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The effects of acute iron overload in wistar rats

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Abstract
Acute iron overload in rats associated with hepatotoxicity, nephrotoxicity and neurotoxicity. Iron toxicity induced labored breathing, abnormal posture and hyperaemia of the ear in treated rats. Body weights were found to be significantly decreased in all the treated groups. Relative organs weights were found to be significantly increased in all the treated groups. There was significant alteration in different antioxidant parameters (MDA, CAT, SOD and GPx). Necropsy revealed various gross alterations in many organs *viz.* liver, kidneys, brain, lungs, and heart with varying intensity include necrosis and congestion in liver and kidney, mild congestion of brain and heart, emphysema and pneumonia in lungs. Histopathological examination of liver, kidney and brain revealed fatty degeneration of hepatocytes, haemorrhage, degeneration and necrosis of kidney tubules, glomerular fragmentation and loss, neuronophagia, gliosis and neuronal degeneration etc.

Keywords: Antioxidant, brain, iron, kidney, liver, rats

1. Introduction
Intentional or accidental ingestion is a common source of iron poisoning. Accidental ingestion of iron-containing compounds is common but iatrogenic overdose via injection of compounds to treat iron deficiency. Both natural and experimental cases of iron intoxication were reported by previous worker (Veldman, 2015; Theelen *et al*., 2019; Aldridge, 2007; Patrick *et al.*, 1996; Khadiga *et al*., 2014; Ackerman *et al*., 2018) [17, 16, 2, 13, 7, 1]. Various pathological alterations *viz.* hepatitis, fibrosis, haemosiderosis, fatty change, vacoular degeneration, congested oedematous kidneys, cellular swelling and necrosis etc. were reported previously (Theelen *et al.*, 2019; Pietrangelo, *et al.*, 1990 and ORUÇ *et al.*, 2009) [16, 14, 11] due to iron intoxication. Iron loading results in the generation of toxic hydroxyl radicals demonstrated *in vivo* in rats (Kadiiska, 1995) [20].

2. Materials and Methods
All the study were carried out in accordance with the guidelines for the use and care of laboratory animals by Institutional Animal Ethical Committee (Approval No. 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/18-19/694). A total of 10 numbers of Wistar albino female rats weighing 150-200 g were procured from PI, DBT MAP Project, Department of Pharmacology and Toxicology, C.V.Sc, AAU. Rats were kept in polypropylene cages in a small group of 5 rats per cage. Animals had free access to standard balanced diet and clean drinking water *ad libitum* and were maintained in a standard laboratory conditions (12:12 hour light/ dark cycle at ambient temperature ranging between (22-25 ^{0}C). Acute toxicity study of flubendiamide was conducted according to Organization for Economic Co-operation and Development (OECD) Guidelines for testing of chemicals, TG 420. Animals were divided into two groups of five animals each. Group A serve as control and group B administered orally iron (Fe) as $FeSO_4.\ 7H_2O$ salt @ 2000 mg/kg B.W with drinking water used as vehicle. Clinical signs of toxicity and mortality were observed and recorded daily for 14 days followed by scarification. Weekly body weights were measured during the period of study and the relative organ weight was calculated to determine the effect of iron on body weight. Different oxidative biomarkers were studied *viz.* MDA, Catalase and GPx. Necropsy was performed and for histopathology samples were collected in 10% neutral buffered formalin.

3. Results and Discussion
Immediately after dosing the treated rats showed labored breathing, difficulty in moving and

hyperaemia of the ear (Fig.1), some animals showed anorexia, dehydration and abnormal posture (Fig.2). Labored breathing may be because of once iron taken up into the cells it targets the mitochondria and interferes with the aerobic cellular respiration (Morse, 1997) [9]. Anorexia and dehydration due to iron intoxication were noted in the present investigation which is in accordance with the previous reports on iron intoxication (Aldridge, 2007 and ORUÇ *et al*., 2009) [2, 11]. Iron ingestion has direct corrosive effect on GI mucosa leading to nausea, vomition and diarrhoea resulting fluid loss and dehydration (Morse, 1997; Baranwal, 2003 and Manoguerra, 2005) [9, 3, 10].

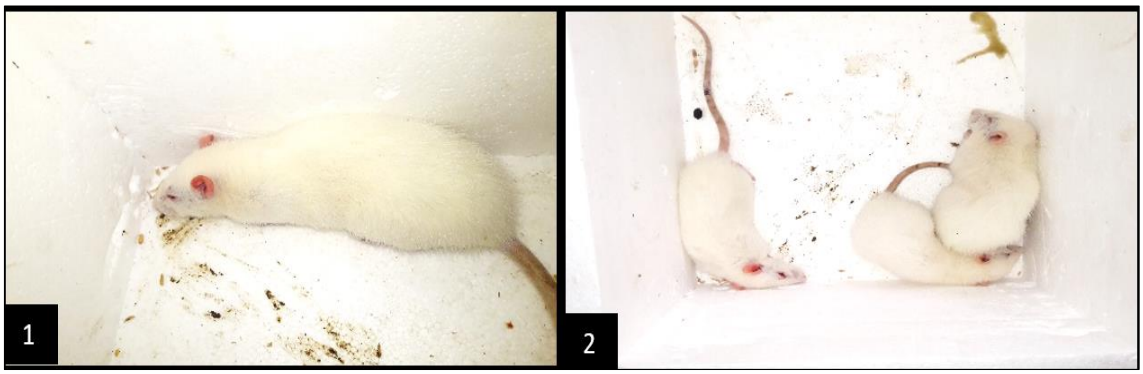

Fig 1: Rat showing hyperaemia of the ear and Fig.2 Rat showed abnormal posture after 10-15 minutes of dosing

Significant decrease in body weight (p<0.05) were recorded in treated group B in comparison to the control group A on different observation days has been presented in table 1and fig.3 Decrease in body weight on different observation days might be due to the toxicity of administered compound resulting in reduced feed intake or increase in wasting due to organ dysfunction (Vemu, 2016) [18].

Table 1: Weekly body weight (gms) variations on acute exposure of iron (Fe) orally in female rats

Group	0 day	7 day	14 day
Group A (Control)	140±14.4^{a}	147.3±15^{a}	149.6±13.2^{a}
Group B (Iron)	132.8±23.2^{a}	128.7±24.7^{a}	125.8±26.7^{a}

Values are given as Mean ± SE (n=6).
Mean with different superscripts (small letters) differs significantly at (p<0.05) level

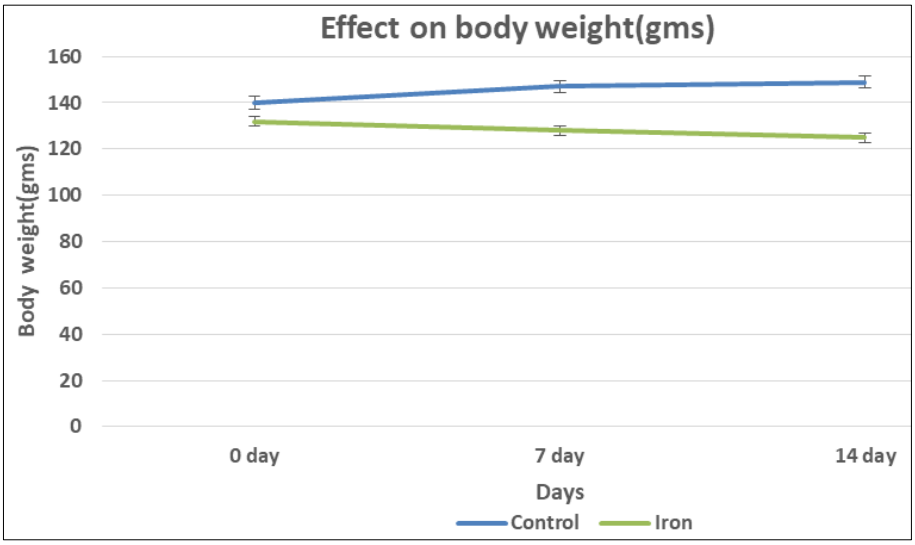

Fig 3: Weekly body weight (gms) on acute exposure of flubendiamide and iron orally in female rat

Significant (p<0.05*) increase in relative organ weight (Liver, kidney and lungs)were recorded in treated groups compare to control has been presented in table 2 and fig.4 Increase in organ weight might be due to reduction in body weight resulting increase in relative organ weight (Vemu, 2016) [18]. A corresponding increase in organ weight might be due to congestion in different organs (Vegad, 2012) [19].

Table 2: Relative organ weight variations on acute exposure of Iron (Fe) orally in female rat

	Liver	Kidney	Lungs	Heart	Brain
Group A (Control)	0.03±0.002^{b}	0.003±0.002^{b}	0.006±0.0005^{b}	0.003±0.0002^{NS}	0.007±0.01^{NS}
Group B (Iron)	0.04± 0.002^{a}	0.004±0.0004^{a}	0.0008±0.0002^{a}	0.003± 0.0002^{NS}	0.009±0.0007^{NS}

Values are given as Mean ± SE (n=6).
Mean with different superscripts (small letters) differs significantly at (p<0.05)

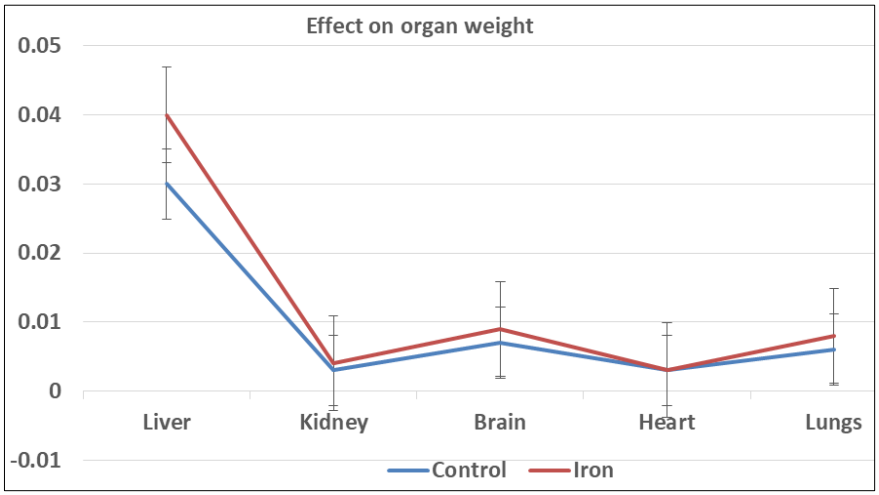

Fig 4: Graphical representation of relative organ weight on acute exposure of flubendiamide and iron orally in female rats

Mean ± S.E. values of liver tissue homogenate for different antioxidant parameters of the experiment are presented in table 3 and fig.5 respectively. Increase in hepatic MDA level were recorded in treated group(0.2±0.04 nM MDA/mg protein) in comparison to control (0.10±0.01 nM MDA/mg protein) but the values were not statistically significant. For catalase activity, highly significant (p<0.001***) increase were recorded in treated group (385.06±16.4 decomposed/min/mg of protein) in comparison to control group (231.78±8.1 decomposed/min/mg of protein). GPx activity were found significantly lower (p<0.01**) in treated group (2.74±0.29 µmoles of GSH oxidized/min/mg of protein) compared to control (4.13±0.23[a] µmoles of GSH oxidized/min/mg of protein). This rise in hepatic MDA level is potentially associated with iron-induced lipid peroxidation and oxidative stress given that iron can promote ROS via the Haber–Weiss reaction (Jomova & Valko 2011) [6]. In turn, ROS are prone to generate increased lipid peroxidation (Halliwell & Gutteridge 1985) [5]. Free ionic iron not bound to proteins can initiate and propagate lipid oxidation via the Fenton reaction in the presence of H_2O_2 and reducing agents such as superoxide anion, ascorbic acid, NADPH and thiols. In iron overload, excess free iron enters inside the cells and concentrates within the mitochondria which disrupts oxidative phosphorylation forms excessive free radicals and ultimately leads to cell death. Because of iron's ability to participate in redox reactions, iron is a powerful catalyst for the formation of toxic hydroxyl radicals (H2O2) (Halliwell, 1994) [4] leading to oxidative stress as a result of which there is an increase in catalase activities. GPx activity was decreased after Fe overloads, it is likely that increased phospholipid peroxidation would include high levels of ROO (Peroxyl radical) that binds to the enzyme and inactivates the reaction centre. The findings of the present investigation was in agreement with the previous findings by Sebio *et al.* (2017) [15].

Table 3: Variations in oxidative parameters on acute exposure to iron orally in female rats

Parameter	Group	Value
LPO nM MDA/mg protein	Group A (Control)	0.10 ± 0.01^{NS}
	Group B (Iron)	0.2 ± 0.04^{NS}
CAT µmoles of H_2O_2 decomposed/min/mg of protein)	Group A (Control)	231.78 ± 8.1^{b}
	Group B (Iron)	385.06 ± 16.4^{a}
GPx (µmoles of GSH oxidized/min/mg of protein)	Group A (Control)	4.13 ± 0.23^{a}
	Group B(Iron)	2.74 ± 0.29^{b}

Values are given as Mean ± SE (n=6).
Mean with different superscripts (small letters) differs significantly at (p<0.01)and (p<0.001) level between groups.

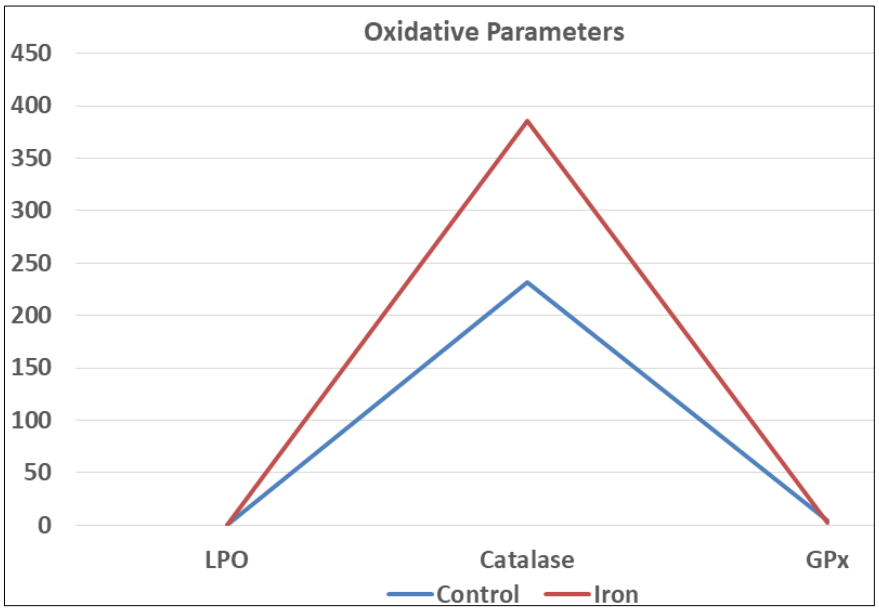

Fig 5: Graphical representation of oxidative parameters on acute exposure of iron orally in female rats

Necropsy revealed various gross pathological alteration. All the liver showed congestion and necrotic spot was recorded only in two livers (Fig.6F). Kidney revealed necrotic spot (Fig.6 G). Brains were mildly congested (Fig.6H). Lungs showed emphysema and pneumonia was present in the apical lobe (Fig.6I). Almost all the heart showed congestion of the coronary blood vessels (Fig.6J) in the treated groups. The findings of the present investigation can be comparable to the report from ORUÇ *et al.* (2009) [11] where the worker noted post-mortem findings including congested oedematous kidneys and congested liver.

Histopathological examination of the liver showed fatty degeneration of the hepatocytes (Fig.7A). Section of the kidney revealed atrophy of the glomerulus, degeneration and necrosis of the kidney tubules (Fig.7B). Neuronophagia, neuronal degeneration (Fig.7C) and gliosis (Fig.7D)were mainly evident in group B rat. Pietrangelo, *et al.*, (1990) [14] documented fatty and vacuolar degeneration in the liver particularly at the latest stage of iron treatment, which is in agreement with the present findings. The histopathological findings reported by ORUÇ *et al.* (2009) [11] in his report were cellular swelling and necrosis of the hepatocytes were also in agreement with the present findings.

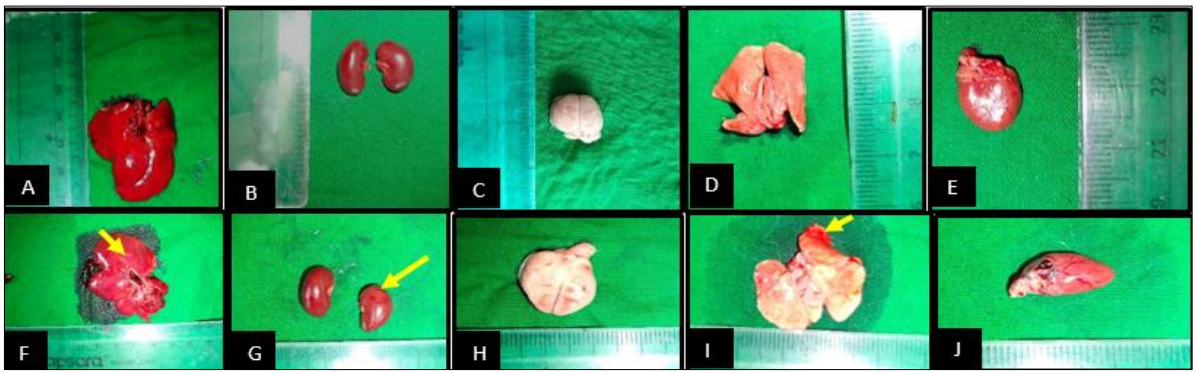

Fig 6: Gross alterations recorded in various organs following acute exposure of Iron 2000mg/kg b.wt. *per os* in female rats.(A-E) Control Group and (F-J)Iron Treated: (F) Livers showing necrotic spot(Arrow) (G)Kidneys showing Necrotic spot (Arrow),(H) Brain showing mild congestion,(I) Lungs appears as highly emphysematous and pneumonia in apical lobe (Arrow)and(J) Heart showing congested coronary blood vessels

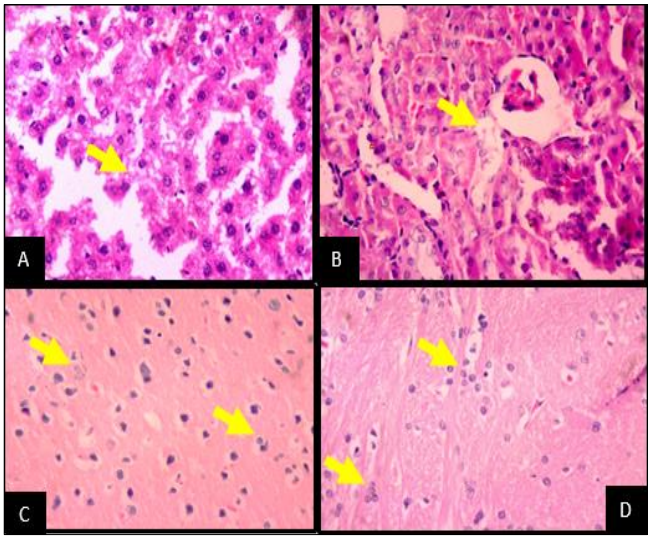

Fig 7: A) Liver section of iron (2000mg/kg b. wt.) exposed group B rat showing fatty degeneration of hepatocytes, (Arrow); (B)Kidney section showing glomerular atrophy, tubular degeneration and necrosis (Arrow); (C) Section of brain showing neuronophagia and neuronal degeneration (Arrow) and (D) gliosis (Arrow), H & E, X 40

4. Conclusion

From the above investigation it can be concluded that iron is a potent hepatotoxic, nephrotoxic and neurotoxic compound in high dose as evident from histopathological studies as well as it can produce oxidative stress to animals as evident from oxidative stress parameters.

5. Acknowledgement

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6. References

1. Ackerman Z, Skarzinski G, Link G, Glazer M, Pappo O, Grozovski M. The effects of chronic iron overload in rats with acute acetaminophen overdose. Toxicol Pathol. 2018;46(5):597-607.
2. Aldridge MD. Acute iron poisoning in children. Dimens Crit Care Nurs. 2007;26(2):43-48.
3. Baranwal AK, Singhi SC. Acute iron poisoning: Management guidelines. Indian Pediatr. 2003;40:534-540.
4. Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. Environ Health Perspect. 1994;102(10):5-12.
5. Halliwell B, Gutteridge JMC. The chemistry of oxygen radicals and other oxygen-derived species. In: Halliwell B, Gutteridge JMC, eds. Free Radicals in Biology and Medicine. Oxford Science Publications, Clarendon Press; c1985. p. 20-64.
6. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283(2-3):65-87.
7. Khadiga G, Adham AA, Alkhalifa M, Manal H, Farhood N, *et al*. Oxidative stress and antioxidant response to subacute and subchronic iron overload in Wistar rat. Biologia. 2014;69(6):817-828.
8. Khadiga. Metabolic response to subacute and subchronic iron overload in a rat model. Acta Biol Hung. 2015;66(4):361-373.
9. Morse SB, Hardwick WE. Fatal iron intoxication in an infant. South Med J. 1997;90(10):1043-1047.
10. Manoguerra AS, Erdman AR, Booze LL. Iron ingestion: an evidence-based consensus guideline for out-of-hospital management. Clin Toxicol. 2005;43:553-570.
11. Oruç HH, Uzunoğlu İ, Cengiz M. Suspected iron toxicity

in dairy cattle. J Fac Vet Med. 2009;28(1):75-77.
12. OECD TG 420. 2001. Guidance document for conducting various tests to assess the toxicity of chemicals. [Internet]. Available from: http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788
13. Patrick GJ, Smith, George CT, Yeoh. Chronic iron overload in rats induces oval cells in the liver. Am J Pathol. 1996;149(2):389-398.
14. Pietwgelo A, Rocchi E, Schiaffonati L, Ventufu E, Cairo G. Liver gene expression during chronic dietary iron overload in rats. Hepatology. 1990;11(5):798-803.
15. Sebio MR, Magriñá SC, Acosta MJ, Boveris A, Repetto MG. Iron and copper toxicity in rat liver: A kinetic and holistic overview. Liver Res Open J. 2017;2(1):9-13.
16. Theelen MJP, Beukers M, Grinwis GCM, Sloet van Oldruitenborgh-Oosterbaan MM. Chronic iron overload causing haemochromatosis and hepatopathy in 21 horses and one donkey. Equine Vet J. 2019;51(3):304-309.
17. Veldman M. Communications Department 2015; Faculty of Veterinary Medicine, Utrecht University, (030) 253 3430, m.m.veldman@uu.nl.
18. Vemu B. Evaluation of flubendiamide toxicity through one generation extended reproductive study in rats. PhD thesis. Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004; c2016.
19. Vegad LJ. A Textbook of Veterinary General Pathology, 2nd Edition. International Book Distributing Co., Khushnuma Complex Basement 7, Meerabai Marg, Lucknow 226001 U.P. (India); c2012.
20. Kadiiska MB, Burkitt MJ, Xiang QH, Mason RP. Iron supplementation generates hydroxyl radical *in vivo*. An ESR spin-trapping investigation. The Journal of clinical investigation. 1995 Sep 1;96(3):1653-1657.