USE OF ACTIVE INGREDIENT OF *NIGELLA SATIVA* TO REDUCE TOXICITY OF SOME TRACE ELEMENTS (FE(III), CR(VI), CU(II), V(IV) AND CO(II))

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Abstract

The aim of the present research is to develop a plant-based method to reduce the toxicity caused by high levels of some essential trace elements in the body. In present work some physical properties of the *Nigella sativa* (NS) seeds were determined and complexation of its principle component thymoquinone (TQ) with some essential trace metals i.e; Fe(III), Cr(VI), Cu(II), V(IV) and Co(II) has been studied using pH-metry. It was observed from pH plots of ligand and complexes that TQ successfully formed complexes with all metals hence could be used as chelating agent in case of toxicity of above mentioned metals. Species distribution curves showed the maximum formation of ML (1:1) complex species of TQ with Fe(III), Cr(VI), Cu(II) and V(IV) within pH 3.00-4.00 and with Co(II) at pH 6.00. But maximum ML_2 (1:2) complex specie formation of TQ with Co (II) seems to occur near pH 11.00, whereas with rest of the four metals within pH 7.00-9.00.

Introduction

Essential elements have important biological and nutritional functions in all living things including human beings. Among these, the elements which are required in low quantities for the body are called essential trace elements. These include iron, chromium, copper, vanadium, cobalt, zinc, manganese, selenium, nickel, tin, and molybdenum (Atkins *et al.*, 1978; Das, 1990). In order to perform their functions they should be in proper balance with respect to one another (Smolin and Grosvenor, 2007).

Trace elements are very important as they perform a variety of essential structural and regulatory roles. They participate in diverse tasks all over the body, each having special duties that only it can perform. So each of the trace elements performs vital roles. If the deficiency of any of them may be fatal, their excess can be equally deadly. Hence our diet should contain just enough of these minerals to maintain health, but the upper level (UL) of their recommended intakes (RDA) should not exceed (Table 1) (Smolin and Grosvenor, 2007; Whitney and Rolfes, 2002). If metal's intake exceeds the UL, it results in metal toxicity which is very dangerous. For example, iron toxicity can damage the intestinal lining and may cause abnormalities in body pH, shock and liver failure. Too much iron promotes the formation of free radicals and causes cell death due to excess oxidation of cellular components. Iron overload can damage iron storing organs, especially heart and liver. Iron toxicity may result in heart diseases, diabetes, arthritis and cancer (Bartfay *et al*., 2000; Klipstein-Grobusch *et al*., 1999). Too much chromium may cause renal failure and other complication. Specially Cr (VI) is a strong irritant and can cause allergic reactions, such as skin rashes. At high levels its inhalation may irritate nose lungs, stomach and intestines, whereas its ingestion can cause stomach upsets and ulcers, convulsions, kidney and liver damage and even death (Stoecker, 1999). Excessive copper intake may cause headache, nausea, abdominal pain and diarrhea. Acute copper toxicity may result in heart problems, liver damage, jaundice, kidney failure, coma and death (Smolin and Grosvenor, 2007). Toxicity symptoms in case of vanadium include stunted growth, diarrhea and stomach cramping. High doses of vanadium even may lead to death (Thompson and Orvig, 2004). Exposure to vanadium dust may result in rhinitis, nose bleeds, conjunctivitis, and pain in chest, sore throat and cough. Vanadium toxicity may decrease fertility and cause complications in pregnancy (Murray, 1996). Large doses of cobalt might stimulate thyroid and bone marrow functions (Food and Nutrition Board, Institute of Medicine, 1998). Metal toxicity is more severe than organic toxicity, because metals retain their identity in the body, whereas organic compounds may decompose.

The most common technique which is used to treat heavy metal toxicity is chelation therapy. It involves administration of chelating agents, such as ethylenediaminetetraacetate (EDTA), dimercaprol (BAL), dimercaptosuccinic acid (DMSA), penicillamine, deferoxamine and deferasirox etc. to remove heavy metals from the body. These chelating agents can be administered intravenously, intramuscularly, or orally (Marsha, 1996). But chelation therapy may produce toxic effects as all chelating agents have both minor and potentially life threatening side effects, including kidney damage, irregular heart beat, and swelling of the veins (Kosnett, 2010). Therapy may also cause nausea, vomiting, diarrhea, temporary lowering of blood pressure, skin peeling and blisters (Howland, 2011). Since treatment removes minerals from the body; it may also remove calcium, magnesium and zinc resulting in hypocalcemia, bone damage and mutations in cells (Marsha, 1996). Chelation therapy may also increase the risk of cancer. Furthermore, as this treatment is often given along with large doses

of vitamins and other minerals, this may initiate the process of free radical formation in the body (Bridges, 2006). Thus this way of treatment inspite of its effectiveness come with a number of serious side effects. On the contrary, plant-based system of medicine being natural does not pose these serious problems (Khan and Jain, 2003). Present research study has been focussed on the use of NS seeds inorder to cure heavy metal toxicity.

Nigella sativa (Family-Ranunculaceae), commonly known as black seed is a cultivated herb. Its seeds are used in indigenous system of medicine and possess carminative, digestive, diuretic and antiseptic properties (Chopra *et al*., 1956). It is also used in the treatment of mild cases of fever, while mixed with sesame oil it is used as an external application in the eruption of skin and in scorpion sting. It is also useful in various body aches and in catarrh (Yousuf *et al*., 1994). The seed increases secretion of milk and is useful in cough, asthma and boils on skin. NS seed is also used in food as spice (Khanna *et al*., 1993).

The multiple uses of NS in folk medicines encouraged many investigators to isolate the possible active components and to conduct in vivo and in vitro studies on laboratory animals and human beings in order to understand its pharmacological actions. It has been concluded from these researches that most of the pharmacological properties of NS seed are due to its principle components. Out of several constituents, thymoquinone (2-methyl-5-isopropyl-1,4-benzoquinone) is the main constituents of NS seed (Al-Saleh *et al.*, 2006), which has been tested for its efficacy against several diseases (Salman *et al*., 2008; Marsik *et al*., 2005).

Among the various bioactivities, examined for thymoquinone (TQ), one of the most important is its antioxidant activity (El-Dakhakhny *et al.*, 2002; Badary *et al*., 2003).The compound was observed to decrease cellular oxidative stress (Mohamed *et al*., 2003).It has a potent chemopreventive potential of inhibiting the process of carcinogenesis (Badary *et al*., 2007). In addition, several research studies have shown its anti-inflammatory (Syed, 2008), anti-tumor (Shoieb *et al*., 2003), antidiabatic (Abdelmeguid *et al*., 2010), antitussive (Hosseinzadeh *et al*., 2008), and antimicrobial (Mohajir *et al*., 1999) activities.

Materials and Methods

Sample Collection: NS seeds were collected from local market. The seeds were cleaned properly to remove dirt and stored in air tight jars.

Reagent and glassware: All the reagents used were of analytical grade, purchased from Merck, Bio Basic Inc., and MP Biochemicals LLC. All glassware used were of standard quality. They were properly cleaned and rinsed with distilled- deionized water and finally dried in oven before use. For determination of dissolved and undissolved solid in the seeds, hydrochloric acid and distilled deionized water were used, whereas for pH-metric studies ferric chloride (hexa hydrate), potassium dichromate, copper sulphate (penta hydrate), vanadyl sulphate (penta hydrate), cobalt acetate (tetra hydrate), thymoquinone, and sodium hydroxide were used.

Instrumentation:
Electrical balance: Shimadzu, Model AX 200 was used for weighing.

Electrical oven*:* Double-walled laboratory oven, Model J-5015-50 was used to remove moisture from glassware and to dry samples.
pH meter: Jenway, Model 3510 pH meter was used for pH metric titrations.

Sample Preparation:
Dissolved and undissolved solids*:* Black seeds were soaked in aqueous (distilled deionized water) and acidic (0.1 M HCl) mediums at room temperature (30±2˚C), for different time intervals.

pH-metric studies: Caliberation buffers of pH 4.00 and 7.00 were prepared using buffer tablets. NaOH (0.1M), $FeCl_3.6H_2O$ (0.005M), $K_2Cr_2O_7$ (0.005M), $CuSO_4.5H_2O$ (0.005M), $VOSO_4.5H_2O$ (0.005M), $Co(CH_3COO)_2.4H_2O$ (0.005M) and TQ (0.005M) were also prepared. NaOH was standardized using oxalic acid as standard solution, every time, before use.

Analysis:
Moisture content: Moisture content was determined at 105 ± 1˚C.
Dissolved (DS) and undissolved (UDS) solids: For this purpose about 1g of cleaned and dried NS seeds were soaked in distilled deionized water for different time intervals i.e; 0.5, 1, 2, 3, 4, and 5 hours. These samples then filtered, and dissolved and undissolved materials were dried separately. Filtrate (DS) was evaporated to dryness on a preheated water bath and then it was kept in oven at 105±1˚C up to dryness, whereas residue (UDS) was also kept in oven, at above mentioned temperature, till it was completely dried. Same procedure was repeated with 0.1M HCl extract.

pH-metric studies*:* All pH metric titrations were done at 25±5˚C. Sodium hydroxide solution was standardized using standard oxalic acid every time before titration of sample solutions. All pH metric titrations were carried out in a double walled glass cell. The temperature was controlled by circulating water through a thermostat (HAAKE, KT 33 Germany). The capacity of the cell was 100 ml. The rubber stopper of the cell contained four holes, one for micro-burette for addition of standard base, another one for purging inert gas (Nitrogen) and the third hole for the removal of oxygen and the forth for glass electrode. The solution completely deareated by passing N_2 gas for 30 minutes in a sealed flask and was protected with atmosphere.

pH-metric titration of ligand (Thymoquinone): For this purpose, 40 mL of TQ solution (0.005M) and 10 mL of distilled deionized water were taken in the pH metric cell containing magnetic bead. Purified nitrogen gas was perged through the solution for half an hour. The temperature was controlled by circulating water (using a thermostat). Then the TQ solution was titrated against 0.1M standard sodium hydroxide solution. NaOH solution was standardized using 0.05M Oxalic acid solution prior to the pH metric titration of TQ.

During titration regular stirring was maintained by means of magnetic stirrer. Standard NaOH was added in sufficiently small increments of 0.05 mL with the help of micro-burette and after each increment pH of the reaction mixture was recorded till pH was not affected by further addition of standard NaOH. pH values were plotted against the added volume of standard NaOH.

pH-metric titrations of metal {Fe(III), Cr(VI), Cu(II), V(IV) and Co(II)}- *Thymoquinone complexes:* For pH metric titration of Fe(III)-Thymoquinone complex, 40 mL of TQ solution (0.005M) and 10 mL of Fe(III) solution (0.005M) were mixed to give 1:4 metal-ligand solution. The resulting reaction mixture was titrated against standard sodium hydroxide solution (0.1M) under the same conditions and in the same manner as previous two titrations, in order to compare pH metric titration of TQ and Fe(III)-Thymoquinone complex.

Similarly, pH metric titrations of other complexes of TQ i.e.; complexes with Cr(VI), Cu(II), V(IV) and Co(II) were performed in the same manner and under the same conditions.

For each titration pH values were plotted against volume of standard NaOH added , which provide valuable information .With the help of pH-metric data species distribution curves were also plotted to find out best pH values for M (free metal), ML (1:1 complex) and ML_2 (1:2 complex) species.

Results and Discussion

Moisture Content: The moisture content of NS seed was found to be 7.932±0.055% (Fig. 1), which is in good agreement with the reported value (Saleem et al., 2007).

Dissolved(DS) and Undissolved Solid(UDS): Results showed higher percentage of dissolved solid in acid (HCl) extract compared to water extract, on the other hand percentage of undissolved solid was found maximum in water extract. This shows that probably seed contains substances which are less soluble in water and more soluble in acid or they contain substances which decompose in acid. Moreover, it was also observed that lesser time is required to extract out maximum dissolved solid in case of acid than water. In case of total solid (DS and UDS) a decrease was noted with time, which may be due to presence of some volatile substances in the seeds (Table 2 &3, Fig. 2).

Thymoquinone and its Complexes: As a reference, initially titration of TQ was performed. In plot of pH against added volume of NaOH only one curve was observed near pH 10.50. Remarkable changes in titration curves of TQ and its complexes were observed (Fig. 3), which indicates complexation between metals and the ligand.

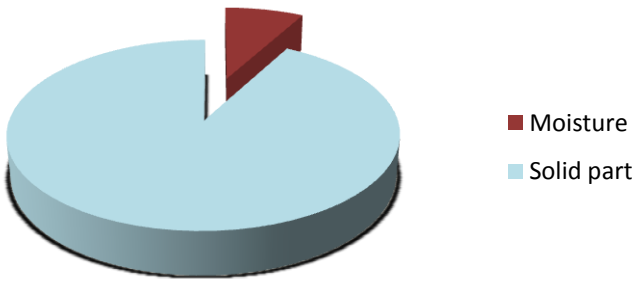


Fig.1. Moisture content in *Nigella sativa*

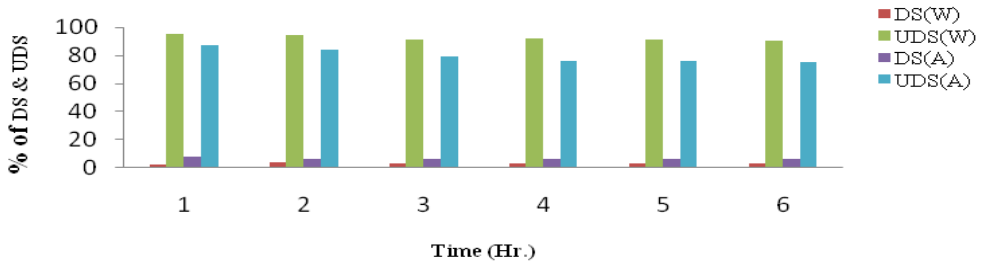


Fig. 2. Comparison of percentages of DS and UDS in water (W) and acid (A) extracts of *Nigella sativa*

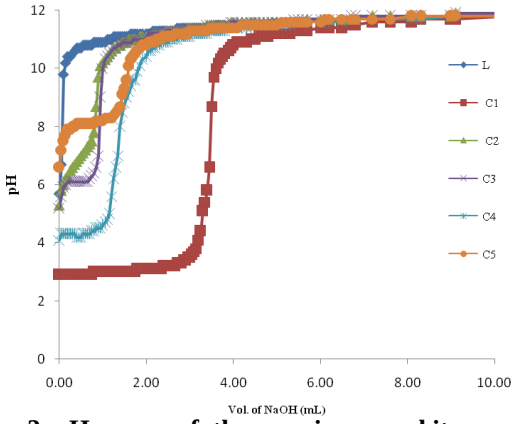
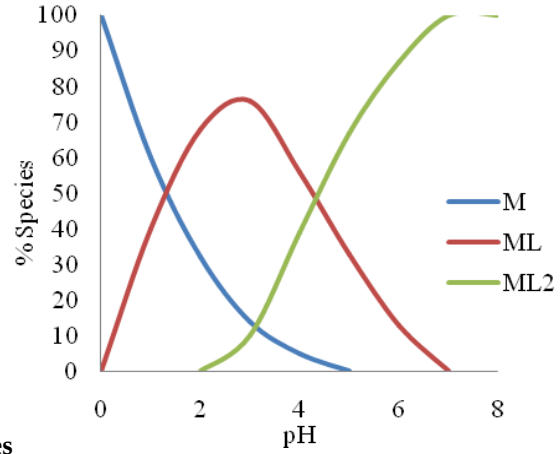
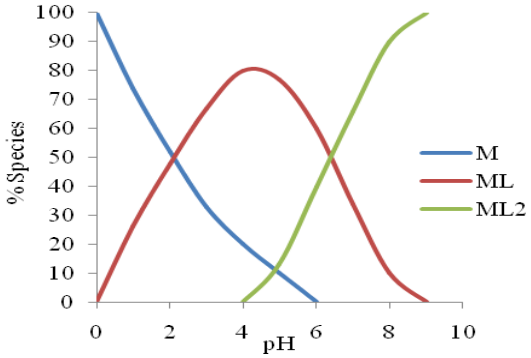


Fig. 3. pH curves of thymoquinone and its complexes

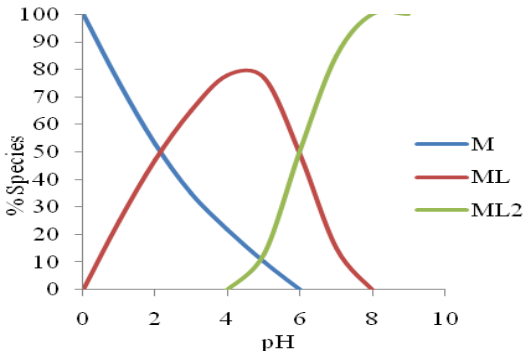
Note: L= Thymoquinone, C_1= Fe(III)-Thymoquinone Complex, C_2= Cr(VI)- Thymoquinone Complex, C_3= Cu(II)-Thymoquinone Complex, C_4= V(IV)-Thymoquinone Complex, C_5= Co(II)-Thymoquinone Complex



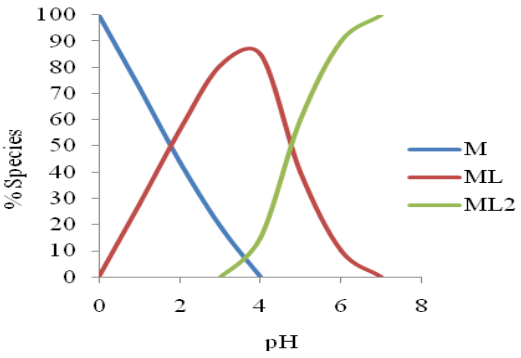
4a. Fe(III)-thymoquinone complex



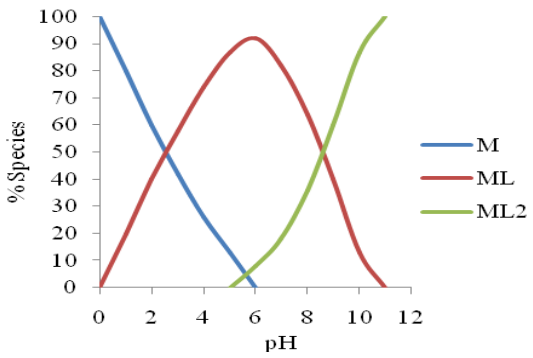
4b. Cr(VI)-thymoquinone complex



4c. Cu(II)-thymoquinone complex



4d. V(IV)-thymoquinone complex complex



4e. Co(II)-thymoquinone complex

Fig.4. Species distribution curves

Table 1. Recommended dietary allowances (RDA), adequate intakes (AI) and tolerable upper intakes levels (UL) for adults.

Parameters	*Fe* *mg/day*	*Cr* *(µg/day)*	*Cu* *(µg/day)*	*V* *(µg/day)*	*Co* *(µg/day)*
RDA	8-18	-	700-900	20-30	5-8
AI	-	25-35	-	-	-
UL	45	-	10,000	18,000	-

Table 2. Percentage of dissolved (DS) and undissolved (UDS) solid in water extract of *Nigella sativa.*

Time (h)	DS (±SD) %	UDS (±SD) %	Total Solid (DS+UDS) %
0.5	1.851±0.1948	95.396±1.3789	97.247
1	3.582±0.0515	94.601±0.7853	98.182
2	3.234±0.1033	91.320±0.447	94.554
3	3.095±0.1402	91.739±0.4644	94.835
4	2.716±0.0986	91.310±0.3856	94.026
5	2.735±0.0478	90.730±0.1777	93.465

Table 3. Percentage of dissolved (DS) and undissolved solid (UDS) in acid extract of *Nigella sativa.*

Time (h)	DS (±SD) %	UDS (±SD) %	Total Solid (DS+UDS) %
0.5	7.579±0.2887	87.199±0.2527	94.778
1	6.399±0.1916	84.196±0.3737	90.595
2	6.208±0.3021	79.122±0.3106	85.330
3	5.994±0.2935	76.023±0.2238	82.017
4	6.089±0.1242	75.928±0.1722	82.018
5	6.083±0.1885	75.550±0.6815	81.633

In case of Fe(III)-Thymoquinone complex, two distinct curves were observed, first near pH 3.00 and second near pH 10.5. First curve indicates the fast consumption of metal, which may be due to formation of ML and ML_2 species; whereas the second curve indicates the neutralization of excess ligand by NaOH. In plot of Cr(VI)-Thymoquinone complex a slight twist was observed at pH 5.80, which may be due to formation of ML specie. Whereas two more curves were observed near pH 7.50 and 10.00.First curve indicates the formation of ML_2specie probably, whereas the second curve shows neutralization of excess ligand. In case of Cu(II)-Complex three prominent curves were observed. First one near pH 6.00 which may be due to formation of ML specie, second curve near pH 6.50 which shows formation of ML_2 species probably. The last curve near pH 10.00 indicates titration of excess ligand. Titration curve of V(VI)-Thymoquinone Complex shows two curves in the plot, first near pH 4.5, whereas second near pH 10.00. First curve starts at pH 4.1 and no significant change in pH was observed up to the addition of 1.00 ml of NaOH, this probably shows that vanadium (IV) forms a stable complex with TQ. The last curve may be due to titration of excess ligand. In case of Co (II)-Thymoquinone Complex two distinct curves were observed in the plot. First one near pH 7.90 whereas second near pH 8.50, which may be due to formation of ML and ML_2 species respectively.

It was observed that out of all metals, Fe(III) formed complex at the lowest pH. V(IV) formed complex at moderate pH, whereas rest of the metals formed complexes at relatively higher pH. As far as stability of these complexes is concerned Fe(III) complex seems most stable, V(IV) complex seems to have moderate stability; whereas rest of the complexes were of relatively lower stabilities. From present research P_{ka} of thymoquinone was found to be 8.5.

Species distribution curves of TQ complexes: Species distribution curves for each complex of TQ were plotted using pH- metric data as it was done in thymol. In case of Fe (III)-Thymoquinone complex the reaction between metal and the ligand seems very fast and maximum ML and ML_2 species formation seems to occur near pH 3.00 and 7.00 (Fig. 4a). For Cr (VI)-Thymoquinone complex maximum percent distribution for these two species was found at pH 4.00 and 9.00 respectively (Fig. 4b). In curve plotted for Cu(II)-Thymoquinone complex maximum ML and ML_2 species formation seems at pH 4.00 and 8.00, whereas plot shows that near pH 6.00, the distribution of each species in the reaction mixture was probably 50% (Fig. 4c). In case of V (IV)-Thymoquinone complex, the plot shows a fast reaction between vanadium and TQ. Maximum percent distributions of ML and ML_2, for this complex, were observed at pH 4.00 and 7.00 respectively, whereas it is evident from the plot that near pH 5.00 the distribution of each species was 50% (Fig. 4d). The curve for Co (II)-Thymoquinone complex clearly shows formation of two complex species (ML and ML_2). Their maximum formation was observed at pH 6.00 and 11.00 respectively, whereas it seems from the plot that each species was 50% between pH ranges 8.00-9.00 (Fig. 4e).

Conclusion: *Nigella sativa* (NS) seeds are an excellent source of many biologically active compounds such as thymoquinone, thymohydroquinone, dithymoquinone and thymol etc. (Benkaci-Ali *et al*., 2010). Out of several constituents, thymoquinone (TQ) has been reported as principle component of the NS seed (Al-Saleh *et al*., 2006).

In present research complexation of TQ with some trace elements has been studied along with some physical properties of the NS seeds. Physical analysis includes determination of moisture content and dissolved (DS) and undissolved (UDS) solids in the seeds. Study on DS and UDS showed maximum dissolved material in acidic medium. This reveals that chlorides of such metals which are insoluble in HCl, for example chlorides of silver, lead, mercury etc., are not present in reasonable quantity in the seed. Results suggest that very short time is required to dissolve the polar part of the seed in acidic environment of stomach.

Complexation of TQ with Fe(III), Cr(VI), Cu(II), V(IV) and Co(II) showed that Fe(III) and V(IV) forms relatively stable complexes than other metals. They forms complexes at low pH hence TQ may chelate them in stomach whereas Cr(VI), Cu(II) and Co(II) forms complexes at higher pH. Among Fe(III) and V(IV), Fe(III) forms most stable complex with TQ. It is clear that these metals are required in trace quantities for the human body and their excess results in heavy metal toxicity, which can cause a wide range of problems such as severe injury to the body organs and to the brain.

From centuries Chelation therapy has been used to treat metal toxicity (Marsha, 1996), which has dangerous side effects as it involves the use of drugs which are potentially toxic (Kosnett, 2010). Their target organs are kidneys, the cardiovascular and the central nervous systems (Howland, 2011). In present research TQ formed complexes with the above mentioned metals successively, hence can be used as chelating agent in chelation therapy.

From results it is revealed that in case of metal toxicity TQ could be helpful in removing heavy metals from the body. It could perform this action by two ways, firstly, by forming complexes with the metals which exceed the toxic level. These metals after formation of complexes will be unable to absorb in the body and then excreted from the body in the complexed form. Secondly, since TQ is an antioxidant (Badary *et al*., 2003) it can reduce these metals, for example Fe (III) to Fe (II), Cr (VI) to Cr (III) etc. and thus converting the toxic metals into reduced form in which they play their active roles in the body (Khan and Fatima, 2006). Hence the intake of NS in case of any of the above mentioned essential metal toxicity could be beneficial without any side effects.

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