi 2$ test. Normality of data was determined using the D'Agostino-Pearsons test and verified using histogram plots. A two-sided P value of 0.05 was considered significant. Statistical analyses were performed using SPSS v.18 (SPSS, Chicago, IL, USA).

Results

As shown in table (1) and figure (1), serum iron level was 0.77700 mcg/dl in group I and 0.68540 mcg/dl in group II with no statistically significant difference between two groups (p=0.087).

In table (1) and figure (2) hepatic tissue iron level was 8.6375 µmol/mg in group I and 8.2320 µmol/mg in group II, with no statistically significant difference between two groups (p=0.776).

Table (1) and figures (3, 4) recorded that there was not statistically difference in alanine transaminase (ALT) and aspartate aminotransferase (AST) between group I and II (p= 0.148 and 0.115) respectively.

As shown in table (1) and figures (5, 6); malondialdehyde in group I was 2.28175 mmol/mg and in group II it was 1.65020 mmol/mg, with statistically significant

difference between two groups (p=0.002). Cyclic adenosine monophosphate in group I was 2.28175 ng/mg tissue, while in group II it was 1.65020 CAMP ng/mg tissue, with statistically significant difference between two groups (p=0.002)..

Table (1) : T- test comparing group I treated with iron and deferoxamine only and group II treated with iron, deferoxamine and intravenous N-Acetyl Cysteine.

	Group 1		Group 2		T test	
	Mean	**SD**	**Mean**	**SD**	**T**	**p value**
Serum iron level mcg/dl	0.77700	0.075024	0.68540	0.028754	2.31	0.087
Hepatic tissue iron level µmol/mg	8.6375	2.60264	8.2320	1.50164	2.95	0.776
ALT IU/L	61.75	6.185	72.20	11.476	1.627	0.148
AST IU/L	264.00	40.158	328.00	60.865	1.8	0.115
MDA mmol/mg tissue	2.28175	0.240510	1.65020	0.163388	4.705	0.002*
CAMP ng/mg tissue	2.28175	0.240510	1.65020	0.163388	4.705	0.002*

Group I; Iron with deferoxamine, Group II = Iron with deferoxamine and NAC; SD; Standard Deviation, ALT; Alanine transaminase, AST; Aspartate Aminotransferase, MDA; Malondialdehyde, mmol; millimol, CAMP; Cyclic adenosine monophosphate , mcg/dl; microgram/deciliter, µmol; micromole/milligram, IU/L; international unit/liter, mmol/mg; millimol/milligram, ng/mg; nanogram /milligram,

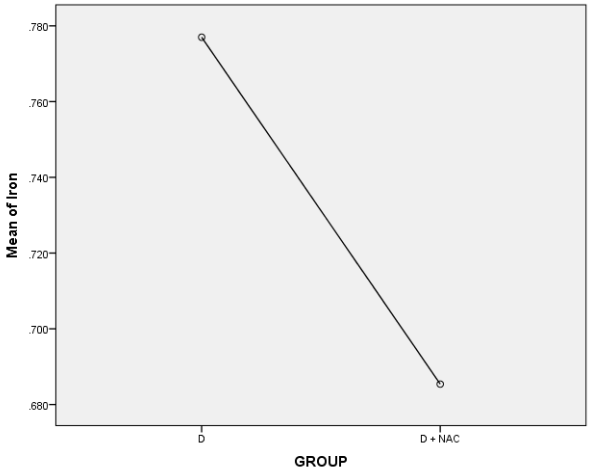

Fig. (1): Correlation of mean of serum iron level between group I and group II.

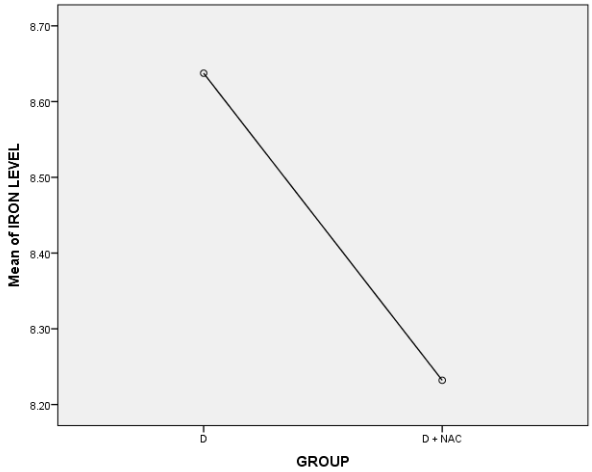

Fig. (2): Correlation of mean of hepatic tissue iron level between group I and group II.

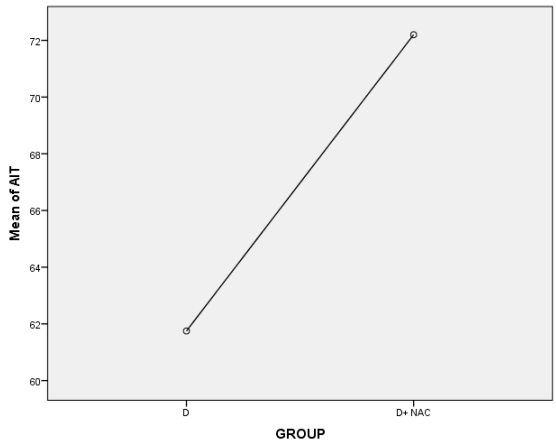

Fig. (3): Correlation of mean of alanine transaminase (ALT) between group I and group II.

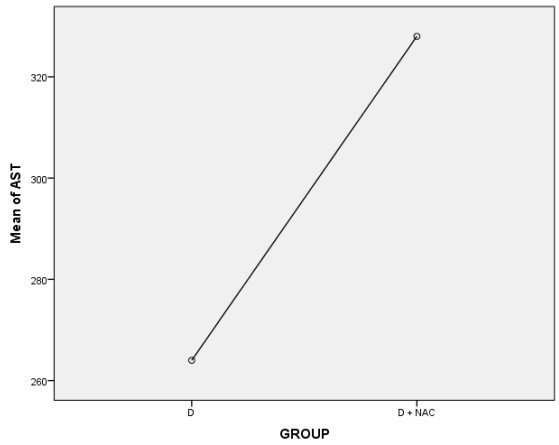

Fig. (4): Correlation of mean of aspartate aminotransferase (AST) between group I and group II.

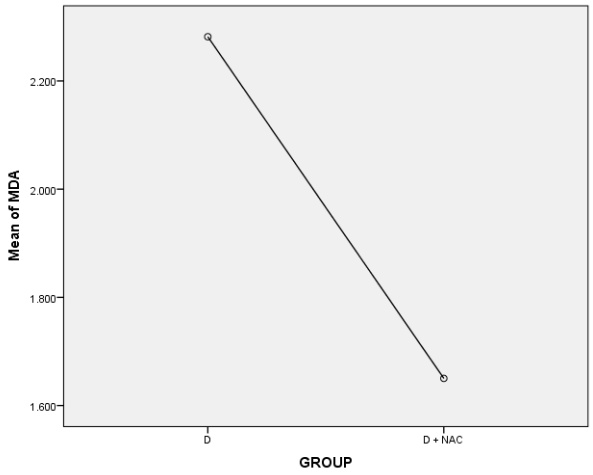

Fig. (5): Correlation of mean of malondialdehyde (MDA) level between group I and group II.

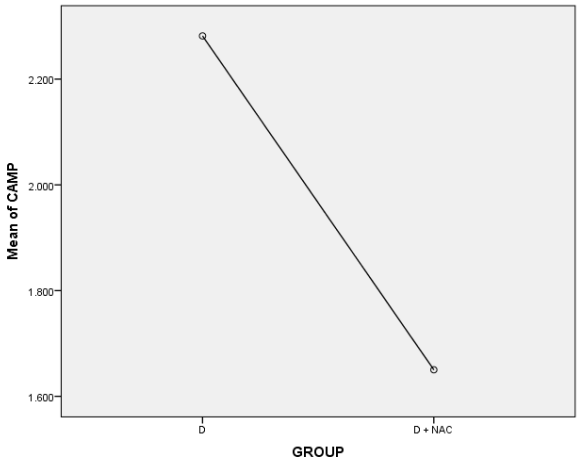

Fig. (6): Correlation of mean of cyclic adenosine monophosphate (CAMP) level between group I and group II

Discussion

Iron is considered an essential element for many cellular processes in living bodies. Elevated tissue iron results in several pathological conditions, especially in hepatic function (Fraga and Oteizab, 2002).

Acute iron toxicity is usually associated with oxidative stress. Oxidative stress results from excess release of free radicles exceeding the antioxidant mechanism capacity. Therefore, studies should be directed towards establishing novel means to limit iron-dependent damage, by minimizing the formation and release of these free radicals (Puntarulo, 2005).

In the current study, it was observed that there was no significant difference between both groups regarding serum iron level and tissue iron level, so adding of the intravenous N-acetylcysteine to the traditional antidotal therapy hasn't an additional effect on increasing iron absorption from gastrointestinal tract. In contrast to Abu-Kishk et al. (2010) who suggested that the oral administration of NAC after acute iron toxicity increases the iron

absorption from the gastrointestinal tract, leading to higher serum iron levels and hence more liver damage and mortality.

Abu-Kishk et al. (2010), Breitbart et al. (2011) and Boveris et al. (2012) discussed that hepatotoxicity induced by acute iron toxicity results from free radical generation and lipid peroxidation. Iron catalyzes hydroxyl radical creation, which is the most potent-free radical. The hydroxyl free radical triggers lipid peroxidation. Being highly reactive, free radicals produce harm at their site of origin which leads to reduced glutathione (GSH) depletion. Due to this affection of GSH system by acute iron toxicity, N-acetylcysteine is suggested as adjuvant treatment. N-acetylcysteine is a well-established potent antioxidant and glutathione substitute, which it widely used as an antidote for various intoxications reducing the lipid peroxidation and enhancing the endogenous antioxidant system (Hundekari et al., 2013).

Because of these mechanisms, it could be observed that, there was significant reduction in level of both malondialdehyde MDA and cyclic adenosine monophosphate CAMP in group II which are treated with the combination of deferoxamine and NAC. Shen et al. (2017) supports the current study by his observation about the improvement of bone marrow damage due to accumulation of reactive oxygen species (ROS) after acute iron toxicity when treated with the combination of deferoxamine and oral NAC. Also, Sripetchwandee et al. (2014) recommended the use of NAC both with chelators for restoring the brain function which affected by iron toxicity**.**

The current study showed insignificant elevation in both alanine transaminase (ALT) and aspartate aminotransferase (AST) in group II versus group I. Abu-Kishk et al. (2010) reported from an experimental study on rats that orally administered NAC significantly elevated liver enzymes. Also fragmentation of livers in all ten rats in group II was noticed during dissection indicating massive damage of liver. It could be explained that changing the dose of NAC could change the response regarding configuration of liver and the level of liver enzymes.

Conclusion:

Finally, it could be concluded that intravenous N-acetylcysteine helps in reduction of oxidative stress caused by acute iron toxicity which was be evident by the reduction of both oxidative stress markers malondialdehyde (MDA) and cyclic adenosine monophosphate (cAMP).

Limitation of study:

More studies must be done by different doses of intravenous N-acetylcysteine to show if there will be a significant correction of serum and hepatic tissue iron levels as well as liver enzymes Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST).

References

Abu-Kishk, I.; Kozer, E.; Goldstein, L.H.; et al. (2010): "Oral N-acetylcysteine has a deleterious effect in acute iron intoxication in rats". Am. J. Emerg. Med., 28 (1): 8-12.

Beeton, C.; Garcia, A. and Chandy, K.G. (2007): "Drawing blood from rats through the saphenous vein and by cardiac puncture". J. Vis. Exp., 7(7):266.

Boveris, A.; Musacco-Sebio, R.; Ferrarotti, N.; et al. (2012): "The acute toxicity of iron and copper: biomolecule oxidation and oxidative damage in rat liver". J. Inorg. Biochem., 116, 63-69.

Breitbart, R.; Abu-Kishk, I.; Kozer, E.; et al. (2011): "Intraperitoneal N-acetylcysteine for acute iron intoxication in rats". Drug Chem. Toxicol., 34(4): 429-432.

Clajus, C.; Becker, J.U.; Stichtenoth, D.O.; et al. (2007): "Acute kidney injury due to deferoxamine in a renal transplant patient". Nephrology Dialysis Transplantation, 23(3):1061-1064.

Draper, H.H.; Csallany, A.S. and Hadley, M. (2001): Urinary aldehydes as indicators of lipid peroxidation in vivo. In: Bio-Assays for Oxidative Stress Status. W.A. Pryor (Ed.), Elsevier. P.P. 184–190.

Fleming, R.E.; Robert, E. and Ponka, P. (2012): "Iron overload in human disease." New England Journal of Medicine, 366(4): 348-359.

Fraga, C.G. and Oteizab, P.I. (2002): "Iron toxicity and antioxidant nutrients". Toxicology, 180 (1): 23-32.

Howland, M.A. (1996): "Risks of parenteral deferoxamine for acute iron poisoning." J. Toxicol. Clinic. Toxicol., 34(5): 491-497.

Hundekari, I.A.; Suryakar, A.N. and Rathi, D.B. (2013): "Acute organo-phosphorus pesticide poisoning in North Karnataka, India: oxidative damage, haemoglobin level and total leukocyte". Afr. Health Sci., 13(1): 129-136.

Jaishankar, M.; Tseten, T.; Anbalagan, N.; et al. (2014): "Toxicity, mechanism and health effects of some heavy metals". Interdisciplinary Toxicology, 7(2):60-72.

Puntarulo, S. (2005): "Iron, oxidative stress and human health". Mol. Aspects Med., 26(4-5): 299-312.

Shen, J.C.; Zhang, Y.C. and Zhao, M.F. (2017): "Protective effects of deferasirox and N-acetyl-L-cysteine on iron overload-injured bone marrow". Braz. J. Med. Biol. Res., 50(12): e6087.

Skoczynska, A.; Kwiecinska, D.; Kielbinski, M.; et al. (2007): "Acute iron poisoning in adult female". Human & Experimental Toxicology, 26:663- 666.

Sripetchwandee, J.; Pipatpiboon, N.; Chattipakorn, N. et al. (2014): "Combined therapy of iron chelator and antioxidant completely restores brain dysfunction induced by iron toxicity". PLoS.One, 9(1): e85115.

Umemura, M.; Kim, J.H.; Aoyama, H.; et al. (2017): "The iron chelating agent, deferoxamine detoxifies Fe (Salen)-induced cytotoxicity". Journal of Pharmacological Sciences, 134: 203-210.

Wessling - Resnick, M. (2017): Iron: Basic nutritional aspects. In: Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals. J. F. Collins (Ed.), Amsterdam, Netherlands, P.P. 161– 173.

Wongjaikam, S.; Kumfu, S.; Khamseekaew, J.; et al. (2016): "Combined iron chelator and antioxidant exerted greater efficacy on cardioprotection than monotherapy in iron-overloaded rats". PLoS One, 11(7): e0159414.

Yassin, M.; Soliman, A.T.; Sanctis, V.D.; et al. (2017): "A young adult with unintended acute intravenous iron intoxication treated with oral chelation: The use of liver ferriscan for diagnosing and monitoring tissue iron load". Mediterr. J. Hematol. Infect. Dis., 9(1): e2017008.

فاعلية إن أسيتيل سيستيين الوريدي كعلاج مساعد في تسمم الحديد الحاد عند الفئران

علياء عبد الحكم هديب[1]، لميس محمد داوود[2] و منار ماهر علي فايد[3]

[1] مدرس السموم الاكلينيكية، قسم الطب الشرعي و السموم الاكلينيكية، كليه الطب جامعه طنطا.

[2] أستاذ مساعد الكيمياء الحيوية الطبية، قسم الكيمياء الحيوية الطبية، كليه الطب جامعه طنطا.

[3] مدرس الطب الشرعي و السموم الاكلينيكية، قسم الطب الشرعي و السموم الاكلينيكية، كلية الطب جامعه طنطا.

يعد الحديد أحد المعادن الثقيلة اللازمة لوظائف الخلايا الحية، التعرض الحاد أو المزمن لجرعات عالية يؤدي إلى تلف مؤكسد. و في الكبد فان الحديد الزائد يضعف تنفس الميتوكوندريا الكبدية. ديفيروكسامين هو الترياق الثابت لسمية الحديد الذي يعمل كخالب. ان أستيل سيستيين هو دواء مضاد للبلغم متاح دون وصفة طبية و يعمل كمضاد للأكسدة و بديل للجلوتاثيون و يستخدم حاليا كترياق لبعض أنواع السموم. الهدف من هذا العمل هو دراسة فاعلية ان أستيل سيستيين عن طريق الحقن الوريدي كعلاج مساعد للأكسدة مع ديفيروكسامين في تسمم الحديد الحاد في الفئران. أجريت هذه الدراسة على عشرين من ذكور الفئران التي تراوحت أوزانها بين ٢٠٠-٢٥٠ جم ومقسمة إلى مجموعتين. الأولى: ١٠ فئران تلقت ٤٠٠ ملجم حديد/ كجم عن طريق الفم تليها ٢٥ ملجم ديفيروكسامين / كجم تحت الجلد والمجموعة الثانية: ١٠ فئران تلقت ٤٠٠ ملجم حديد/ كجم عن طريق الفم يليه ١٥٠ ملجم ان أستيل سيستيين / كجم وريديا و٢٥ ملجم ديفيروكسامين / كجم تحت الجلد. أظهرت النتائج أن استخدام إن أسيتيل سيستئين قد خفض بشكل كبير علامات الإجهاد التأكسدي مالونديهيد و سيكليك أدينوسين مونوفوسفات. و علي الجانب الاخر فان انخفاض مستوي الحديد في الدم و نسيج الكبد و زياده انزيمات الكبد الألانين ترانس أميناز و أسبرتات ترانس أميناز ليس لهم دلالة إحصائية. الخلاصة أن إعطاء إن أستيل سيستيين وريديا يمكن أن يساعد في الحد من الإجهاد التأكسدي الناجم عن سمية الحديد الحادة .