ORIGINAL ARTICLE

NIGELLA SATIVA: ROLE IN IRON OVERLOAD

Maryam Naeem, Muneeba Razzaq, Uzma Naeem*, Saima Rafique**, **Khalida Ajmal****, Tasneem Murad***

Department of Zoology, Government College University, Lahore, Department of Pharmacology, *Islamic International Medical College, Rawalpindi, **Wah Medical College, Wah Cantt, Pakistan

Background: In thalassemia patients, iron is overloaded because of multiple blood transfusions. This overloaded iron can damage the organs badly which is a major cause of death in these patients. Iron overload in thalassemia major can only be treated by removing the excessive iron through different chelating agents. Commercially available chelating agents are costly and associated with multiple adverse effects. Effectiveness of naturally present chelators is under study. This study was conducted to explore the chelating effect of one of the commonly used herb *Nigella sativa*. **Methods:** This experimental, randomized controlled trial was conducted in Zoology Department, Government College University, Lahore in its multidisciplinary laboratory. A total of 36 male albino mice were divided into three groups, 12 in each group. Iron was overloaded in groups 2 and 3 by intravenous injections of iron dextran (0.1 ml/g body weight) for 15 days on daily basis. After 15 days, iron dextran injection was discontinued, and mice were allowed to feed on *Nigella sativa* (200 mg/Kg body weight) for further 15 days. Blood sampling was done at baseline, 15, and 30 days to analyse iron concentration in serum, heart, kidney, and liver. Data was analysed using Student's *t*-test. **Results:** The artificially administered iron in mice increased the iron levels in serum, liver, heart and kidneys to a significant level while administration of *N. sativa* significantly decreased these levels. **Conclusion:** We propose clinical trials of *Nigella sativa* in thalassemia major patients as an adjunct therapy to explore its efficacy and safety.

Keywords: Thalassemia, Iron overload, *Nigella sativa*, Mice, Clinical trial

Pak J Physiol 2021;17(2):20–3

INTRODUCTION

Thalassemias are genetic disorders that are prevalent worldwide with highest death rates in West and South Asia. The current management of thalassemia patients is blood transfusion along with chelating agents to prevent iron overload.[1] Almost 200–250 mg of iron is present in a unit of transfused blood leading to damage of important organs such as liver, kidney, and heart as a result of iron overload.[2] In the absence of active remedies this iron accumulation can cause death due to cardiac failure or arrhythmias in the patients of thalassemia major.[3]

Most effective remedy for removal of this overloaded iron is the chelation therapy by using chelating agents. These agents remove iron by forming the unwavering resolvable multiplexes and are then removed from the body in urine or faeces. Desferrioxamine is one of the commonly used chelators but its cost, availability, and association with multiple adverse effects makes the management of iron overload difficult.[4] In developing countries, all these factors lead to high mortality due to iron induced organ damage.[5]

Because of these limitations of chelating agents, investigators are focusing on beneficial plants and herbs for chelating therapy. *Nigella sativa* is a wonder herb with a wide range of pharmacological effects and remarkable religious background. A number of research studies have proven the efficacy of *Nigella sativa* seeds in various diseases like cough, bronchitis, and influenza.[5] The activity of an essential element of *Nigella sativa*, thymol, has been investigated by Kishwar *et al*. They concluded that thymol could reduce the toxicity of iron and other metals by forming the complexes with these metals.[6]

This experimental study was aimed to determine the *Nigella sativa* seed's protective effects against organ damage caused by iron overload and in elimination of excessive iron from the body as a possible treatment option for iron overload in patients of thalassemia major.

MATERIAL AND METHODS

This experimental, randomized controlled trial was carried out in the Zoology Department, Government College University, Lahore, in collaboration with its multidisciplinary laboratory.

For this experimental study, two months old, healthy male albino mice weighing 25–50 grams were obtained and kept in the animal house of Government College University Lahore. Fresh water and commercial pelleted diet were provided daily. Mice cages were placed in a well-ventilated room with constant temperature of 24 °C, humidity 50–70% and 12-hour light/dark cycle. *Nigella sativa* seeds were obtained and certified by Botany Department, Government College University, Lahore, through proper taxonomical rules. Seeds were crushed into powder form and calculated form of the powder was mixed with mice feed.

Mice were divided into three groups; Group 1 was normal control group while groups 2 and 3 were experimental groups each comprising of twelve mice. Group 1 mice were fed on normal diet throughout the experiment and injected with the salt solution; concentration of solution was like that present in iron dextran injection. Groups 2 and 3 were fed on normal diet and injected through tail vein with iron dextran injection 0.1 ml/g body weight for 15 days on daily basis. Then two mice were sacrificed with an interval of five days to determine whether the iron was being overloaded or not. After confirmation (by blood tests) group 3 mice were allowed to be fed on *Nigella sativa* (200 mg/Kg body weight) mixed feed for 15 days.

Blood samples from all mice were taken three times, at days 0, 15, and 30. Intra cardiac technique was used for blood sampling under chloroform anaesthesia. This blood was than centrifuged at 6,000 rpm and obtained serum was collected through micropipette and stored at -8 ºC. After 30 days mice were sacrificed, and organs (heart, kidney, and liver) were removed. Every organ was divided into three equal parts. One was preserved in 10% formalin for microtome while two were stored at -86 ºC for iron analysis and protein carbonyl content determination. For iron analysis, serum and frozen organs were first digested in aqua regia. Aqua regia (1 ml/organ) was added to organs and serum in separate test tubes and was left overnight at room temperature. Serum and organs were dissolved in aqua regia. That mixture was then allowed to boil for 2–3 minutes. After boiling, 1 ml distilled water was added, and mixture was filtered to remove any impurities. Then optical density was measured using atomic absorption spectrophotometer to determine the amount of accumulated iron. Student's *t*-test was applied to compare the individual parameters in different groups.

Samples were assigned different numbers for identification, described, and placed in small plastic cassettes. After fixation and embedding, eosin and haematoxylin stain was used for histological sections. Slides were examined under ×10 and ×40 power of light microscope. The microscopic qualitative parameters including necrosis of cells and size of nucleus were observed.

RESULTS

Artificially administered iron in mice led to significantly increased levels of iron in serum, liver, heart, and kidneys as compared to normal control group (Table-1).

After 15 days of *N. sativa* treatment of iron overloaded mice, concentration of iron in serum, liver, kidney, and heart was decreased (Table-2, Figures 1–4).

Histopathological analysis reinforced the above findings. Iron overload induced organ damage was evident under the microscope in the form of necrosed cells, increased size of nucleus, absent or scanty cytoplasm in groups 2 and 3 (Figures 1b, 2b, and 3b). In group 3 after herb treatment significant improvement in cells of kidney, liver and heart was observed (Figures 1c, 2c and 3c).

Table-1: Comparison of iron levels in serum, liver, kidney, and heart between control and iron overloaded groups (Mean±SEM, mg/ml)

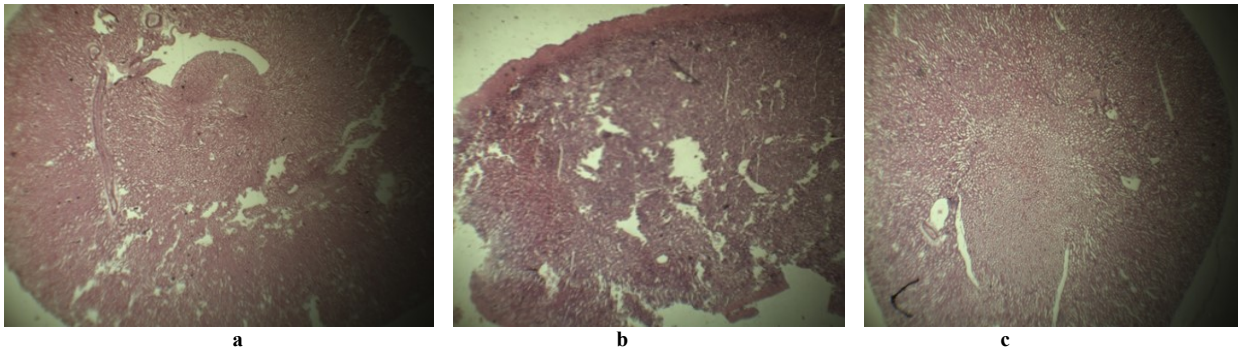
Groups	Control	Iron overloaded	*p*
Serum iron concentration	0.003295 ±0.002145	0.017641 ±0.001141	0.003*
Liver iron concentration	0.00260 ±0.00078	0.02287 ±0.00143	0.05*
Kidney iron concentration	0.00269 ±0.00069	0.01896 ±0.00043	0.05*
Heart iron concentration	0.00317 ±0.00113	0.03010 ±0.00111	0.03*

*Significant

Table-2: Comparison of serum iron concentration in herb treated group with control group and iron overloaded control group after 30 days (Mean±SEM, mg/ml)

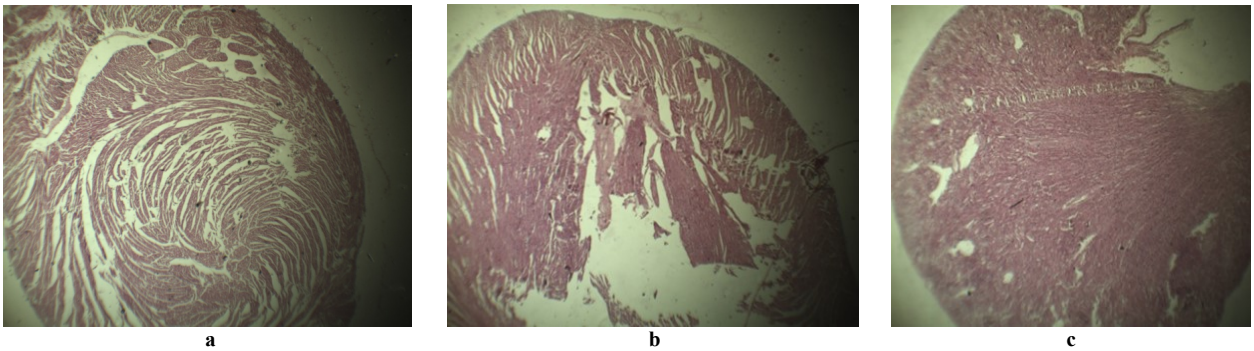
Groups	Iron overloaded	Iron overloaded +herb treated	*p*
Serum iron concentration	0.0256920 ±0.000121	0.0042744 ±0.0061561	0.05*
Liver iron concentration	0.0210143 ±0.0000111	0.0056160 ±0.0004352	0.04*
Kidney iron concentration	0.0351089 ±0,0096451	0.0048415 ±0.0000321	0.03*
Heart iron concentration	0.0339128 ±0.0023141	0.0032690 ±0.0010061	0.01*

*Significant



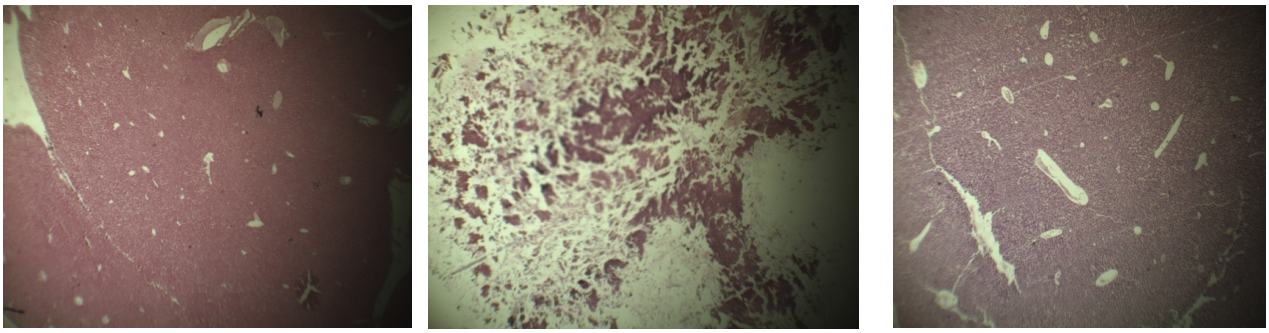
a b c

Figure-1: Comparison of kidney tissues (a) control kidney having low iron level (b) iron overloaded kidney, cells damaged by iron (c) iron overloaded+herb treated kidney, cells are repaired



a b c

Figure-2: Comparison of heart tissues (a) control heart having low iron level (b) iron overloaded heart, cells damaged by iron (c) iron overloaded+herb treated heart, cells are repaired



a b c

Figure-3: Comparison of liver tissues (a) control liver having low iron level (b) iron overloaded liver, cells damaged by iron (c) iron overloaded+herb treated liver, cells are repaired

DISCUSSION

The present study was conducted to observe the effect of *N. sativa* on artificially iron overloaded mice. After 15 days iron overload was confirmed by comparing with the control group. In a previous study serum iron concentration in normal men and women was compared with that of iron overloaded patients. Serum iron level in normal men and women was reported as 6–186 ng/ml and 3–162 ng/ml respectively while in iron overloaded patients the iron concentration was increased to a range of 940–4,240 ng/ml.[7] Das *et al*[8] also conducted a study on mice with iron overload and serum iron concentration was increased to double the amount of iron in the serum of normal mice. The serum iron level in iron overloaded+herb treated mice was very close to the control value, i.e., 0.0033 mg/ml. Another study proved that *Medicago sativa* and *Allium porrum* extracts reduced the iron concentration to significant levels.[9]

Extraneously overloaded iron was also accumulated in liver, kidneys, and heart. Liver iron concentration in control mice was 0.0021 mg/ml which was increased to 0.0228 mg/ml after giving iron injections. Das *et al*[8] also reported the increased liver iron concentration (685.79%) in iron overloaded mice. *N. sativa* treatment significantly decreased the iron level of liver. Another study conducted by Danladi *et al*[10] observed the effect of *N. sativa* on the liver antioxidant enzyme activities which were decreased by CCl4 treatment and subsequently increased by *N. sativa*.

After overloading iron in mice, a rapid increase in the kidney iron concentration was also observed. High concentration of iron in kidney was decreased by the *N. sativa* treatment. It was observed from the present study that iron level was decreased to 0.0047 mg/ml after treating the iron overloaded mice with *N. sativa*. Chelating ability of another plant extract known as *Tetracarpidium conophorum* was also reported in a previous study. Chelating ability of *T. conophorum* for ferrous iron was observed as more than 70%.[11] Iron level in heart was increased to 0.0301 mg/ml in iron overloaded mice after 15 days of iron accumulation while the iron concentration in control group was 0.0031 mg/ml. Wongjaikam *et al*[12] demonstrated iron overloading in rats and found significantly ($p<0.05$) increased cardiac iron concentration as compared to the control rats.

N. sativa treated mice had a decreased iron concentration of 0.0036 mg/ml in heart which is very low as compared to iron overloaded control group, i.e., 0.0301 mg/ml. Zaoui[13] reported that *N. sativa* oil (1 ml/Kg/day) oral administration in rats for 12 weeks could significantly reduced serum cholesterol, glucose, triglyceride levels, platelet counts and leukocytes by 15–30% as compared to control group.

Histopathological analysis of liver, kidney and heart in the current study reported that there was a clear difference between the tissues of control group, iron overloaded group and iron overloaded+herb treated group. Tissues of the control group mice were very healthy whereas iron overload showed liver, kidney, and heart defects as necrosis of the cells. The size of the nucleus was also increased as compared to normal mice. Cytoplasm was absent in the cells of iron overloaded mice. In a previous histopathological study, it was demonstrated that iron overload damaged the tissues and hepatic necrosis and other morphological changes were observed in iron overloaded rats.[14]

CONCLUSION

Our study demonstrated beneficial effect of *N. Sativa* in iron overloaded mice. Future studies are recommended to replicate these in thalassemia major patients receiving blood transfusions.

REFERENCES

1. Sharma DC, Arya A, Kishor P, Woike P, Bindal J. Overview on thalasemias: A review article. Med Res Chronicles 2017;4(3):325–37.
2. Jones G, Goswami SK, Kang H, Choi HS, Kim J. Combating iron overload: a case for deferoxamine-based nanochelators. Nanomedicine (Lond) 2020;15(13):1341–56.
3. Piperno A, Pelucchi S, Mariani R. Inherited iron overload disorders. Transl Gastroenterol Hepatol 2020;5:25.
4. Kontoghiorghe CN, Kontoghiorghes GJ. Efficacy and safety of iron-chelation therapy with deferoxamine, deferiprone, and deferasirox for the treatment of iron-loaded patients with nontransfusion-dependent thalassemia syndromes. Drug Des Devel Ther 2016;10:465–81.
5. Hoffbrand AV, Cohen A, Hershko C. Role of deferiprone in chelation therapy for transfusional iron overload. Blood 2003;102(1):17–24.
6. Kishwar F, Mahmood I, Mahmood T, Qamar-ul-Haq. Thymol, an active constituent of nigella sativa, could reduce toxicity of some trace metals Fe(III),Cr(VI),Cu(II),V(IV) and Co(II). Eur Res J 2015;1(6):1110–25.
7. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. BMJ 1972;4(5834):206–8.
8. Das A, Chaudhuri D, Ghate NB, Panja S, Chatterjee A, Mandal N. Protective effect of *Clerodendrum colebrookianum* leaves against iron-induced oxidative stress and hepatotoxicity in Swiss albino mice. Indian J Exp Biol 2015;53(5):281–91.
9. Mirzaei A, Delaviz H, Mirzaei M, Tolooei M. The effects of *Medicago sativa* and *Allium porrum* on iron overload in rats. Glob J health Sci 2015;7 (7 Spec No):137–42.
10. Danladi J, Ahmed SA, Akpulu SP, Owolagba GK, Iduh MU, Mairiga AA. Protective effect of cool extraction of black seed (*Nigella Sativa*) oil against CCl4-induced oxidative damages in wistar rats testis. IOSR-J Pharm Bio Sci 2013;5(2):68–74.
11. Olabinri, BM, Eniyansoro OO, Okoronkwo CO, Olabinri PF, Olaleye MT. Evaluation of chelating ability of aqueous extract of *Tetracarpidium conophorum* (African walnut) *in vitro*. Int J App Res Nat Prod 2010;3(3):13–8.
12. Wongjaikam S, Kumfu S, Khamseekaew J, Sripetchwandee J, Srichairatanakool S, Fucharoen S, Chattipakorn SC, *et al*. Combined iron chelator and antioxidant exerted greater efficacy on cardioprotection than monotherapy in iron-overloaded rats. PLoS ONE 2016;11(7):0159414.
13. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine 2002;9(1):69–74.
14. Karlsson M, Ekstedt M, Dahlström N, Forsgren MF, Ignatova S, Norén B, *et al*. Liver R2* is affected by both iron and fat: A dual biopsy-validated study of chronic liver disease. J Magn Reson Imaging 2019;50(1):325–33.

Address for Correspondence:

Dr. Uzma Naeem, Associate Professor, Department of Pharmacology, Islamic International Medical College, Rawalpindi, Pakistan. **Cell:** +92-300-9196818

Email: uzma.naeem@riphah.edu.pk

Received: 15 Feb 2020 **Reviewed: 26 Mar 2021** **Accepted: 29 Mar 2021**

Contribution of Authors:

MN: Concept, data acquisition and analysis, final approval
MR: Concept, data acquisition and analysis, final approval
UN: Manuscript writing, Revision of the project, critical review of intellectual content, final approval
SR: Revision of the project, critical review of intellectual content, final approval
KA: Revision of the project, critical review of intellectual content, final approval
TM: Revision of the project, critical review of intellectual content, final approval

Funding source: None
Conflict of interest: None