Mentha extract consumption (*Mentha piperita L*) reduced blood iron concentration and increased TIBC levels in broiler chickens

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Abstract: The aim of this study was to evaluate the effects of Mentha extract (*Mentha piperita*) on Blood Hemoglobin (Hb), Hematocrit (Hct), Red Blood Cell (RBC), Iron and TIBC (total iron binding capacity) in broiler chickens. A total of 160 one-day old Ross (308) broiler chicks were randomly assigned into 4 treatment groups with 4 replicates of 10 chicks each were fed a same starter and grower diets. From 1 to 42 d of age, broiler drinking water supplemented with 0 (ZM), 0.2 (LM), 0.4 (MM) and 0.6% (HM) Mentha extract. No significant differences in blood Hb, HTC and RBC between the treatments were observed, but blood iron concentration of 0.4 and 0.6 (%) Mentha extract received birds were lower as compared to that of control birds (ZM) at day 42 of age ($P<0.05$). The HM birds had the lower blood iron than all of treatments ($P<0.05$). Blood TIBC of the Mentha extract received birds was higher ($P<0.05$) as compared to that of ZM birds. Furthermore, HM birds had the higher TIBC value ($P<0.05$) as compared to LM and MM birds. Significant negative correlation ($P<0.01$) was found between Mentha extract supplementation and blood iron, and positive correlation ($P<0.01$) between Mentha extract supplementation and blood TIBC. A negative linear regression ($P<0.0003$) existed between Mentha extract consumption with blood iron concentration and a positive linear regression ($P<0.0001$) between the Mentha extract consumption and blood TIBC at whole of experiment period.

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1. Introduction

Iron is important for all forms of life and considered to be a principal oligo-element of the human organism. Iron is a chemical element that has several vital function in the body. It is major role being as an oxygen carrier in blood haemoglobin and muscle myoglobin. In addition it is a component of many enzymes and required for a number metabolic processes (Quintana *et al.*, 2006). It is present at 65% at the hemoglobin level, 5% at the myoglobin level, 0.3% in enzymes and cytochrome and 30% in ferritin and hemosiderine form. Iron is the metal which carries oxygen to all the cells. It also well assumes a metabolic action at the cellular level through the activation of catalase and peroxydase. It is the most abundant metal in the brain and participates in the main neuronal processes, including neurotransmitter synthesis and myelination of axons. In the brain, it has distinct regional and cellular distributions (Connor *et al.*, 1990).

Dietary iron deficiency is the most common nutritional deficiency in the world and can ultimately result in anaemia (cook, 1990). Iron deficiency affects approximately 2 billion people worldwide (Stoltzfus, 2001) and is the most prevalent nutritional deficiency in the world and affects up to two-thirds of children in most developing countries (World health organization, 2000). Typical homemade complementary foods used in developing countries are poor sources of bioavailable iron and thus are inadequate to meet infants' high iron requirements for rapid growth and blood volume expansion after 6 months of age (Gibson *et al.*, 1998).

Iron forms of foods divided in Heme and non Heme iron. Heme iron which exists in meat and highly bioavailable. The absorption of this form is not affected by other factors presented in foods. Non Heme iron is the other form of iron present in vegetable, cereals and dairy product (Saltzman and Russell, 1998). Adversely with Heme iron, this form have a low absorption and markedly affected by gastro intestinal acidity, tannins, polyphenols, phytates, calcium and phosphate (Hurrell *et al.*, 1999; Davidsson *et al.*, 2001). The presence of inhibitors of absorption such as phytic acid or polyphenol compounds in plant foods is a major cause of iron deficiency (Fairweather and Hurrell, 1996). Polyphenol compounds are widely present in the human diet as components of fruits, vegetable, spices, cereals, tea, coffee, red wine, cacao and different herb teas (Hurrell *et al.*, 1999). The penolic compounds are released from the foods and beverage during digestion process and can complex with iron and making it unavailable for absorption (Hurrell *et*

al., 1999). The limiting factors consumption does not influence iron absorption in western society where the most people have a adequate iron store concentration, but in undeveloped countries with marginal iron status there seem to be negative association between limiting factors likely polyphenolic component and iron status (Temme and van hoydonck, 2002).

Some previous studies have shown inhibitory effects of drinking tea on non Heme iron absorption (Hallberg and Rossander, 1982; Brune *et al.,* 1989). Tannins of tea are the main factors that inhibited iron absorption (Kaltwasser *et al.,* 1998). In the som way, consumption of 20 g/L of *Mentha piperitae* tea by rats during 30 days has decreased the serum iron and ferritin and increased the unsaturated iron-binding capacity (Akdogan *et al.,* 2004). Oral administration of *Cuminum cyminum* for 6 weeks in diabetic rats has resulted in significant increase of total hemoglobin and glycosylated hemoglobin (Dhandapani *et al.,* 2002)

Mentha piperita is one of the world's oldest medicinal herbs, and is used in both Eastern and Western traditions. Ancient Greek, Roman, and Egyptian cultures used the herb in cooking and medicine. Peppermint is currently one of the most economically important aromatic and medicinal crops produced in the U.S. The world production of peppermint oil is about 8000 tons per year (Eccles,1994). Herbalists consider peppermint an astringent, antiseptic, antipruritic, antispasmodic, antiemetic, carminative, diaphoretic, mild bitter, analgesic, anticatarrhal, antimicrobial, rubefacient, stimulant, and emmenagogue (Hoffman and Bove., 1996). Mentha piperita is a perennial plant, in *Lamiaceae* family and contains about 1.2-1.5% *essential oil*. The principal components of the oil are menthol (35-55%), menthone (15-30%), and menthyl acetate (3-10%). Other compounds found in the peppermint are flavonoids (12%), polymerized polyphenols (19%), carotenes, tocopherols, saponin, and choline (Murray,1995). Negative effects of phenolic compounds such as black tea (Thankschan *et al.,* 2008), grape seed extract (King *et al.,* 2008), spinach and aubergine (Gilloolg *et al.,* 1983) on iron absorption has been shown. but there is not sufficient information regarding the effects of Mentha plant or extract on iron absorption. Mentha extract does have essential oil, tannins, glycosides, saponins and other components (Escop, 2003). Menthol and Menthone are the main phenolic components in oil of *Mentha piperita* (Escop, 2003) and it was hypothesized that Mentha extract may changes the iron absorption.. So Mentha extract supplementation in drinking water were evaluated on the amounts of blood iron, Hemoglobin (Hb), red blood cell (RBC), Hematocrit (Hct) and total iron binding capacity (TIBC) in broiler chickens. According the previous suggestions of Conway et al. (2006), plasma iron concentration was used as an indicator of iron absorption in recent experiment. The aims of this study was to determaine a potential influence of Mentha extract comsumption in drinking water on the iron absorption in broiler chickens.

2. Material and Methods

A total of 160 mixed one-day-old broiler chicks (Ross 308) were purchased from a local hatchery, weighed on arrival and randomly allocated to 16 pens (1x1 m) of 10 birds each, with equal numbers of male and females (four replicates per each treatment). Water and feed were available ad libitum. All chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1), but received 0.0%, 0.2%, 0.4% and 0.6% alcoholic extract of Mentha piperita (0.03% menthol and pH=5.5) in drinking water during the experimental period, and defined as ZM, LM, MM and HM treatments, respectively. Mentha piperita alcoholic extract was prepared using a standard maceration method (Zhang et al., 2005). For this purpose, vegetative parts of the shade dried Mentha piperita full bloom stage were crushed and soaked in ethanol 80% in 1: 5 ratios (w/v) for 72 h on a shaker. The extract strained afterwards and its menthol content was determined by TLC (thin layer chromatography) method, the pH value by using a pH meter instrument (HQ40D, Hach Co., Loveland, CO, USA). At days 21 and 42 of age, two birds per pen (a male and a female) were selected, weighed and killed by decapitation to obtain the blood samples. Blood samples were collected in anticoagulant tubes (citrate sodium 3.6% solution). Blood in microcapillary tubes were used to blood hematocrit (Hct) measurements after centrifugation (5000 rpm) for 7 min. A commercial kit (Zist-Shimi Company, Iran) was used for hemoglobin determination. In this method, ferrous ions of hemoglobin were oxidized to the ferric state by potassium ferricyanide to form hemiglobin (methemoglobin). Hemiglobin reacts with cyanide to form hemiglobincyanide (cyanmethemoglobin) that can be measured spectrophotometry. Blood iron concentration and TIBC were determined colorimetrically with a commercial kit (Zist-Shimi Company, Iran) using a spectrophotometer (Unico 2100, Japon). Red blood cells were determined by a hemocytometer manually.

The data were subjected to one way analyses of variance using SAS statistical package (version 9.1) and analyzed based a completely randomized design using the General Linear Model procedure.

Table 1. Composition of experimental diets

Ingredients (%)	Starter (0-21 d)	Grower (21-42 d)
Corn	54.87	61.78
Soybean meal (44 % protein)	36.72	26.36
Fish meal	1.31	4.50
Vegtable oil	3.00	4.00
Limestone	1.15	1.05
Dicalcium phosphate	1.94	1.49
Vit. and min. premix[1]	0.50	0.50
Salt	0.30	0.30
DL-methionine	0.21	0.02
Total	100.00	100.00
Calculated analysis		
ME (kcal/kg)	2937	3100
CP (%)	21.44	19.37
Calcium (%)	1.05	1.00
A. Phosphorus (%)	0.51	0.50
Sodium (%)	0.16	0.14
Arginine (%)	1.41	1.23
Methionine + Cystine (%)	0.91	0.69
Lysine (%)	1.20	1.10
Tryptophan (%)	0.31	0.26

[1] provide per kilogram of diet: vitamin A, 15000 IU; vitamin D_3,8000 IU; vitamin K3, 3 mg; B_{12}, 15 µg; niacin, 32 mg; choline, 840 mg; biotin, 40 µg; thiamine, 4 mg; B_2 (riboflavin), 6.6 mg; pyridoxine, 5 mg; folic Acid, 1 mg; Zn, 80 mg; Mn, 100 mg; Se, 200 mg; Fe, 80 mg; Mg (magnesium oxide), 12; Cu, 10 mg; Ca (calcium pontatenate), 15 mg; iodeine,1 m

Tukey–Kramer Multiple Comparison Test at significance level of 0.05 was used to compare the mean values. Correlation coefficients between the Mentha consumption and Blood Hemoglobin (Hb), Hematocrit (Hct), Red Blood Cell (RBC), Iron and TIBC (total iron binding capacity) were calculated by the CORR procedure of SAS (Trampel et al., 2005). In addition, regression models (linear) were performed to show the changes of blood iron and TIBC by Mentha extract supplementation at whole of experiment.

3. Results

The effects of Mentha extract on the Blood iron, TIBC, Hb, Hct and RBC showen in table 2. No significant differences in blood Hb, HTC and RBC between the treatments were observed, but blood iron concentration of 0.4 and 0.6 (%) Mentha extract received birds were lower as compared to that of control birds (ZM) at day 21 and 42 of age ($P<0.05$). The HM birds had the lower blood iron than all of treatments ($P<0.05$) at days 21 and 42 of age. Blood TIBC of the Mentha extract received birds was higher ($P<0.05$) as compared to that of ZM birds at both of age. Furthermore, there was no difference between the blood TIBC of Mentha received birds at day 21 of age but at day 42 of age, HM birds had the higher TIBC value ($P<0.05$) as compared to LM and MM birds. Significant negative correlation ($P<0.01$) was found between Mentha extract supplementation and blood iron, and positive correlation ($P<0.01$) between Mentha extract supplementation and blood TIBC (table 3). Mentha extract consumption significantly affected the blood iron concentration and additional negative response was observed at greater Mentha levels (Figure 1). A negative linear regression existed between Mentha extract supplementation with blood iron concentration at whole of experiment (liner regression Y = 15.14 - 0.7727X, P<0003, R2 = 0.352) (Figure 1). Positive response of TIBC percentage was observed at greater Mentha extract levels (Figure 2). In addition, a positive linear regression existed between Mentha extract supplementation and blood TIBC (liner regression, Y= -11.78 + 0.3376X, P<0001, R2 = 0.854) at whole of experiment (Figure 2).

Table 2. Blood Hematocrit (Hct), Hemoglobin (Hb), Red Blood Cell (RBC), Iron and Total Iron Binding Capacity (TIBC) of Broiler chickens[1] received free Mentha extract water (ZM) or 0.2 (LM), 0.4 (MM) and 0.6% (HM) of Mentha extract in drinking water

Treatment	HCT(%)	Hb(g/dl)	RBC(x10^6 cells/mm3)	Iron (mg/dl)	TIBC(%)
ZM	28.50	9.50	3.17	16.88^{a}	35.50^{c}
LM	27.75	9.13	3.08	15.50^{ab}	43.2^{b}
MM	27.75	9.25	3.08	14.13^{bc}	44.3^{b}
HM	27.00	8.96	2.99	12.63^{c}	52.00^{a}
Pooled SEM	0.48	0.15	0.05	0.35	1.10
P Value	0.76	0.65	0.72	0.0001	0.0001

[-] [ab]Means with no common superscript letter in each columns differ significantly ($P<0.05$)
[1] blood samples of eight chickens per treatment were used for these determinations.

Table 3. Pearson correlation coefficients for Mentha extract, hematocrit, hemoglobin, RBC, blood iron and TIBC in broiler chickens

	Mentha extract	HCT	Hb	RBC	Iron	TIBC
Mentha extract		-0.2	-0.213	-0.215	-0.564	0.92
P.value	1.000	0.28	0.248	0.243	**<0009**	**<0001**
HCT	-0.2		0.979	0.997	0.024	-0.184
P.value	0.28	1.000	**<0001**	**<0001**	0.894	0.319
Hb	-0.213	0.979		0.98	0.02	-0.218
P.value	0.248	**<0001**	1.000	**<0001**	0.913	0.232
RBC	-0.215	0.997	0.98		0.021	-0.195
P.value	0.243	**<0001**	**<0001**	1.000	0.9	0.291
Iron	-0.564	0.024	0.02	0.021		-0.527
P.value	**<0009**	0.894	0.913	0.9	1.000	**<002**
TIBC	0.92	-0.184	-0.218	-0.195	-0.527	
P.value	**<0001**	0.319	0.232	0.291	**<002**	1.000

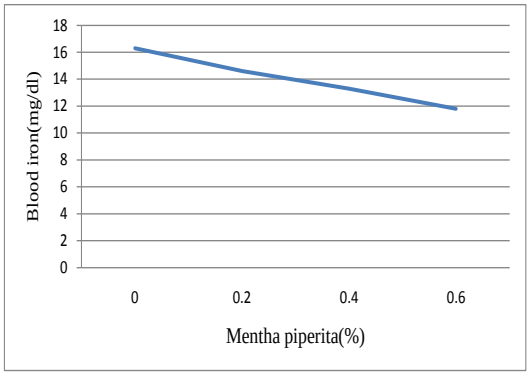

Figure 1. The relationship between the level (%) of menthe piperita supplementation on drinking water and the blood iron level in the whole of experiment(liner regression, Y = 15.14 - 0.7727X, P<0003, R2 = 0.352) in broiler chickens.

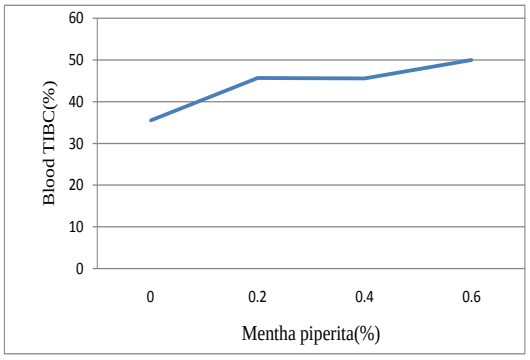

Figure 2. The relationship between the level (%) of menthe piperita supplementation in drinking water and blood TIBC in the whole of experiment (liner regression,Y = -11.78 + 0.3376X, P<0001, R2 = 0.854) in broiler chickens.

4. Discussions

Some researchers used the animal models such as piglets, rats and chicks for the studies of anemia or iron absorption (Furugouri,1972; Zhang ,1989; Zeyuan, *et a*l. 1998 ; Greger and Lyle ,1988) According to the examples mentioned we used the chicken model to study the Mentha effects on iron metabolism. Nevertheless chicken has not been used for this purpose but it was used as a model to study the cystic fibrosis (Craig-Schmidt *et al.*, 1986) and proposed to study the cause of Kashin-Beck disease (Cook, 2000) in human. Although Mentha piperita extract caused a decrease in blood iron (P<0.05) and caused a increase in blood TIBC percentage (P<0.05) but no effects on the amounts of blood Hct and Hb and RBC in our study. Consistently results have been published regarding the nonexistence effects of phenolic compounds on these indices. But in contrast result, No effects of red (high polyphenol contain) and white (low polyphenol contain) beans (*Pha seolus vulgaris L*) on iron bioavailibilities in pigs diets (Tako *et al.*, 2009), no relationship between serum ferritin and consumption of black, green and herbal tea in French adult men and women (Mennen *et al.*, 2007) and no association between black tea consumption and blood hemoglobin and ferritin concentrations in adult South African (Hogenkamp *et al.*, 2008) have been reported. In a same way with current study results, drinking of 200 ml tea (prepared from 5 g dry tea) by individuals inhibited the iron absorption from solutions of $Fecl_3$ and $Feso_4$, bread, a meal of rice with potato and onion soup. This inhibition was connected to the tannin contents of tea and the formation of insoluble iron-tannin complex in the intestinal lumen (Disler *et al.*, 1975). Hurnell *et al.* (1999) observed the inhibited iron absorption by tea from a bread meal by 50-90% depending on the kind of the tea and the

concentration of polyphenols in the brewed tea. Gibson (1999) reported a significant adverse association between tea consumption and serum ferritin (r =-0.09) in preschool children. Thankschan *et al.* (2008) investigated the effects of drinking tea (1 or 2 cup black tea) on iron absorption in iron deficient and iron adequate foods with rice meal in women and observed an inhibited iron absorption by a similar amount in both groups and connected it to low bioavailability of iron from plant-based diet containing mineral absorption inhibitors such as polyphenols and phytates. Mehta *et al.* (1992) reported that consumption of tea and coffee was negatively associated with risk of anemia in United States. Gilloolg *et al.* (1983) indicated a negative correlation between amount of spinach and aubergine (rich in polyphenols) with iron absorption in men. As same as tea, spinach, aubergine and coffe, Mentha extract does have essential oils (rich in polyphenols) (Escop, 2003). Menthol and menthon are a main phenolic compounds in Mentha extract and have been used for traditional medicine (Akdogan, 2004). The functional group of polyphenol compounds (menthol and menthon) is an aromatic ring structure with one or more hydroxyl groups and combines with iron and causes destruction of iron absorption. Phenolic compounds influence iron absorption by complexing iron in the intestinal lumen or by altering intestinal permeability (Harborue, 1986). King *et al.* (2008) examined the influence of the dietary polyphenols epigallocatechin-3-gallate (EGCG) and grape seed extract (GSE) on transepithelial iron transport in Caco-2 intestinal cells and reported the inhibited non heme iron absorption by polyphenol compounds from apical iron import in intestinal cells. Besides Mentha have other components such as tannins, glycosides and saponins (Escop, 2003) that affect the iron absorption. It was suggested that saponins may interfere with iron metabolism either by forming complexes with the dietary iron thereby unavailable for absorption or by producing changes in mucosal function with long-term consumption thus reducing the efficiency nutrients absorption (Southon *et al.*, 1988ab). Siddiqi (1994) reported the reduced iron absorption by *Cicer arietinum* consumption (rich in polyphenol and saponin). They also indicated the insoluble iron-polyphenols complex formation at the stomach pH. Low pH of the Mentha extract may be other reason of low iron absorption in our study. We used a low pH Mentha piperita extract (pH=5.5) that possibly caused the greater decrease in gastrointestinal pH and so the easier formation of complex between polyphenols and iron in intestinal lumen. An opposite relations for blood TIBC and iron with the Mentha extract supplementation were found in our experiment. This shows that Mentha supplementation decreases the blood iron concentration and increases the blood iron requirements for maximum saturation of blood transferrin. Because TIBC is the blood capacity to bind iron with transferrin or the amount of iron needed to 100% saturation of transferrin. Blood TIBC often increases in iron deficiency and decreases in choronic inflammatory disorders and hematochromatosis (Tietz, 1999). In the same way, Furugouri (1972) observed higher blood TIBC at 10 and 20 days of age in the pigs with anemia and unsupplemented ferrous fumarate deits than in pigs receiving ferrous fumarate.

In conclusion, the results obtained in the present study reveal that an addition of Mentha extract in drinking water led to an decrease in the blood iron concentration and increase in blood TIBC (%) in broiler chickens and possibly in human. Inhibited absorption of iron by phenolic compounds and low pH in Mentha extract is the possible reason.

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