



**IN VITRO STUDY OF IRON CHELATING ACTIVITY OF INDIAN WALNUT
(*JUGLANS REGIA*) AND COFFEE (*COFFEA ARABICA* L.) IN VARIOUS EXTRACT.**

Pallavi P. Lahane, Sangita S. Kunjwani, Zia H. Khan* and Nazia D. Khan

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.)

***Author for Correspondence: Dr. Ziaulhasan Khan**

Department of Biochemistry, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.), India 444003.

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ABSTRACT

Metals with normal concentration have essential roles in body metabolism, however in higher concentration they can induce severe toxicity. Treatment with chelating agents is a useful practice to reduce metal toxicity in a live organism. The fruit of Indian walnut (*Juglans regia*) and Coffee (*Coffea arabica* L.) were taken with the aim of evaluating the chelating activities. The extract in three different solvents viz. aqueous, methanol and petroleum ether were prepared. The chelating ability was studied by using Benzie and Strain method. EDTA was used as a standard for this purpose. Methanolic extract was found to be the most effective iron chelator. Highest activity was found in 1000 µg/ml concentration of walnut plant i.e. 89.71% and coffee shows 90.32% that of standard EDTA was found to be 97.90%. In conclusion the methanolic extract of Indian walnut (*Juglans regia*) and Coffee (*Coffea arabica* L.) could be used in the treatment of iron overload diseases.

KEYWORDS: Indian walnut (*Juglans regia*), Coffee (*Coffea arabica* L.), Iron chelating activity, EDTA.

INTRODUCTION

Metals are very important to carry metabolic activity of the body, but sometimes its concentration is raised above normal value which could be harmful. Chelating agents are proved very useful to reduce such metals from the body. Chelating agents form a complex with metal ions, especially with copper and iron, and are able to delay or retard oxidation reactions. (Pokorny J., 2001 and Shahidi F., 2000).

DFO (Deferoxamine) was certified and confirmed by the European Union as a drug for thalassemia to reduce iron overload in 2006. These synthetic chelators strongly bind to metal ions and promote their excretion. However, such synthetic drugs have many side effects, so it is necessary to study some natural chelating agents for iron excretion as they have a low risk of side effects (Ali Mirzaei, Raheleh Khatmi, 2013).

Indian walnut (*Juglans regia*) belongs to the family *Juglandaceae*. It is mostly cultivated for nuts but is also very useful for health. It gives 2738Kj (654Kcal)/100gms. It is nutrient dense with protein and essential fatty acids. It contains phenolics (7 phenolic compounds), Omega-3 fatty acid, and amino acid L-arginine. It also has Alpha linolenic acid (ALA) which 50% lowers the risk of sudden cardiac death. It also has an unusual form of vit. E which is Neuron protective in

nature. It also has tanning, melatonin etc. In India, walnut is mostly cultivated in Kashmir.

Coffee (*Coffea arabica* L.) belongs to the family Rubiaceae. It is mainly used as 'drink'. Coffee has important organic compounds and nutrients including B-complex vitamin, riboflavin. Coffee also contains other substances such as sugar, kaffic acid and citric acid (Ky *et al.*, 2001). Coffee has many polyphenolic compounds, coffee polyphenols form a non-soluble complex with copper and iron. This ligand causes the absorbance lack of iron and copper in the stomach (Gordon and Kohen, 2004).

The effect of phenolic compounds on the absorbance of these metals has been studied and indicated that phenolic monomers and polyphenols form complexes with metals. *Juglans regia* and *Coffea arabica* shows great activity of iron chelation which can be an important tool for the treatment of various diseases like thalassemia. Thalassemia patients usually show underproduction of normal globin proteins (Maggie *et al.*, 2002). Iron chelation can be used to improve the quality of life in such diseases. The aim of the current work is to evaluate the chelating ability of *Juglans regia* and *Coffea arabica* against iron overload by *in vitro* techniques so we can get to know its metal chelating potential for iron.

MATERIALS AND METHODOLOGY

Collection of material

The Indian walnuts and Coffee beans were purchased from local market of Akola city, Maharashtra. The identification was made from Botany department of College. The seeds were grinded by grinder.

Preparation of extract

Walnut fruit and coffee beans were grinded by mechanical grinder and subjected to Soxhlet apparatus for extract preparations. Various concentration of aqueous, methanolic and petroleum ether extract were prepared such as 125ug/ml, 250ug/ml, 500ug/ml and 1000 ug/ml.

Iron chelating activity

The iron chelating activity of walnut and coffee was determined by method of Benzie and strain (1996). The principal is based on formation of 1, 10 phenanthroline-Fe complex and its disruption in presence of chelating agent. The reaction mixture containing 1ml of 0.05% 1,10 phenanthroline in methanol, 2ml ferric chloride (200uM) and 2ml of various concentration of walnut fruit extract (aqueous, methanolic and petroleum ether) such as 125ug/ml, 250ug/ml, 500ug/ml and 1000 ug/ml was incubated at room temperature for 10mins and absorbance of same was measured at 510 nm on spectrophotometer. Similar process repeated with Coffee beans. EDTA was used as a classical metal chelator.

Observation

Table no.1: Effect of different extract of *Juglans Regia* on iron chelation.

Concentration in ug/ml	Aqueous extract (% activity)	Methanolic extract(% activity)	Petroleum ether extract (% activity)	EDTA as standard (% activity)
125ug/ml	20.28	31.84	29.22	56.68
250ug/ml	29.11	56.79	32.00	65.87
500ug/ml	33.80	75.71	49.00	83.83
1000 ug/ml	42.00	89.71	56.13	97.90

Table No. 2 : Effect of different extract of *Coffea arabica* on iron chelation.

Concentration in ug/ml	Aqueous extract (% activity)	Methanolic extract (% activity)	Petroleum ether extract (% activity)	EDTA as standard (% activity)
125ug/ml	12.95	27.46	21.24	56.68
250ug/ml	26.20	41.97	41.96	65.87
500ug/ml	27.46	66.15	77.70	83.83
1000 ug/ml	75.82	90.32	76.94	97.90

CONCLUSION

Indian walnut (*Juglans regia*) shows good iron chelation activity. Coffee (*Coffea arabica*) shows more iron chelating activity as compare to walnut. Thus natural source could be used as good iron chelators especially for thalassemic patients who are usually depend on transfusion therapy and synthetic iron chelators.

REFERENCES

1. Ali Mirzaei, Raheleh Khatmi : Antioxidant and Iron chelating activity of coriander sativum and petroselinum crispum. *Bulletin of Environmental Pharmacology and Life Sciences.*, 2013; 2(7).
2. Benzie and Strain: The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.*, 1996; 239(1): 70-6.
3. Maggio A, D'Amico G, Morabito A, Capra M, Ciaccio C, Cianciulli P, Gregorio FD, Groupon G, Malinga R, Magnano C, Mangiagli A, Quarta G, Rizzo M, D'Asiolo DG, Rizzo A, Midiri M.: Deferiprone versus deferoxamine In patients with thalassemia major: a randomized clinical trial. *Blood cell Mol Dis.* 2002; 281(2): 196-208.
4. Khorsandmanesh S., Gharachorloo M., Bazayr B.: The stabilizing and chelating effects of green and roasted coffee extracts. *Journal of Food Biosciences and Technology*, 2012; 2: 9 – 12.
5. Pokorny J., Yanishlieva N. and Gordon M.: Antioxidants in food. Practical applications, CRC Press LLC, Boca Raton, FL. U S App., 2001; 7-84.
6. Shahidi, F.: Antioxidants in food and food antioxidants. *Mol. Nutr. Food Res.*, 2000; 44: 158-111.