IJPSR (2016), Vol. 7, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 April, 2016; received in revised form, 27 May, 2016; accepted, 08 August, 2016; published 01 September, 2016

SALVIA VIRGATA JACQ. AND *SILIBUM MARIANUM* L. GAERTN DISPLAY SIGNIFICANT IRON-CHELATING ACTIVITY

Mohammad Ali Ebrahimzadeh[1], Masoumeh Khalili *[2], Mohammad Azadbakht [1] and Masoud Azadbakht [1]

Pharmaceutical Science Research Center [1], School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
Golestan Research Center of Gastroenterology and Hepatology (GRCGH) [2], Golestan University of Medical Sciences, Gorgan, Iran.

Keywords:

Silibum marianum; *Salvia virgata*; Iron overload; Desferrioxamine; Ferozine; Liver

Correspondence to Author:
Masoumeh Khalili

Golestan Research Center of Gastroenterology and Hepatology (GRCGH), Golestan University of Medical Sciences, Gorgan, Iran.

Email: mkhalili_ps@yahoo.com

ABSTRACT: In some disease like thalassemia major long-term transfusion alone inexorably produces the clinical problem of iron overload. Danshen and Milk thistle has been widely prescribed in traditional folk medicine for treatment of liver diseases. In this study we selected eight medicinal plants whose efficiencies had been previously demonstrated in treatment of liver diseases in ancient medicine were studied for their *in vitro* chelating activity. After preliminary evaluation of these plants *in vitro* condition, two most efficient plants were nominated for further evaluation in *in vivo* condition on iron-overloaded mice. The extracts with high iron chelation activity were injected intraperitoneally. Liver sections were stained by haematoxylin and eosin and Perls' stain. Danshen and Milk thistle had maximum iron chelation activity at *in vitro* condition. The iron-overloaded mice treated with the extracts showed a significant decrease in plasma iron level and in plasma Fe^{3+} content. Perl' stain improved sensitivity of the test significantly in order to determine the low amount of deposited iron in the liver of iron-overloaded mice treated by the extract. As evidenced by H&E staining, lever sections treated with the extracts displayed a significant decrease in extent of necrotic hepatocytes, fibrous tissues and pseudo lobules. Danshen and Milk thistle extracts exhibited satisfactory potency regarding chelating of excessive iron in iron-overloaded mice.

INTRODUCTION: Long-term treatment with red-cell transfusion effectively prevents stroke and other complications of sickle cell anemia1and can sustain patients with chronic congenital and acquired refractory anemia, including thalassemia major, Diamond–Blackfananemia, myelodysplastic syndromes, myelofibrosis, aplastic anemia, and other disorders [1, 2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(9).3756-63

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (9).3756-63

Long-term transfusion alone inexorably produces the clinical problem of iron overload. In patients with thalassemia who undergo transfusion from infancy, iron-induced liver disease and endocrine disorders develop during childhood and are almost inevitably followed in adolescence by death from iron-induced cardiomyopathy.

In patients with sickle cell anemia, although iron-induced complications appear to develop later, eventually, liver disease with cirrhosis as well as cardiac and pancreatic iron deposition can develop [1]. Iron plays a pivotal role in oxidative reactions, resulting in formation of hydroxyl free radicals from peroxidases during Fenton or Haber-Weiss

chemistry reaction [3, 4], which are involved in development of cardiovascular diseases. Iron - chelating compounds can circumvent this process [5]. In the Fenton reaction, the hydroxyl radical ($OH^{\bullet}$) is produced from hydrogen peroxide (H_2O_2):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

The superoxide radical ($O_2^{\bullet-}$) acts to reduce ferric iron, which then reacts with hydrogen peroxide by Fenton chemistry to yield the hydroxyl radical:

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$$

Net reaction: $O_2^{\bullet-} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^{-}$ Fe catalyst

Desferal (deferoxaminemesylate (DFO)) and Deferiporine are two drugs extensively used to chelate iron in patients with thalassemia major; however, their significant side effects has made researchers to consider natural compounds, which relatively exhibit lower degrees of deleterious side effects [5, 6]. Chelating capacity has been reported for numerous plants and mushrooms, Including *Echium vulgare* L. and *Echium rubrum* L. [7], *Quercusinfectoria* [8], *salvia*sp [9, 10, 11, 12, 13], *Glycyrrhiza glabra* L. [14], *Origanum vulgare* L. [12, 15], *Citrus* sp[16, 17], *Diospyroslotus* [18, 19, 20], *Silybum marianum* [21, 22, 23, 24] and *Cantharellu scibarius* [25, 26, 27] and *Pleurotusporrigens* [26, 28]. Because of their potentially lower risk for human, herbal extracts are of great interest.

Eight medicinal plants whose efficiencies had been previously demonstrated in treatment of liver diseases in ancient medicine were studied for their *in vitro* chelating activity (**Table 1**). After preliminary evaluation of these plants *in vitro* condition, two most efficient plants were nominated for further evaluation in *in vivo* condition on iron-overloaded mice.

MATERIALS AND METHODS:

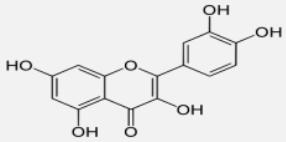
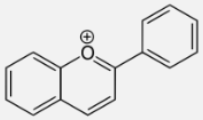
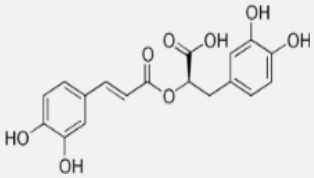
Chemicals and standards: Methanol (MeOH), Dichloromethane, Hexane and ferrozinewas obtained from Merck Co. (Darmstadt, Germany). Distilled deionized water was prepared by Ultrapure™ water purification system.

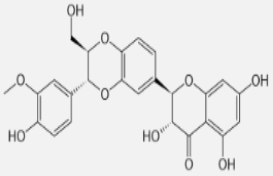
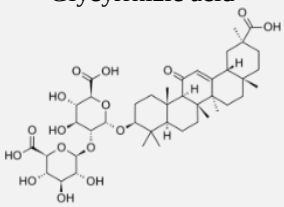
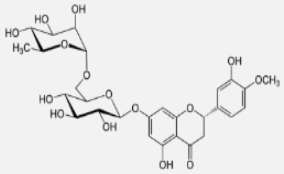
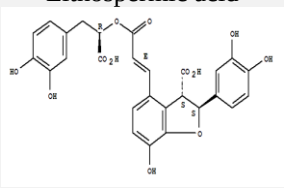
Plant materials:

The species were identified by Dr. Masood Azad bakht and deposited in the School of Pharmacy herbarium of Mazandaran University of Medical Sciences. Details on the selected plants and their herbarium numbers are given in **Table 1**.

The samples were transported to the laboratory and were dried in the oven at 45-6 °C for 48 hour.

TABLE 1: THE NAMES AND CHARACTERISTICS OF A NUMBER OF MEDICAL PLANTS WITH IRON-CHELATING ACTIVITY

Sample name	Coll. dates &Localities	Herb. numbers	Tissue	Effective compound	Extraction method	Ref
Quercuscasta neifolia	October 2012, Mazandaran province, forest of Sari	H 1032	Fruit	Quercetin 	Hot distilled water (3 h)	[8, 29]
Echiumamoen um	July 2013, Mazandaran province, Local market of Sari	K 1043	Flower	Antosianin 	Ethanol 96% (maceration)**	[7]
Origanumvul gare L.	July 2013, Local market	H 1260	Shoot	Rosmarinic acid 	Ethanol 50% (maceration)**	[12, 15, 30]
Silibummaria num L.	Jun 2013, Mazandaran province, forest of Sari	J 2012	Seed	Silybin	Methanol 80% (Soxhlet)***	[21, 22, 23, 24,

Gaertn						31
Glycyrrhiza glabra L.	July 2013, Local market	K 1044	Root	Glycyrrhizic acid 	Distilled Water 50°C (maceration)*	32
Citrus bigaradia	November 2012, Mazandaran province, Local market of Sari	H 1033	Peel	Hesperidin 	Methanol 80% (maceration)**	16, 17
Salvia virgata Jacq.	Jun 2013, Mazandaran province, forest of Neka	F 2031	Shoot	Lithospermic acid 	Dichloromethane (maceration)**	9, 10, 11, 13, 33
Diospyros lotus	October 2012, Mazandaran province, forest of Sari	K 2011	Fruit	---	Distilled water (maceration)**	18, 19, 20

* Materials were extracted for 72 h, repeated three times.
** Materials were extracted for 72 h at room temperature, repeated three times.
***Materials were delipidized using hexane in a decanter at room temperature. After delepidization, the sample was excluded from hexane, allowed to dry for six h, transferred to decanter and subjected to methanol 80% for 72h at room temperature to obtain its hydroalcohol extract.

Plant Extracts:

Plant extracts were prepared as described in **Table 1.** Extracts were concentrated using a rotary evaporator (under reduced pressure at 40°C) to obtain crude solid extracts, which then freeze-dried (MPS-55 Freeze-drier, Operon, South Korea) to completely remove solvents [26].

Metal Chelating Activity Assay:

To assay metal chelating activity, 0.5 ml of 2 mM $FeCl_2$ solution was added to 1 ml of each fraction (800 µg mL^{-1}). The reaction was initiated by adding 5 mM ferrozine (0.2 mL), and the absorbance of the solution was measured by spectrophotometer at 562 nm. EDTA was used as a standard. The ratio of inhibition of ferrozine–Fe^{2+} complex formation was calculated using the following equation:

$$I\ (\%) = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of fraction [26].

Animals and experimental design:

Thirty five male mice strain NMRI (20- 25g) were purchased from Pastor Institute (Amol, Iran) and housed in polypropylene cages at an ambient temperature, 23 ± 1°C and 45-55% relative humidity, with a 12 h light: 12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water. Experiments were conducted between 8:00 and 14:00 h. All the experimental procedures were conducted in accordance with the NIH guidelines of the Care and Use of Laboratory Animals. The mice were divided into 5 groups as: (A) control, (B) iron-overloaded, (C) DFO, *S. virgata* and (D) *S. marianum*. To induce iron overloading, the mice were given 100 mg/kg/day iron by *i.p.* injections of iron dextran with frequency of 5 days a week for 4 subsequent

weeks and then left for 1 month to equilibrate the excessive iron. Mice in the DFO group, as the positive control, were given DFO *i.p.* injections of iron dextran with frequency of 5 days a week for 4subsequent week with a dose of 1mg/kg/day. To evaluate the iron-chelation capacity of plant extract, the extracts were applied as *i.p.* injection with a frequency of 5 times a week at concentrations of 200 mg/kg/day for 4 weeks. Normal saline instead was given to the control iron-overloaded group, with the same frequency and duration [9, 34, 35].

At the end of the experiment, the mice were euthanized by diethyl ether. Blood samples obtained directly from the heart chamber of the anaesthetized mice. Their plasma was separated. Livers were removed and preserved in 10% buffered formalin specified for histopathological study.

Determination of iron in plasma by atomic absorption spectroscopy:

The iron content was determined by atomic absorption spectroscopy at 248.3 nm using an air/acetylene flame (Perkin–Elmer AAS 100 Wellesley, MA). Iron standard solutions for calibration were prepared from single-element stock solutions (Merck, Darmstadt, Germany) in 0.2% (w/v) nitric acid. The mice plasma samples were analysed directly after 5 times dilution ultrapure water for iron contents [35, 36]. Iron was expressed as µg/L.

Determination of plasma ferric ion (Fe^{3+}):

Plasma Fe^{3+} content was determined by kit (ZiestChem, Iran). Ferric content was expressed as µmol/L.

Histology:

Formalin-fixed liver specimens for histology were embedded in paraffin wax. Tissue sections were cut at 5µm and stained by haematoxylin and eosin (H&E) and Perls' stain.

Statistical analysis:

Statistical analysis was performed using GraphPad Prism 5 for Windows. The results are expressed as Means ± SD. One way analysis of variance (ANOVA) was performed. Newman-Keuls multiple comparison test was used to determine the differences in means. All *P* values less than 0.05 were regarded as significant.

RESULTS:

Ferrous ion (Fe^{2+}) Chelating Activity Assay:

The chelating activity was measured by monitoring the colour reduction of the red Fe^{2+}/ferrozine complex. The highest iron inhibition was found in *S. virgata* and then in *S. marianum*, 94.28.13±0.53 and 84.49±0.69 %, respectively (**Fig. 1**).

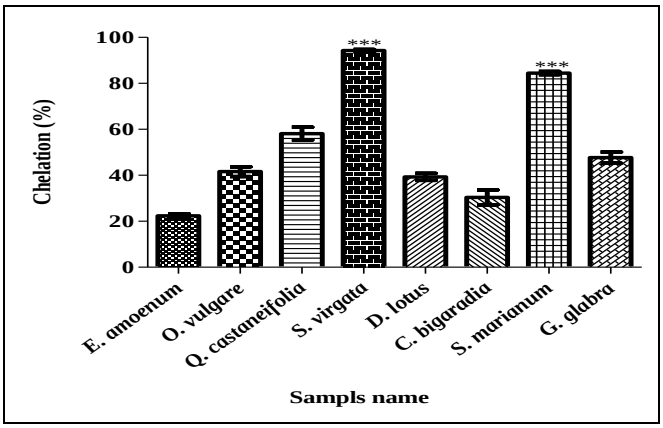


FIG. 1: PERCENT IRON CHELATION ACTIVITY OF SAMPLES AT CONCENTRATION 800 µg ml^{-1}EDTA WAS USED AS CONTROL (IC_{50} =7.93±0.09 µg ml^{-1}). (EACH VALUE IS THE MEAN OF THREE REPLICATE DETERMINATIONS ± SD).

Content of total iron in the plasma of iron overloaded and control mice:

Fig.2 presents the plasma Total plasma iron content (µg/l) (registered with Atomic Absorption Spectroscopy) in different treatments.

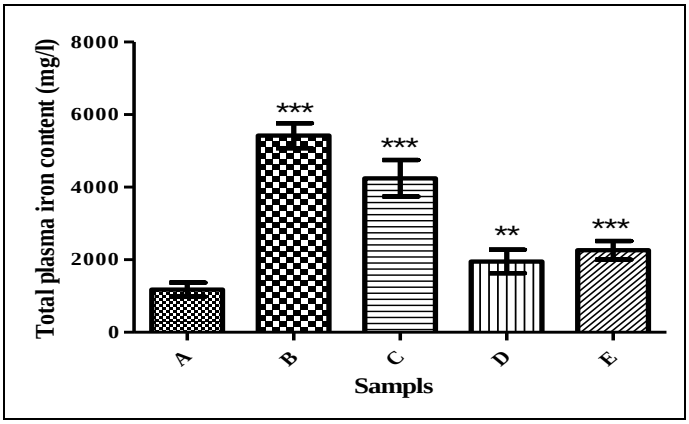


FIG. 2: TOTAL IRON PLASMA IRON CONCENTRATION THAT WAS DETERMINED BY ATOMIC ABSORPTION ((A) CONTROL GROUP, (B) IRON OVERLOADED GROUP, (C) DFO GROUP, (D) *S. MARIANUM* EXTRACT GROUP AND (E) *S. VIRGATA* EXTRACT GROUP) (MEANS ± SD, *P*<0.05)

The iron overloaded group displayed the maximum iron absorbance (5420±346.3µ/l), showing a significant difference with the control group ($p< 0.001$) as well as with the other treatments (*S. virgata* extract, *S. marianume* and DFO). There was no significant difference between the *S. Virgata* extract and *S. marianume* groups, while both displayed significant differences with the DFO group ($p< 0.001$)

Plasma level of Fe^{3+} in iron- overloaded mice: The plasma level of Fe^{3+} have been found to increase in iron-overloaded mice, rising from 38.48±12.32µmol/L in control group to 120.1±10.64µmol/l in iron overloaded mice, indicating a significant increase at statistical level of $P< 0.001$. There was a significant difference in the plasma iron content between the DFO and the iron-overloaded mice, but not between the DFO and the control groups. In addition, plasma iron content was found to decrease upon treatment with the extracts of *S. marianum* and *S. virgata*, showing significant differences with the control group (**Fig.3**).

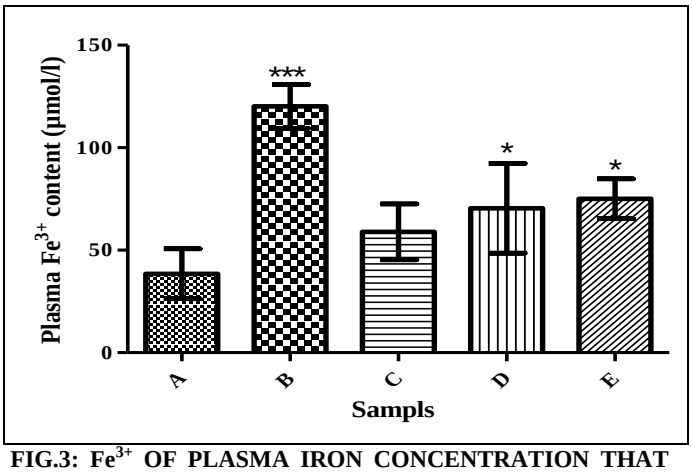


FIG.3: Fe^{3+} OF PLASMA IRON CONCENTRATION THAT WAS DETERMINED BY IRON KIT ((A) CONTROL GROUP, (B) IRON OVERLOADED GROUP, (C) DFO GROUP, (D) S. MARIANUM EXTRACT GROUP AND (E) *S. VIRGATE* EXTRACT GROUP) (MEANS ± SD, P<0.05)

Study of morphological changes:

(1) Morphological changes of the liver observed by H&E staining: Observation of H&E stained liver sections under an optical microscope showed that the hepatocellular plates in the control group were spoke wheel shaped with the central veins the center and distributed radially outward (**Fig. 4A**). H&E- stained liver tissues of iron overloaded mice are shown if **Fig. 4B**. Periportal and parenchymal inflammation, hepatics sinusoids elongation, central veins hyperemia, increased number of brown pigments surrounding the port, and focal necrosis are observed in liver parenchymal tissues. The result of H&E staining indicated that periportal inflammation, focal necrosis and genesis of brown pigments occur in the mice receiving DFO as well, but in less degree than that observed in iron-overloaded mice (**Fig. 4C**).

Fig. 4-D and **E** indicate the results of H&E staining of mice liver tissues treated with the extracts of *S. marianum* extract and *S. virgata* extract, respectively. Reduced inflammation occurs in both groups; however, a trivial inflammation happens in some portal areas.

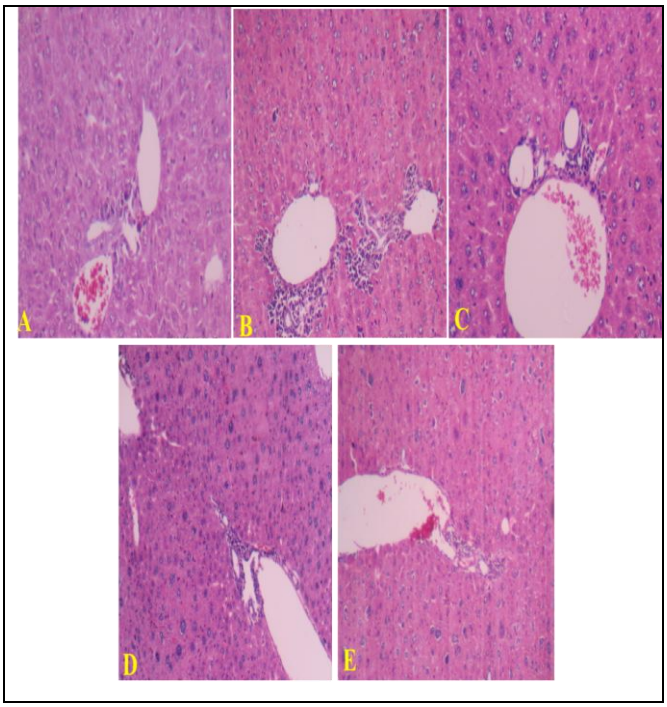


FIG. 4: EFFECTS OF EXTRACTS ON IRON DEPOSITION IN MOUSE LIVER WITH H&E. REPRESENTATIVE MICROSCOPIC PHOTOGRAPHS OF LIVERS STAINED WITH H&E (MAGNIFICATION 100×). ((A) CONTROL GROUP, (B) IRON OVERLOADED GROUP, (C) DFO GROUP, (D) *S. MARIANUM* EXTRACT GROUP AND (E) *S. VIRGATA* EXTRACT GROUP)

(2) Morphological changes of the liver observed by Prussian blue staining: Fig. 5 displays the results of Prussian blue staining of liver tissues in different groups. No iron accumulation is observed in the control group (**Fig. 5A**). Periportal inflammation, hepaticsinusoids elongation, central veins hyperemia, accumulation

of iron pigments around the port and parenchymal tissue, and focal necrosis in liver parenchymal tissue occur in iron- overloaded mice (**Fig.5B**). As seen in **Fig. 5C,** DFO treatment decreases the iron accumulation in liver tissue. In comparison to the iron-overloaded group, the mice treated with the extracts of *S. marianum* and *S. virgata* show striking decrease in iron content (**Fig. 5D** and **5E**). The minimum iron accumulation, and therefore the minimum tissue injury, occurs in *S. virgata* treated group (**Fig. 5E**).

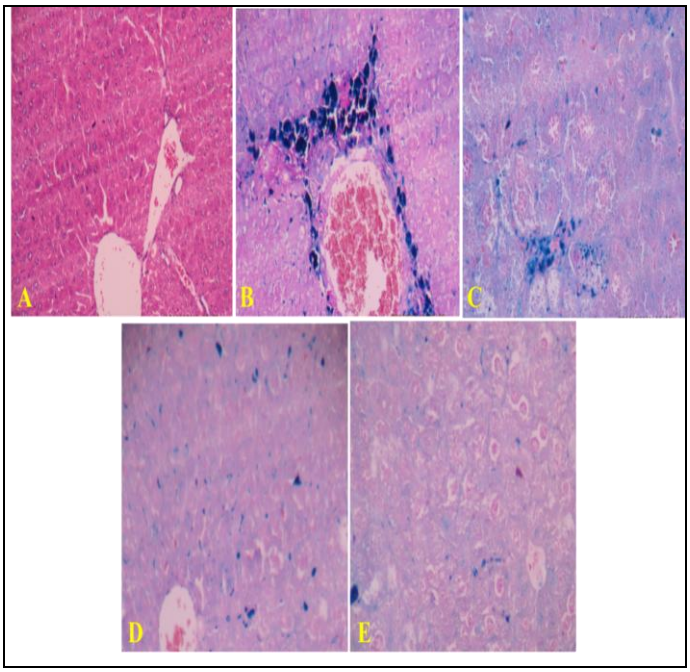


FIG.5: EFFECTS OF EXTRACT ON IRON DEPOSITION IN MOUSE LIVER. REPRESENTATIVE MICROSCOPIC PHOTOGRAPHS OF LIVERS STAINED WITH PRUSSIAN BLUE (MAGNIFICATION 100×). ((A) CONTROL GROUP, (B) IRON OVERLOADED GROUP, (C) DFO GROUP, (D) *S. MARIANUM* EXTRACT GROUP AND (E) *S. VIRGATA* EXTRACT GROUP)

DISCUSSION: Based on the history of Danshen and Milk thistle in treatment of liver diseases in ancient medicine in European and Asia countries, and also for their approved anti-inflammation capacities, we evaluated the iron chelating activities of these plants in iron-overloaded mice [9, 10, 13, 24, 37, 38]. Excess iron level in plasma has been reported to cause free radical production, lipid peroxidation and oxidative damages, resulting in iron precipitation and necrosis in liver [9, 13]. In this study, we evaluated the hydro alcohol extract of *S. marianum* and dichloromethane extract of *S. virgata* on iron overloaded mice and found that they significantly decreased the total plasma content of Fe^{3+}. This result was further confirmed by pathological analysis what indicated that iron precipitation, inflammation and necrosis had been reduced in hepatocytes upon treatment with the extracts. *S. marianum*' s flavonolignans, well known as silimarins (Silybin (SBN), Isosylibin (ISBN), Silydianin (SDN), Silycristin (SCN) and Taxifolin (TAX)) [31, 39], contribute to liver health through different mechanisms including the activation of DNA polymerases, increase of cell membrane stability, inhibition of free radicals, and increment of glutathione cellular levels. Silimarin inhibits lipid peroxidation as well [40, 41, 42].

We showed that hydroalcoholic extract of *S. marianum* decreases the Fe^{3+} content in iron-overloaded mice. Our finding in this case corresponds to the results of Gharagozloo et al (2008) and Borsari et al (2001) [21, 22].

Salvia has been found to contain a variety of natural compounds with significant antioxidant activity, including phenolic acids (such as rasmic acid, rosmarinic acid, cafeic acid and lithospermic acid), flavonoids and proanthocyanidins. Among these compounds, lithospermic acid has been proven to be efficient in treatment of liver diseases [43]. As evidenced by H&E and perlsstainings, the extract of salvia was able to decrease iron precipitation, and tissue necrosis and inflammation. These findings correspond to the results of Gao et al (2013) and Zhang et al (2013) [9]. Therefore, reduced inflammation and increased iron chelating in mice liver tissues upon treatment with the extracts of these plant may be due to the presence of silimarin and other natural compounds, like phenols. However, more studies are needed to certify these findings.

CONCLUSION: The extracts of *S. marianum* and *S. virgata* exhibit significant iron chelating activity and are able to reduce inflammation and necrosis in mouse liver tissues. More studies are needed to exactly determine which compounds are responsible for these biological activities.

REFERENCES:

1. Brittenham Gary M, Iron-chelating therapy for transfusional iron overload; New England Journal of Medicine, 2011; 364 (2): 146-156.

2. Fenaux Pierre, Rose Christian, Impact of iron overload in myelodysplastic syndromes; Blood Reviews, 2009; 23, Supplement 1 (0): S15-S19.
3. Britton Robert S, Leicester Katherine L, Bacon Bruce R, Iron toxicity and chelation therapy; International journal of hematology, 2002; 76 (3): 219-228.
4. Crisponi Guido; Remelli Maurizio, Iron chelating agents for the treatment of iron overload; Coordination Chemistry Reviews, 2008; 252 (10): 1225-1240.
5. Flora Swaran JS, Pachauri Vidhu, Chelation in metal intoxication; International journal of environmental research and public health, 2010; 7 (7): 2745-2788.
6. Sharpe Philip C, Richardson Des R, Kalinowski Danuta S, Bernhardt Paul V, Synthetic and natural products as iron chelators; Current Topics in Medicinal Chemistry, 2011; 11 (5): 591-607.
7. Nićiforović N, Mihailović V, Mašković P, Solujić S, Stojković A, Muratspahić D Pavlović, Antioxidant activity of selected plant species; potential new sources of natural antioxidants; Food and Chemical Toxicology, 2010; 48 (11): 3125-3130.
8. Kaur Gurpreet, Athar Mohammad, Alam M. Sarwar, Quercus infectoria galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages; Chemico-Biological Interactions, 2008; 171 (3): 272-282.
9. Gao Yonggang, Wang Na, Zhang Ying, Ma Zhihong, Guan Peng, Ma Juanjuan, Zhang Yuanyuan, Zhang Xuejing, Wang Jiangyan, Zhang Jianping, Mechanism of protective effects of Danshen against iron overload-induced injury in mice; Journal of ethnopharmacology, 2013; 145 (1): 254-260.
10. Lima Cristovao F., Andrade Paula B., Seabra Rosa M., Fernandes-Ferreira Manuel, Pereira-Wilson Cristina, The drinking of a Salvia officinalis infusion improves liver antioxidant status in mice and rats; Journal of Ethnopharmacology, 2005; 97 (2): 383-389.
11. Orhan Ilkay Erdogan, Senol Fatma Sezer, Ercetin Tugba, Kahraman Ahmet, Celep Ferhat, Akaydin Galip, Sener Bilge, Dogan Musa, Assessment of anticholinesterase and antioxidant properties of selected sage (Salvia) species with their total phenol and flavonoid contents; Industrial Crops and Products, 2013; 41 (0): 21-30.
12. Orhan I Erdogan, Belhattab R, Şenol FS, Gülpinar AR, Hoşbaş S, Kartal M, Profiling of cholinesterase inhibitory and antioxidant activities of Artemisia absinthium, A. herba-alba, A. fragrans, Marrubium vulgare, M. astranicum, Origanum vulgare subsp. glandulossum and essential oil analysis of two Artemisia species; Industrial Crops and Products, 2010; 32 (3): 566-571.
13. Zhang Ying, Zhang Yuanyuan, Xie Yun, Gao Yonggang, Ma Juanjuan, Yuan Jie, Li Juan, Wang Jiangyan, Li Lei, Zhang Jianping, Multitargeted inhibition of hepatic fibrosis in chronic iron-overloaded mice by Salvia miltiorrhiza; Journal of ethnopharmacology, 2013; 148 (2): 671-681.
14. Belinky Paula A., Aviram Michael, Fuhrman Bianca, Rosenblat Mira, Vaya Jacob, The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation; Atherosclerosis, 1998; 137 (1): 49-61.
15. Ding Hsiou-Yu, Chou Tzung-Han, Liang Chia-Hua, Antioxidant and antimelanogenic properties of rosmarinic acid methyl ester from Origanum vulgare; Food Chemistry, 2010; 123 (2): 254-262.
16. Ramful Deena, Bahorun Theeshan, Bourdon Emmanuel, Tarnus Evelyne, Aruoma Okezie I., Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application; Toxicology, 2010; 278 (1): 75-87.
17. Su Min-Sheng, Shyu Yuan-Tay, Chien Po-Jung, Antioxidant activities of citrus herbal product extracts; Food Chemistry, 2008; 111 (4): 892-896.
18. Moghaddam A. H., Nabavi S. M., Nabavi S. F., Bigdellou R., Mohammadzadeh S., Ebrahimzadeh M. A., Antioxidant, antihemolytic and nephroprotective activity of aqueous extract of Diospyros lotus seeds; Acta Poloniae Pharmaceutica - Drug Research, 2012; 69 (4): 687-692.
19. Nabavi S.M., Ebrahimzadeh M.A., Nabavi S.F., Fazelian M., Eslami B., In vitro antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in Iran; Pharmacognosy magazine, 2009; 5 (18): 122-126.
20. Jungmin Oh, Heonjoo Jo, Ah Reum Cho, Sung-Jin Kim, Jaejoon Han, Antioxidant and antimicrobial activities of various leafy herbal teas; Food Control, 2012.
21. Borsari Marco, Gabbi Cristina, Ghelfi Franco, Grandi Romano, Saladini Monica, Severi Stefano, Borella Fabio, Silybin, a new iron-chelating agent; Journal of Inorganic Biochemistry, 2001; 85 (2–3): 123-129.
22. Gharagozloo Marjan, Khoshdel Zahra, Amirghofran Zahra, The effect of an iron (III) chelator, silybin, on the proliferation and cell cycle of Jurkat cells: A comparison with desferrioxamine; European Journal of Pharmacology, 2008; 589 (1–3): 1-7.
23. Ligeret Heidi, Brault Antoine, Vallerand Diane, Haddad Yara, Haddad Pierre S, Antioxidant and mitochondrial protective effects of silibinin in cold preservation–warm reperfusion liver injury; Journal of Ethnopharmacology, 2008; 115 (3): 507-514.
24. Wu Jhy-Wen, Lin Lie-Chwen, Tsai Tung-Hu, Drug–drug interactions of silymarin on the perspective of pharmacokinetics; Journal of Ethnopharmacology, 2009; 121 (2): 185-193.
25. Khalili Masoumeh, Ebrahimzadeh Mohammad Ali, Omrani Farnoosh, Karami Mohammad, Antihypoxic Activities of the Golden Chanterelle Mushroom, Cantharellus cibarius (Higher Basidiomycetes); International Journal of Medicinal Mushrooms, 2014; 16 (4).
26. Ebrahimzadeh Mohammad Ali, Nabavi Seyyed Mohammad, Nabavi Seyyed Fazel, Eslami Shahram, Antioxidant and free radical scavenging activities of culinary-medicinal mushrooms, golden chanterelle Cantharellus cibarius and Angel's wings Pleurotus porrigens; International Journal of Medicinal Mushrooms, 2010; 12 (3): 265-272.
27. Khalili Masoumeh, Ebrahimzadeh Mohammad Ali, Kosaryan Mehrnoush, Abbasi Ali, Azadbakht Mohammad, Iron chelation and liver disease healing activity of edible mushroom (Cantharellus cibarius), in vitro and in vivo assays; RSC Advances, 2015; 5 (7): 4804-4810.
28. Khalili Masoumeh, Ebrahimzadeh Mohammad Ali, Kosaryan Mehrnoush, In Vivo Iron-Chelating Activity and Phenolic Profiles of the Angel's Wings Mushroom, Pleurotus porrigens (Higher Basidiomycetes); International journal of medicinal mushrooms, 2015; 17 (9).
29. Bahador Nima, Baserisalehi Majid, The effect of Quercus castaneifolia extract on pathogenic enteric bacteria; Anaerobe, 2011; 17 (6): 358-360.
30. Oktar Süleyman, Yönden Zafer, Aydin Mehmet, Ilhan Selçuk, Alçin E, Öztürk Oktay Hasan, Protective effects of caffeic acid phenethyl ester on iron-induced liver damage in rats; J Physiol Biochem, 2009; 65 (4): 339-344.

31. Khalili Masumeh, Hasanloo Tahereh, Kazemi Tabar Seyyed Kamal, Rahnama Hassan, Influence of exogenous salicylic acid on flavonolignans and lipoxygenase activity in the hairy root cultures of Silybum marianum; Cell biology international, 2009; 33 (9): 988-994.
32. Belinky Paula A., Aviram Michael, Mahmood Saeed, Vaya Jacob, Structural Aspects of The Inhibitory Effect of Glabridin on LDL Oxidation; Free Radical Biology and Medicine, 1998; 24 (9): 1419-1429.
33. Şenol F. Sezer, Orhan Ilkay, Celep Ferhat, Kahraman Ahmet, Doğan Musa, Yilmaz Gülderen, Şener Bilge, Survey of 55 Turkish Salvia taxa for their acetylcholinesterase inhibitory and antioxidant activities; Food Chemistry, 2010; 120 (1): 34-43.
34. Gao Xueshan, Qian Mingwei, Campian Jian Li, Marshall James, Zhou Zhanxiang, Roberts Andrew M., Kang Y. James, Prabhu Sumanth D., Sun Xiao-Feng, Eaton John W., Mitochondrial dysfunction may explain the cardiomyopathy of chronic iron overload; Free Radical Biology and Medicine, 2010; 49 (3): 401-407.
35. Porter JB, Hoyes KP, Abeysinghe RD, Brooks PN, Huehns ER, Hider RC, Comparison of the subacute toxicity and efficacy of 3-hydroxypyridin-4- one iron chelators in overloaded and nonoverloaded mice; Blood, 1991; 78 (10): 2727-2734.
36. Ziyatdinova Guzel K, Voloshin Alexandr V, Gilmutdinov Albert Kh, Budnikov Herman C, Ganeev Talgat S, Application of constant-current coulometry for estimation of plasma total antioxidant capacity and its relationship with transition metal contents; Journal of Pharmaceutical and Biomedical Analysis, 2006; 40 (4): 958-963.
37. Hu Yangyang, Wang Shengpeng, Wu Xu, Zhang Jinming, Chen Ruie, Chen Meiwan, Wang Yitao, Chinese herbal medicine-derived compounds for cancer therapy: A focus on hepatocellular carcinoma; Journal of Ethnopharmacology, 2013; 149 (3): 601-612.
38. Shu Tao, Pang Mao, Rong Limin, Zhou Wei, Wang Juan, Liu Chang, Wang Xuan, Effects of Salvia miltiorrhiza on neural differentiation of induced pluripotent stem cells; Journal of Ethnopharmacology, 2014; 153 (1): 233-241.
39. Khalili Masoumeh, Hasanloo Tahereh, Kazemi Tabar Seyyed Kamal, Ag {+} Enhanced Silymarin Production in Hairy Root Cultures of'Silybum Marianum'(L.) Gaertn; Plant Omics, 2010; 3 (4): 109.
40. Altorjay István, Dalmi L., Sari B., Imre Semsei, Balla György, The effect of silibinin (Legalon) on the the free radical scavenger mechanisms of human erythrocytes in vitro; Acta Physiologica Hungarica, 1992; 80 (1-4): 375.
41. Batakov EA, Effect of Silybum marianum oil and legalon on lipid peroxidation and liver antioxidant systems in rats intoxicated with carbon tetrachloride; Eksperimental'naia i klinicheskaia farmakologiia, 2001; 64 (4): 53-55.
42. Dehmlow Carola, Murawski Niels, de Groot Herbert, Scavenging of reactive oxygen species and inhibition of arachidonic acid metabolism by silibinin in human cells; Life sciences, 1996; 58 (18): 1591-1600.
43. Lu Yinrong, Yeap Foo L., Polyphenolics of Salvia—a review; Phytochemistry, 2002; 59 (2): 117-140.

How to cite this article:
Md. Ebrahimzadeh A, Khalili M, Mod. Azadbakht and Azadbakht M: *Salvia Virgata* Jacq. and *Silibum Marianum* L. Gaertndisplay Significant Iron-Chelating Activity. Int J Pharm Sci Res 2016; 7(9): 3756-63.doi: 10.13040/IJPSR.0975-8232.7(9).3756-63.