

The Effects of *Allium porrum* and *Medicago sativa* on Iron Concentration in Thalassemia SerumsAli Mirzaei¹, Mojtaba Abbasi², Somayyeh Sepehri³, Mahsa Mirzaei^{1*}¹Medicinal plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.²Veterinary Department, Islamic Azad University, Shahrkord Branch, Shahrkord, Iran.³Department of Biotechnology, Ferguson College, Pune University – 414001, Maharashtra, India.*Corresponding Author: Mahsa.mirzaee@rocket.com.

Abstract: Iron overload is a major problem in Beta thalassemia patient due to regular blood transfusions. Regularly iron chelation is more recommended after 10-20 blood transfusions practice or when serum ferritin is more than 1000 ng/ml. The aim of present study was evaluated of chelating activity of *Allium porrum* and *Medicago sativa*. **Materials and Methods:** The total phenol, antioxidant activity and chelation capacity of plant extracts were estimated. For in vitro study of iron chelating, plant extracts were expose with serum thalassemic for 2h. **Results:** Total phenolic content and antioxidant activity in Hydro- alcoholic and phosphate buffer extracts were reported with maximum and minimum contents respectively. Iron content in thalassemia and control serums was estimated 181.6 ± 18.1 and 87 ± 17.3 respectively. A significant difference was found between iron concentrations in absence and presence extracts $p < 0.001$. Iron levels in normal and thalassemia significantly reduced when exposed with plants extracts especially in *Medicago sativa* in phosphate buffer $p < 0.001$. Iron concentration in serums control (86.9) after mixed with *Medicago sativa* phosphate buffer extract 66.2 ± 4.07 and *Allium porrum* phosphate buffer extract 75.2 ± 6.8 were reported. **Conclusion:** *Medicago sativa* and *allium porrum* with high iron chelating activity was a good plant chelator for in vitro study. These extracts can be a candidate for treatment of iron overload. [Ali Mirzaei, Mojtaba Abbasi, Somayyeh sepehri, Mahsa Mirzaei. **The Effects of *Allium porrum* and *Medicago sativa* on Iron Concentration in Thalassemia Serums.** *Life Sci J* 2013;10(11s):27-31]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 5

Key words: β - Thalassemia major, iron chelating, Iron overload, *Medicago sativa*, *Allium porrum***Introduction**

β - Thalassemia major is a congenital disease that characterized by severe anemia which dependence to regular blood transfusion in early of life. The disorder is more prevalent in Asia particularly in Mediterranean countries (Flint, 1998). Precipitation of hemoglobin in β - Thalassemia major was induced and consequently release of reactive iron was occurred in blood circulation. Iron toxicity or Hemosiderosis may be formed due to regular blood transfusions or high intestinal iron absorption (Milena, 2010). Iron excretion in human body has uni-directional cycle, because unable to loss by excretory systems. Consequently, iron excess deposited in heart, liver, brain, spleen and endocrine systems and induced a number of complications. (Taher, 2006; Rund, 2005; Loukopoulos, 2005).

Nowadays, synthetic iron chelators were used for iron excretion in iron overload state. Chelators are small molecule that powerfully formed a complex by metal ions in organisms so; they called iron binders in literatures. Regularly iron chelation more recommended after 10-20 blood transfusions practice or when amount of serum ferritin more than 1000 ng/ml (Thalassemia International Federation, 2008). Iron chelating method is useful assay for estimation of plant chelating potential on in vitro state. Chelating activity in medicinal plants mostly is

depending to phytochemical constituent especially flavonoids substances (Gupta et al., 2011). Deferoxamine is synthetic iron chelator drug which useful for iron overload treatment. Extensive use of deferoxamine by injection route was limited its application in clinics. In addition, short half-life of this drug limited its efficiency in practice (Borgna-Pignatti and Galanello, 2004; Yasser et al., 2008). Deferiprone is another iron chelator which applied for treat of iron overload conditions. It is recommended for treat of myocardial siderosis in iron toxicity. Reduced white blood cell count chiefly neutrophil was reported in literatures due to long timed application. Currently, there is no effective and choice drug without side effects in practice for the treatment of iron excess in thalassemia and other iron storages disease, except chelation therapy is the only alternate method for iron excretion. Thus, scientists are more focused on medicinal plants rich in phytochemical compounds with high antioxidant activity for the removed of iron in blood thalassemia. The Present in vitro work was performed for iron chelation and antioxidant activity of *Allium porrum* and *Medicago sativa* in thalassemia. In present work for evaluation of iron chelating in test tube (in vitro), different plant extracts were expose with human serums either in thalassemia patient and normal conditions. *Allium porrum* (common name leek)

belonging to the family *Alliaceae* that rich in vitamins C,E,B, potassium, iron and copper. They also contain carotenoids and chlorophyll, glycosides, total phenols, Flavonoid, high levels of phenolics with antioxidant activity and anti-blood clotting Potential (Rana, 2012). *Medicago sativa* is from Fabaceae family which its sprouts are often consumed as vegetable salad. Plant contains total phenol, flavonoids, alkaloids, coumarins, triterpenes and phytosterols. *Medicago sativa* possesses different potential such as antioxidant, antidiabetic, Anti-rheumatic, Anti-Cancer, Cardiotonic activity and lowering cholesterol (Rana et al, 2010; Kundan et al, 2011).

Examination on plant extracts Collection and extraction of plant material

Aerial parts of *Allium porrum* and *Medicago sativa* were collected in Yasuj, Iran. Samples were recognized and a voucher specimen was collected. The aqueous, hydro- alcoholic and phosphate buffer (in physiologic pH) extractions were performed by maceration method. Extractions was concentrated using rotary evaporator (Heideolph model 4000; Germany).

Determination of total phenolic compounds

The total phenolic content extract was determined and Gallic acid used as a standard (Karim et al., 2011).

Antioxidant activity of Dipheny-picrylhydrazyl (DPPH)

The antioxidant activity of extract assessed (Mirzaei and Mirzaei, 2013).

Metal chelating activity

The iron chelation and IC_{50} by extracts was determined using modified method of Dinis (Dinis et al., 1994).

Examination on thalassemia and control serums

This research was performed on 60 person, who comprised 30 age and sex matched (18 males and 12 females) normal individual or (controls) and 30 (16 males and 14 females) as β - thalassemia patient, who were earlier identified by electrophoretic exams. All the patients were receiving blood transfusion regularly every month. For exclusion of study thalassemia patient with history of cardiovascular, hypertension, diabetes mellitus and thyroids disease were considered. For analysis of data a total of three ml of blood was collected by every participant in fasting state. The serum was separated by centrifuged at 2500 rpm for 10 min. For measuring of iron, local clinical chemistry standards

Pars Azemon local company kits was used. Serum iron evaluated on thalassemia patient and control groups in presence and absence of plant extracts. In a test tube 0.5 ml of extracts at concentration (4mg/ml) was mixed with 0.5 ml thalassemia and control serums in 37 °C for 2 h. After incubation period, reaction mixture was centrifuge at 1500 rpm for 10 min and then iron concentration was measured and compare with iron level before expose with plant extracts. All data were expressed as mean \pm SD. The paired sample t test was used for the comparison of data. $P < 0.05$ was described as significant whereas $p < 0.001$ was identified as highly significant.

Results

In total phenolic content and antioxidant activity in term of diphenylpicryl hydrazyl (DPPH) in Hydro- alcoholic and phosphate buffer extracts were reported with maximum and minimum contents respectively. *Medicago sativa* plant in all extracts with maximum concentration of total phenol and DPPH activity was reported (Figure 1, 2). A significant difference between phenol content and antioxidant activity in *Medicago sativa* total and *Allium porrum* extracts was seen $P < 0.001$. In present study in primary screening for iron chelating *Medicago sativa* in all three extracts was reported with maximum activity. An IC_{50} value of iron chelating power in phosphate buffer (0.01mg/ ml), Hydro alcoholic (0.6 mg/ ml) and aqueous extracts (0.72 mg/ ml) was determined. However in *Allium porrum* iron chelating potential in phosphate buffer (1.2mg/ ml), Hydro alcoholic (1.9 mg/ ml) and aqueous extracts (2. mg/ ml) was reported (Figure3). For evaluation of secondary iron chelating activity of plants extracts, thalassemia and control serums were used also. The patients were aged between 1-12 years and the average hemoglobin concentration ranged between 7.7 – 8.7 gm /dl (data not shown). *Allium porrum* and *Medicago sativa* extracts were exposed with serum patient in a test tube as an in vitro model. In presence of extracts a significant decrease was reported in iron thalassemia serum after incubation of 2 h. at 37 °C, compared to the iron thalassemia serum in absence of extract $p < 0.001$. Iron content in thalassemia and control serums was estimated 181.6 ± 18.1 and 87 ± 17.3 respectively (Figure 4-7). A significant differences between patient and control serums in terms of iron content $p < 0.001$. Iron concentration of thalassemia after mixture with phosphate buffer of *Medicago sativa* and *Allium porrum* extracts were estimated 91.8 ± 17.3 and 109.5 ± 11.96 respectively (Figure 4). However, iron level in Hydro alcoholic extract of *Medicago sativa* and *Allium porrum* was reported 93.6 ± 13.8 and 117 ± 22.6 respectively (Figure 5).

In aqueous extracts iron content in *Medicago sativa* (100.5 ± 12.1) and in *Allium porrum* extracts (121.5 ± 8.1) was estimated (Figure 6).

A significant difference was found between iron concentrations in absence and presence of all extracts $p < 0.001$. Iron levels in normal and thalassemia significantly reduced when exposed with plants extracts especially in *Medicago sativa* of phosphate buffer $p < 0.001$. The analysis revealed statistically significant differences between *Medicago sativa* and *Allium porrum* extracts in terms of iron chelating potential in all extracts (phosphate, Hydro alcoholic and aqueous) $p < 0.001$. Iron concentration in serums controls after mixed with *Medicago sativa* phosphate buffer extract 66.2 ± 4.07 and *Allium porrum* phosphate buffer extract 75.2 ± 6.8 were reported (Figure 7).

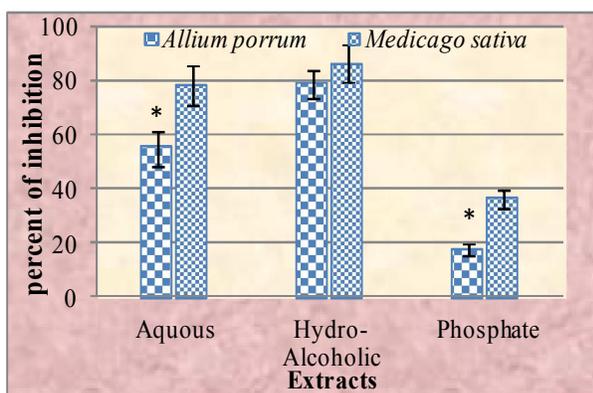


Figure 1: Total phenolic content in different extracts in *Medicago sativa* and *Allium porrum*.

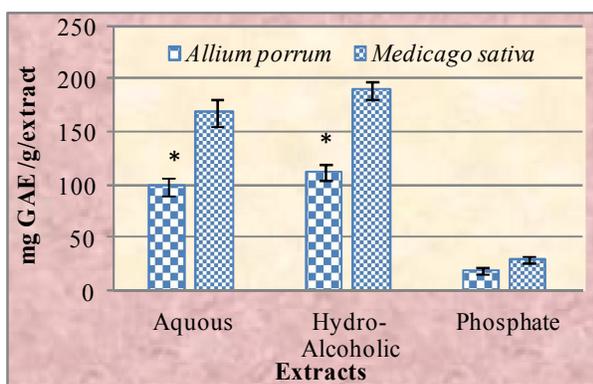


Figure 2: Antioxidant activity of different extracts in *Medicago sativa* and *Allium porrum*. * Statistically significant difference at ($P < 0.001$).

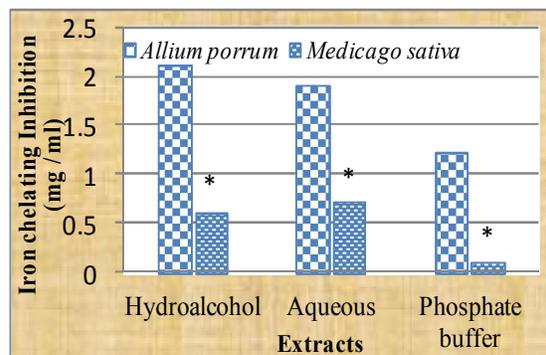


Figure 3: Iron chelating potential in different extracts in *Medicago sativa* and *Allium porrum*. * Statistically significant difference at ($P < 0.001$).

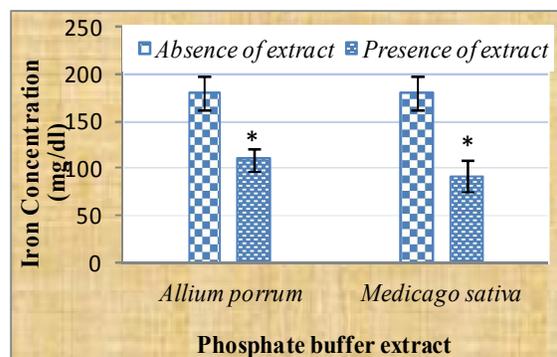


Figure 4: Iron content in thalassemia patient when expose with *Medicago sativa* and *Allium porrum* phosphate buffer extracts. * Statistically significant difference at ($P < 0.001$).

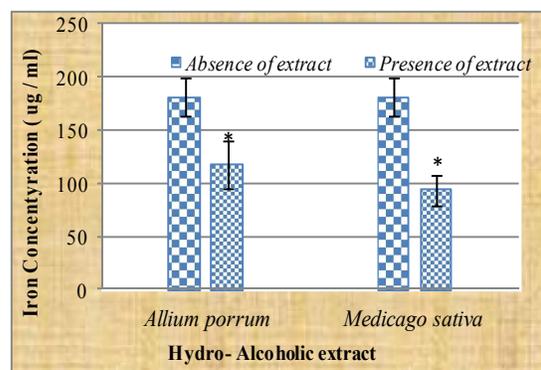


Figure 5: Iron content in thalassemia patient when expose with *Medicago sativa* and *Allium porrum* hydro-alcoholic extract. * Statistically significant difference at ($P < 0.001$).

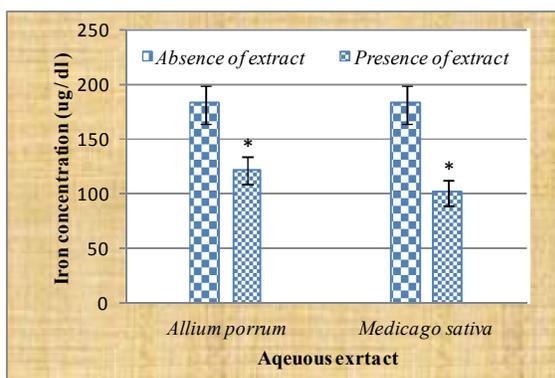


Figure 6: Iron content in thalassemia patient when expose with *Medicago sativa* and *Allium porrum* aqueous extract. * Statistically significant difference at ($P < 0.001$).

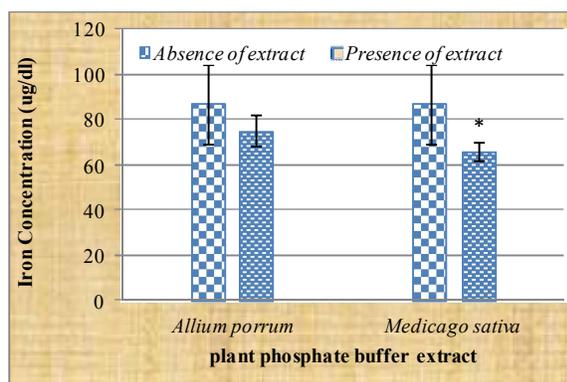


Figure 7: Iron content in serum controls when expose with *Medicago sativa* and *Allium porrum* phosphate buffer extract. * Statistically significant difference at ($P < 0.001$).

Discussion

Plants are rich sources of phytochemicals with antioxidant potential that mostly related with phenolic substances mainly flavonoids (Piett, 2000). In present work for introduce a drug with chelating potential; screening of iron chelating was carried out. For efficacy of plant chelators, different plant extracts were exposed with β -thalassemia and control serums. In present paper iron concentration in thalassemia patient serums was significantly reduced in presence of plants extracts compare to control serums $P < 0.001$. Chelating potential is associates with antioxidant activity or phytochemical content (Mira et al., 2002). The interaction between extract phytochemicals especially flavonoids and iron has been described by researchers in literatures (Mira et al., 2002).

In recent work it was considered a significant reduction rate in iron thalassemia by *Medicago sativa* extracts in buffer phosphate (49.5 %), aqueous (44.6%) and hydro –alcoholic (48.4%) $P < 0.001$. There was also a significant reduction rate in iron thalassemia by *Allium porrum* extracts in phosphate buffer (39.7%), aqueous (33%) and hydro –alcoholic (35.5%) were reported $P < 0.001$.

However, iron reduction rate in control serums was recorded 23.8 and 13.4 in presence of *Medicago sativa* and *Allium porrum* extracts respectively ($P < 0.001$). Iron level on thalassemia was more decreased than serum control which is important for treat of iron overload. This difference could be due to presence of high levels of free radicals in thalassemia patients. *Medicago sativa* extracts more affected iron concentration on thalassemia compare to *Allium porrum* extracts $p < 0.05$. This difference could be due to antioxidant potential and phytochemical contents.

There are many factors such as: plasma components, enzyme antioxidant, reducing substances, hormones, blood pH, body temperature that effect on iron chelating in living organisms. Our knowledge up to now not well known what is the fate of iron in presence of mentioned factors in blood. Thus, a new procedure is essential for determination of iron concentration in vivo state. In present paper *Medicago sativa* and *allium porrum* extracts were good chelator for iron reductin at in vitro state. This method represent a useful, cheap and more practical to treat of iron toxicity in different disorders especially β -thalassemia major if correlation will be present between in vitro and in vivo study. We believed this solution will aid researchers to find a novel drug for iron overload process.

In general, plants with high concentration of phenolic substances have a good iron chelating potential; hence this extract can be use as an alternate chelator for treat of thalassemia patient serum in future (Ebrahimzadeh et al., 2008d). We believed this solution will aid researchers to find a novel drug for iron overload process. Thus, we managed a study for effects of *Medicago sativa* and *allium porrum* extracts on iron overlaod that induced by iron dextran on rats.

Conclusion

Medicago sativa and *allium porrum* with high iron chelating activity was a good plant chelator for in vitro study. These extracts must be more study for treatment of iron overload in rats.

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