Iron Chelating potential of *Zataria multiflora* and *Matricaria chamomilla* on Thalassemic Serums *in Vitro* model.

Ali Mirzaei[1], Narges Nikpay Hossein abad[2], Mojtaba Abbasi[3], Reza Ghafarian Shirazi[4], Nooshin Mirzaei [1]*

[1]Medicinal plants Research Center, Yasuj University of Medical Sciences, Yasuj, Iran.
[2]Student committee research center,Yasuj University of Medical Sciences, Yasuj, Iran
[3]Veterinary Department, Science, Islamic Azad University, Shahrkord branch, Iran.
[4]Department of Chemistry, University of Eastern Finland, Joensuu, Finland.
*Corresponding Author: nooshin.mirzaee@yahoo.com

Abstract: Iron overload in β- Thalassemia major induced mostly by regular blood transfusions.The in vitro study was designated for Iron chelation and antioxidant activity of *Zataria multiflora* and *Matricaria chamomilla* plant extracts in thalassemic and healthy serums. **Materials and Methods**: Iron chelation, antioxidant activity, total phenol and flavonoids contents of *Zataria multiflora* and *Matricaria chamomilla* plant extracts were determined. For in vitro study of iron chelating, plant, extracts were exposed with serum thalassemic for 2h. Malondialdehyde (MDA) in blood serum and Superoxide Dismutase(SOD) in red blood cells were determined. Serum iron before and post expose with plant extracts was determined. **Results:** There was significant increase in iron concentration in β - thalassaemia major compare to control serums. Iron level in thalassemic and control serums were reported 178 ±8.2 and 89 ± 12 respectively. Iron concentration in thalassemic serums after exposed with *Z.multiflora and M. chamomilla* extracts in 2 hour incubation at in vitro state were decreased 27 and 33 % respectively. However, iron content in serum controls after expose with *Z. multiflora and M. chamomilla* extracts were decreased 16.8% and 23.6 % respectively. **Conclusion:** MDA and iron in blood serum and SOD in erythrocyte of thalassemic samples was significantly higher than healthy persons. However serum vitamin E was lower than healthy person.
[Ali Mirzaei, Narges Nikpay Hossein abad, Mojtaba Abbasi, Reza Ghafarian Shirazi, Nooshin Mirzaei. **Iron Chelating potential of *Zataria multiflora* and *Matricaria chamomilla* on Thalassemic Serums *in Vitro* model.** *Life Sci J* 2013;10(9s): 230-235](ISSN:1097-8135). http://www.lifesciencesite.com. 30

Key words: β- Thalassemia major,iron chelating, *Zataria multiflora, Matricaria chamomilla*

1.Introduction

β- Thalassemia major is a hereditary hemolytic disease that displays by severe anemia in early of life (Weatherall, 1981). The disorder affects approximately 150 million persons in the world (WHO, 1983) and it common in Southeast Asia particularly in Mediterranean populations (Flint, 1998). Hemoglobin is a hemo - chromo protein in erythrocytes which compose of two alpha and two beta subunits with iron for O_2 and CO_2 transport in center. In β-thalassemia major, precipitates of highly unstable free α-globin was occurred due to absent of β-globin chain and result in, release of reactive iron was formed (Scott,86). Hemosiderosis or iron overload also induced via regular blood transfusions and increased of iron absorption in intestines (Milena, 2010). Iron metabolism in human has uni-directional, because unable to eliminated by excretory route. Therefore, excess of iron ion deposited in vital organs such as heart, liver, spleen and endocrine organs. Iron toxicity in major β-thalassemia produces some complications including heart, liver disease, diabetes and hypothyroidism, finally early death (Taher, 2006; Rund, 2005; Loukopoulos, 2005). In addition, iron deposition cause generation of a variety of free radicals such as superoxide, hydroxyl and hydrogen peroxide. Oxidative stress formed when production of free radicals exceeds, the ability of antioxidants defense systems to neutralize of these free radicals (Milena, 2010; Repka et al. 1993). Iron chelators or iron binders are agents that used for removing iron in iron overload or Iron toxicity by iron excretion through the urine or stool route. Generally, iron chelation is applied after 10-20 transfusions practice or when serum ferritin concentration was upper 1000 ng /ml (Thalassemia International Federation, 2008).Iron chelating assay can used in vitro condition for evaluation of plant chelator activity. In this test chelator property is depending to phytochemical component particularly flavonoids content or antioxidant activity (Gupta et al., 2011). Recently, deferoxamine and deferiprone are useful drugs which can be applied for treat of iron chelation in thalassemia major. Moderate Morbidity and mortality rate of these drugs in patient who that receive regular prolonged infusions are reported in literature (Borgna-Pignatti and Galanello, 2004; Yasser et al., 2008). Now, there is no effective and choice drug in the clinic for the treatment of iron overload in thalassemia, except chelation therapy is the only practice which increases quality of life.So many

scientists used variety of medicine and plant products as a plant chelator or antioxidant for the iron excretion and inhibited of oxidative stress in thalassemic patient respectively. The Present in vitro study was designated for iron chelalation and antioxidant activity of *Zataria multiflora* and *Matricaria chamomilla* in thalassemic serums. *Zataria multiflora* Boiss belongs to *Lamiaceae* family and is native to Iran which has displayed significant antioxidant activity. In herbal folk medicine its essential oil has been suggested for treat of respiratory and gastrointestinal disease and anticoagulant therapy. Total phenol and flavonoids was the major phytochemical component in literature (Derakhshanfar et al. 2011). German chamomile *(Matricaria chamomilla* belongs to the family compositae (*Asteraceae*) has a widespread usage. In herbal medicine its infusion preparations are used for treat of some diseases. Particularly inflammatory and digestive disorders. Active phytochemical components are reported phenolic compounds and essential oils (Ghavami et al., 2012).

2.Material and Methods

2.1.Collection and extraction of plant material

Aerial parts of *Matricaria chamomilla* (Asteraceae) and *Zataria multiflora(*miaceae) were collected in Yasuj Iran. Authentication samples were carried out and a voucher specimen was deposited in the biochemistry laboratory, Yasuj University of Medical Sciences, Yasuj, Iran.The aerial parts were washed, shade-dried, and the aqueous extractions was carried out by maceration method. Extraction was concentrated and dried using a rotary evaporator (Heideolph model 4000; Germany). The dried extract was kept in the refrigerator for further studies.

2.2.Examination on Plant Extracts

2.2.1.Determination of total phenolic compounds

The total phenolic contents of extracts were determined using Folin-Ciocalteau method with some modifications. Total phenol was expressed as Gallic acid equivalent (GAE) / g extract (Karim et al.,2011).

2.2.2.Determination of total flavonoid content

The total flavonoid content was determined with aluminum chloride. The total flavonoid values were determined in terms of rutin equivalents/g extract (Kosalec et al., 2004).

2.2.3.Antioxidant activity of Dipheny-picrylhydrazyl (DPPH)

The antioxidant activity of extract assessed with little modification. Percent of inhibition was calculated as follow: % Inhibition = $[(A_0 - A_1)/A0] \times 100$. A_0 is the absorbance of control and A_1 is the absorbance of the plant extracts (Mirzaei and Mirzaei, 2013).

2.2.4.Metal chelating activity

The chelation of Fe^{2+} ions by extracts was determined using modified method of Dinis (Dinis et al., 1994). IC_{50} or Inhibition concentration in 50% also was calculated. IC_{50} is the maximal concentration of extract to cause 50% inhibition of free radicals activity. The serum concentration of malondialdehyde (MDA), were estimated by a thiobarbituric reaction (Satoh, 1979). The SOD enzyme activity in red blood cell was measured by Kajari Das' method based on superoxide radicals (Das, 2000). Vitamin-E in serum was measured based on oxidation of α tocopherol by ferric ions. (Mehbooali and perwaiz iqbal 2008).

2.2.Examination on thalassemic and control serums

This study was carried out on 140 subjects, who included 70 age and sex matched (30 males and 40 females) healthy controls and 70 (28 males and 42 females) β- thalassemia major patient, who were previously diagnosed by electrophoretic tests. All the patients were taken blood transfusion monthly and under the regular observation of medical specialists during these period. Thalassemic patient serums with history of heart and thyroid diseases, hypertension, and diabetes mellitus were excluded from the research. After sign a written consent and well explanation about the study for all the subjects, a total of 5ml of blood was collected. One ml blood with anticoagulant.

2.3.Ethylenediamine tetra acetic acid

(EDTA) was selected for hemoglobin and activity SOD assay and 4 ml blood without anticoagulant was collected for estimation of MDA, iron and vitamin-E. For preparation of red blood cell (RBC) and serum the blood samples were centrifuged at 2500 rpm for 10 min. The separated serum was deposited in fridge at -20^0C. The estimation of iron was carried out according to accepted local clinical chemistry standards using Pars Azemon local company kits. Serum iron carried out on thalassemic patient and control groups in presence and absence of plant extracts. The effect of aerial part extracts by *Zataria multiflora* and *Matricaria chamomilla* were estimated as plant chelator on serums iron.In a test tube 0.5 ml of extracts at concentration (4mg/ml) was exposed with 0.5 ml thalassemic and control serum for 120 min in 37 ^{0}C. Iron analysis was carried out after centrifuge at 1500 rpm for 10 min, as iron after expose and compare with iron level before expose with plant extracts.

2.4.Statistical analysis

All data were reported as mean ± SD. Analysis of variance was used for the comparison of data. $P<0.05$ was recognized as significant while $p<0.001$ was considered as highly significant.

3.Results

The total phenolic concentration of the plant extracts were measured using the Folin–Ciocalteau method. The Z. multiflora extract had the highest total phenolic and flavonoids contents (214 mg gallic /g extract) and 23.3.2mg Rutin/g extract respectively. Total phenolic and flavonoid contents *M. chamomilla* extract reported 81.3 mg gallic/g extract and 10.6mg Rutin /g extract respectively (Table 1).

The maximum chelating activity (% of inhibition) *of M. chamomilla* extracts (71.8) was reported as higher than that of *Z. multiflora* extracts (50.33%) in concentration at 4 mg/ml.The IC_{50} values of *M. chamomilla* and *Z. multiflora* for Chelating activity showed 1.25±0.08 and 2.8± 0.0.1 mg/ml respectively (Table 1). The DPPH free radical scavenging activity in *M. chamomilla* (65 %) and *Z. multiflora* extract (88 %) was reported in concentration 4 mg/ml (Table 1).

The patients were aged between 1-18 years and the average hemoglobin concentration ranged between 6.5 – 8.9 gm/dl (data not shown).MDA and iron in serum and SOD in red blood cell of thalassemic serums were significantly higher than healthy person ($p<0.001$).However serum vitamin E were lower than healthy person $p<0.001$) (Figure 1). In this study, it was reported that there was a significantly increased in iron concentration in β - thalassaemia major compare to control $p<0.001$.

Z. multiflora and *M. chamomilla* extracts were expose with serum of thalassemic patient in vitro state. A significant decrease was reported in iron thalassemic serum in presence of extracts after 120 min at 37 ^{0}C, compared to the iron thalassemic serum in absence of extract $p<0.001$. Iron concentration in thalassemic and control serum was reported 178 ±8.2 and 89 ± 12 respectively. Iron levels in thalassemic serums after combination with Z. multiflora and M. chamomilla extracts were decreased 27 and 33 % respectively. However, iron content in serums controls after expose with Z. multiflora and M. chamomilla extracts were decreased 16.8% and 23.6 % respectively (Figure 2) (Figure 3).

Table 1: Antioxidant activity and Total phenol and flavonoids contents of *Zataria multiflora* and *Matricaria chamomilla Zataria multiflora, Matricaria chamomilla.*< Iron Chelating (IC) Dipheny-picrylhydrazyl (DPPH**),** Total phenol(TP), IC50=Inhibitory concentration $_{50}$, Data are expressed as mean ± S.D; (n = 3)

Plant name	IC	TP mg GAE/g extract	Flavonoids mg Rutin/g extract	DPPH % Inhibition / mg extract	IC_{50} Iron Chelating
Z. multiflora	50.3 ± 4.6	214 ±11.3	23.3 ±1.3	88 ±11	2.8
M.chamomilla	71.83± 5.7	81.3 ±8.4	10.6 ±1.1	65 ±11.5	1.25

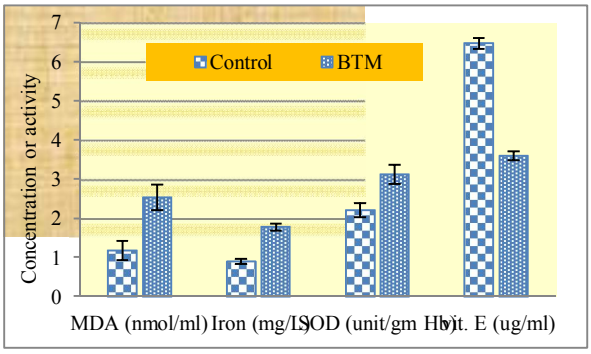

Figure 1: Antioxidant activity and iron concentration in thalassemic patient (Data was shown as mean ± SD). (n =70 male and female) and control (70 male and female) serums). Malondialdehyde (MDA), were estimated by thiobarbituric reaction, the superoxide dismutase (SOD) enzyme activity in red blood cell was measured based on superoxide radicals, Vitamin-E in serum was estimated based on oxidation of α tocopherol by ferric ions and iron by local Iranian kit on serum was analyzed. The values of MDA, iron and SOD in thalassemia serums was significant difference compare to control serum ($P<0.05$). BTM= Beta thalassemia major.

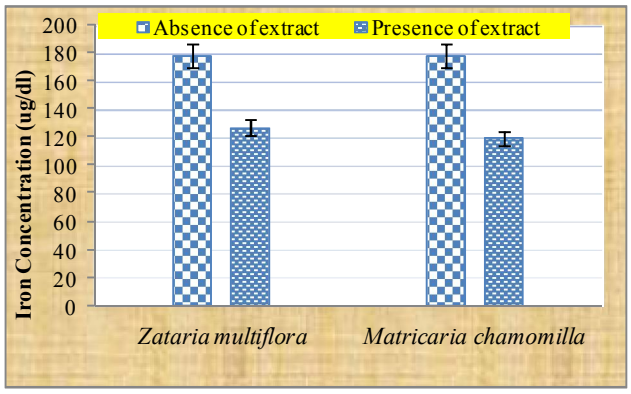

Figure 2: comparing of iron concentration in thalassemic serum samples in absence and presence of aqueous plants extracts. In a test tube 0.5 ml of extracts at concentration(4mg/ml) was exposed with 0.5 ml serum of thalassemic patient for 120 min in 37 ^{0}C. In end of reaction after centrifuge at 1500 rpm, iron analysis was carried out as iron after expose and compare with iron level before expose with plant extracts.

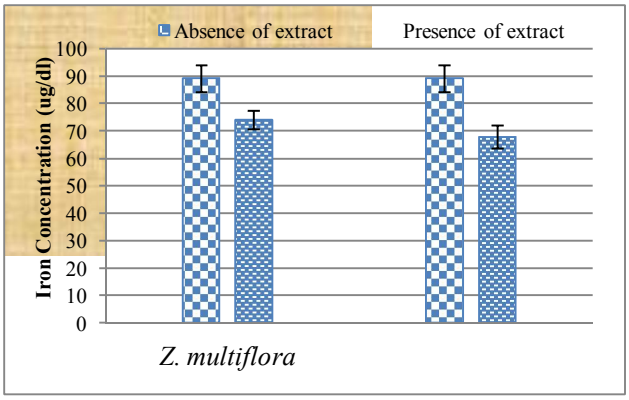

Figure 3: Comparing of iron concentration in control serum samples in absence and presence of plants extracts. In a test tube 0.5 ml of extracts at concentration (4mg/ml) was exposed with 0.5 ml serum of thalassemic patient or control for 120 min in 37 ^{0}C. In end of reaction after centrifuge at 1500 rpm, iron analysis was carried out as iron after expose and compare with iron level before expose with plant extracts.

4.Discussion

In β -thalassaemia major, reactive oxygen species are mostly formed by deposited iron ions. Iron excess in reactive form is major complication which increased by precipitation of unstable free α-globins, repeated blood transfusions and intestinal iron absorption (Milena, 2010; Scott, 1986). *Z. multiflora* and *M. chamomilla* as iron chelators in present study significantly removed iron in thalassemic serums by forming soluble and stable (interactions with flavonoids after incubation(Mira et al., 2002). In present study chelation therapy was significant reduced iron content (30%) in thalassemic patient serums compare to control P<0.05. chelating potential was depends on antioxidant activity or flavonoid structures (Mira et al., 2002). The reaction between flavonoids compounds and iron metal has been reported in literatures (Mira et al.,2002).Chelation therapy decreases iron-related complications in iron overload state and thus increases quality of life (Hebbel et al., 1990).

In recent study it was found a significant difference in rate of iron removed in thalassemic patient (27 and 33%) and control serums (16.8 and 23.6) by *Z. multiflora* and *M. chamomilla* plant extracts respectively(P<0.05). Iron chelating on thalassemic serums more affected than serum control which essential key role for treat of iron toxicity.

This mechanism is may be due to high level of free radicals, MDA marker and low activity of non- enzymatic antioxidant such as vitamin E in blood thalassemic patient. Medicinal plants are rich sources of natural phytochemicals with antioxidant activity that mainly associated with phenolic compounds particularly flavonoids (Piett, 2000).

Generally, plant extracts with high levels of phenolic compounds revealed a good iron chelating; therefore such kind of herbal extract can be use as a choice chelator for treatment of thalassemic patient in future (Ebrahimzadeh et al., 2008d). Flavonoids are a major class of phenolic compounds with hydroxyl functional group in their structures. They have antioxidant activity due to free radical termination activity and therefore great important on human nutrition (Kessleret al., 2003).It is well known that radical scavenging of flavonoid substances primarily depended to hydroxyl position in molecule (Mensor et al., 2001; Hou et al., 2003). In present research moderate relationship was reported between chelating property and the phenol and flavonoid compounds, however, in some studies there were a direct association between chelating potential and phenol and flavonoid contents was described. Some plants with high concentration of phenol and flavonoid displayed very weak chelating potential in literature (Ghasemi et al., 2009; Nickavar et al., 2007). In another study in *Tetracarpidium conophorum* plant extract chelating capacity was significantly related to their total phenol and total flavonoid content (Olabinri et al.,2010). Malondialdehyde (MDA) is a marker for estimation of lipid peroxidation process, tissue damage and oxidative stress which widely induced in mammalians. MDA concentration increased in excess amounts in thalassemia due to iron excess in erythrocytes (Diczfalusy et al., 1997). In present study increased of plasma MDA level (106%) was reported in beta-thalassemia patients compare to control. Antioxidant activity is a significant factor of tissue damage, particularly in patients with increased oxidant stress (Patric et al., 2006).Vitamin E is a lipid soluble antioxidant with chain breaking property and powerful peroxyl radical scavenger (Attia et al., 2011).Investigators were found important differences in antioxidant potential in terms of tocopherol activity. In present research similar some study, vitamin E was decreased in thalassemic serum compare to control p <0.05 (De Luca et al., 1999; Kassab-Chekir et al.,2003).The decrease of Vitamin E(53%) was been found due to increased of MDA and free radicals concentration (Attia et al., 2011).

In addition, activity of SOD significantly increased (41%) in thalassemia patient compare to control p<0.05). Present findings supported by Sonali et al who observed that the increased of SOD in β –thalassemia major (Sonali et al.,2011).High level of erythrocyte SOD activity in β –thalassemia major is a compensatory response due to lipid peroxidation and

production of younger red blood cells.The repeated blood transfusion was responsible for synthesis of immature erythrocyte (Gupta et al., 2011).

Conclusion

According to present data, *Z. multiflora* and *M. chamomilla* with high levels of antioxidant activity and iron chelating potential was a good plant chelator for in vitro studies. These extracts maybe use as a choice for treatment of iron overload in thalassemic patient or iron toxicity as a new hypothesis for iron excretion.

Acknowledgment

The author deeply acknowledges administration of Yasuj Blood transfusion and Yasuj Shahid Behshty Hospital for allowing collecting the samples and funding present research**.**

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7-28/2013