



## Effects of Enalapril on Growth Performance, Ascites Mortality, Antioxidant Status and Blood Parameters in Broiler Chickens Under Cold-Induced Ascites

Fathi M, Haydari M & Tanha T

Department of Animal Science, College of Agriculture, Payame Noor University, Tehran, Iran

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### Corresponding author

Mokhtar Fathi  
[fathi\\_mokhtar@yahoo.com](mailto:fathi_mokhtar@yahoo.com)

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### Abstract

Six hundred 1-d-old male broilers (Ross 308) were assigned to four experimental groups; each was composed of 5 floor pen replications of 30 birds including control (no enalapril), 15, 30 and 60 ppm enalapril in the drinking water. From d 21 to 49, all the chicks were exposed to low ambient temperature to induce ascites. Mortalities were inspected to determine the cause of death and diagnose of ascites. At the end of the experiment (wk 7), 2 chickens from each replicate were randomly selected and slaughtered. Body weight gain, feed intake and feed conversion ratio were calculated. Plasma protein, glucose, red blood cell, white blood cell, triglyceride, high-density lipoprotein, malondialdehyde, the activity of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatine kinase, total antioxidant capacity, superoxide dismutase, and glutathione peroxidase were also determined. Results showed that enalapril for 30 and 60 ppm, significantly improved feed conversion ratio and enhanced body weight gain when measured at day 49. These levels of enalapril compared to the other groups, significantly reduced malondialdehyde level and glutathione peroxidase activity, but increased total antioxidant capacity and superoxide dismutase activity in plasma. Moreover, enalapril at levels of 30 and 60 ppm, significantly reduced aspartate aminotransferase, alkaline phosphatase and creatine kinase activities in plasma. Mortality due to ascites and right to total ventricular weight ratio were significantly low in groups received enalapril at greater levels ( $\geq 30$  ppm). Compared to the control, enalapril increased high-density lipoprotein. In conclusion, enalapril could improve growth performance and reduced mortality in broilers.

### Introduction

Ascites or PHS (pulmonary hypertension syndrome) is a most common metabolic syndrome associated with rapid growth in modern broiler chickens and it is an important cause of mortality in broiler chickens that has been reported from many parts of the world (Wideman, 1988). It is proposed that increased

blood pressure in the pulmonary circulation (pulmonary hypertension) can cause enlargement of the right ventricle (RV) and eventually congestive heart failure (Julian, 1993; Wideman and Bottje, 1993; Franco, 2012). It has proposed that ascites and endothelial function disorder might be associated with oxidative

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stress induced by reactive oxygen species (ROS) and other oxidants (Ruiz-Feria, 2009). Oxidative stress occurs when tissue depleted of antioxidants (Iqbal *et al.*, 2002). Hypoxia can induce ROS production and endothelial damage through impairing endothelial nitric oxide (NO) synthesis by ROS radicals and subsequently increase pulmonary arterial pressure. The ROS causes a loss of NO bioavailability, so that, reducing the potential for endothelial vasodilatation and subsequently, high pulmonary arterial pressure and ascites prevalence. Moreover, the reactions of ROS like superoxide anion with NO leads to the production of peroxynitrite, a powerful oxidant agent for endothelial damage (Ruiz-Feria, 2009).

It is believed that drug administration can reduce blood pressure as hypertension contributes to cell damage antihypertensive. Moreover, some studies have reported that the beneficial assistance of antihypertensive drugs including enalapril could be in part due to their antioxidant effects whereby there is the reserve of free radical production. Several reports have demonstrated that some of the antihypertensive drugs, such as the angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers, reduce blood pressure and improve oxidative status (Wiemer *et al.*, 1997; Mantle *et al.*, 2000; Bayorh *et al.*, 2003).

Enalapril may have positive effects as an angiotensin converting enzyme inhibitors class of antihypertensive drug on the control of hypertension and improved antioxidant defense mechanisms in hypertension (Chandran *et al.*, 2014). The primary objective of this study was to assess the efficacy of enalapril in drinking water in the prevention of ascites mortality in broilers under induced ascites.

## Materials and Methods

### Diets and birds

Six hundred 1-d-old male broiler chickens (Ross 308) were allocated randomly to 4 treatments groups with 5 replicates and 30 chicks per each. All chicks were fed a basal corn-soybean meal diet for starter (1 to 21 d) and grower (22 to 49 d) periods (Table 1). Birds had free access to feed and water, with 23 hour light per day throughout the experimental period. From d 7, the water was supplemented with 0, 15, 30 and 60 ppm of Enalapril Drug (ENALAPRIL PURSINA 10MG TAB).

**Table 1.** Composition of basal diet

Ingredients (%)	Starter (1 to 21d)	Grower (22 to 49d)
Corn	54.47	59.25
Soybean meal (44% protein)	22.50	20.75
Corn gluten meal	7.00	8.00
Fish meal	6.16	3.00
Soybean oil	6.00	5.70
Dicalcium phosphate	1.72	1.22
Limestone	1.20	1.30
Vitamin premix <sup>1</sup>	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25
Salt	0.25	0.25
DL-Methionine	0.20	----
L-Lysine	----	0.03
<i>Calculated analysis</i>		
ME (kcal/kg)	3010	3175
CP (%)	23	21
Calcium (%)	1.00	0.90
Available phosphorus (%)	0.5	0.45
Lysine (%)	1.44	1.23
Methionine (%)	0.51	0.45
Methionine + Cystine (%)	1.09	0.95

<sup>1</sup>Supplied per kilogram of premix: vitamin A, 11,000 IU; vitamin D3, 5,000 IU; vitamin E, 40 IU; vitamin K, 4 mg; riboflavin, 5 mg; vitamin B6, 4 mg; vitamin B12, 0.011 mg; niacin, 50 mg; biotin, 0.01 mg; thiamine, 3 mg;

<sup>2</sup>Supplied per kilogram of premix: zinc 80 mg; manganese oxide, 100 mg; selenium, 10 mg; iron sulfate 80 mg.

### Growth performance and ascites evaluation

Body weight gain (BWG) and feed intake (FI) were measured weekly and corrected feed conversion ratio (FCR) based on mortality, was calculated. On days 49, eight birds from each group were selected, slaughtered after 8 h of feed deprivation and then hearts were removed. The pericardium, marginal adipose tissues and atriums were removed from the hearts. The left and right ventricles were separated and their individual weights were measured. The right ventricle / total ventricle (RV / TV) as a simple measure of ascites incidence was determined (Ruiz-Feria, 2009).

### Ascites induction program

The experimental ascites was induced using cold temperature model (Ruiz-Feria, 2009). All birds were housed (1,260 m altitude) under 32°C and 30°C during the first and second week of age, respectively. Then the house temperature was decreased to 15°C during wk 3 and maintained between 10 °C and 15°C for the rest of the study. Mortalities were recorded daily and all of the dead birds inspected for judgment of ascites. Diagnosis of ascites generally depends on

observation of the following one or several symptoms including right ventricle hypertrophy, swollen and colloidal fluid in the abdominal activity (Geng *et al.*, 2004).

#### Blood and biochemical parameters sampling

At the end of the study (day 49), two birds per replicate were selected and weighed after 8 h of feed deprivation. Whole blood samples were collected by vein puncture into heparin anticoagulation tubes for measuring red blood cell (RBC), white blood cell (WBC), hematocrit and hemoglobin (Sysmex KX-21 N Automatic blood analyzer, Kobe, Japan). Another set of blood samples (1 mL/bird) were collected into heparin, then transferred to the laboratory for analysis within two hours of collection and centrifuged (3000 g, for 10 min at room temperature). Then, plasma was collected and stored at  $-20^{\circ}\text{C}$  until measurement of the other enzymatic and chemical analysis. The concentrations of plasma metabolites were measured using standard kits (Sigma Chemical Co, St. Louis, MO 63178-9916, USA).

Enzymatic and chemical tests included glucose, total protein, triglyceride, cholesterol, high-density lipoprotein (HDL), alkaline phosphates (ALP), aspartate aminotransferase (AST), creatine kinase (CK) and alanine aminotransferase (ALT) activities, were measured (Autolab, PM 4000, Auto analyzer Medical System, Rome, Italy).

#### Antioxidant indices and measuring malondialdehyde concentration in plasma

At day 49, ten birds per experimental group were slaughtered. Whole blood samples were drawn from slaughtered birds. Erythrocytes are susceptible to oxidative stress as a result of the

high polyunsaturated fatty acid content of their membranes (Igbal *et al.*, 2002). Whole heparinized blood was assayed for glutathione peroxidase (GPx) activity. GPx activity was determined via commercially available enzyme kit (Ransel, RANDOX/RS-504 supplied by Randox Laboratories, Crumlin, UK). Erythrocyte hemolysate was used for Superoxide dismutase (SOD) activity. Lipid peroxidation was measured by the thiobarbituric acid method (Rezar *et al.*, 2007). This method evaluates oxidative stress by measuring malondialdehyde (MDA), the last product of lipid breakdown caused by oxidative stress. Total antioxidant capacity (T-AOC), was measured using commercial kits (Randox, Pars Azmoon Co. Tehran, Iran) and auto analyzer (Alcyon 300, USA).

#### Statistical analysis

The data were analyzed based on a completely randomized design (CRD) with four treatments and five replicates per treatment using the GLM procedure of SAS (2003). Contrasts between treatments means were evaluated by Tukey's test at a significance level of 5%.

#### Results

##### Growth performance and ascites index and mortality due to ascites

The results of this study showed improvement in BWG and FCR, as shown in Table 2. Two upper levels of enalapril supplementation improve the growth performance of broilers under induced ascites.

These results showed that at day 49, broilers in 30 and 60 ppm enalapril group had significantly higher BWG and FCR than that of the control birds ( $P < 0.05$ ).

**Table 2.** Effects of different levels of enalapril on performance of broiler chickens (49 d)

Performance traits	Enalapril (ppm)				SEM	P-value
	0	15	30	60		
Body weight gain (g)	2760 <sup>b</sup>	2782 <sup>b</sup>	2900 <sup>a</sup>	2897 <sup>a</sup>	25.4	0.001
Feed intake (g)	5684 <sup>a</sup>	5600 <sup>a</sup>	5219 <sup>b</sup>	5216 <sup>b</sup>	112.5	0.001
Feed conversion ratio	2.05 <sup>a</sup>	2.02 <sup>a</sup>	1.79 <sup>b</sup>	1.80 <sup>b</sup>	0.05	0.001

Means in a row without common superscripts differ significantly ( $P < 0.05$ ).

In the present study, it was clearly demonstrated that enalapril supplementation at 30 and 60 ppm levels, significantly reduced

ascites mortality and ascites index (RV / TV) in broilers (Table 3).

**Table 3.** The effect of enalapril on Mortality due to ascites and RV / TV<sup>1</sup> of broiler chickens

Enalapril (ppm)	Mortality (%)	RV/TV <sup>1</sup>
0	14.0 <sup>a</sup>	0.36 <sup>a</sup>
15	13.0 <sup>a</sup>	0.34 <sup>a</sup>
30	6.0 <sup>b</sup>	0.24 <sup>b</sup>
60	7.5 <sup>b</sup>	0.25 <sup>b</sup>
SEM	0.053	0.02
P-value	0.018	0.001

<sup>1</sup>Right ventricle / total ventricle.Means in a column without common superscripts differ significantly ( $P < 0.05$ )**Antioxidant enzymes and MDA concentration in plasma**

The effects of different levels of enalapril on antioxidant enzymes activity and MDA concentration in plasma are presented in Table 4. As shown, compared to other groups, 30 and 60 ppm enalapril, significantly increased SOD activity and T-AOC in plasma ( $P < 0.05$ ). Altogether, MDA in plasma and GPx activity was reduced by 30 and 60 ppm of enalapril.

**Table 4.** Effects of different levels of enalapril on concentrations of MDA<sup>1</sup>, T-AOC<sup>4</sup> and antioxidant enzymes activities of broiler chickens

Enalapril (ppm)	MDA <sup>1</sup> (nmol/L)	SOD <sup>2</sup> (U/gHb)	GPx <sup>3</sup> (U/gHg)	T-AOC <sup>4</sup> (nmol/L)
0	3.17 <sup>a</sup>	3111 <sup>b</sup>	222 <sup>a</sup>	0.98 <sup>b</sup>
15	2.90 <sup>b</sup>	3500 <sup>b</sup>	229 <sup>a</sup>	1.10 <sup>b</sup>
30	1.85 <sup>c</sup>	7210 <sup>a</sup>	210 <sup>b</sup>	2.57 <sup>a</sup>
60	1.98 <sup>c</sup>	7680 <sup>a</sup>	205 <sup>b</sup>	2.95 <sup>a</sup>
SEM	0.52	520	9.5	0.24
P-value	0.001	0.012	0.001	0.010

<sup>1</sup>Malondialdehyde; <sup>2</sup>Glutathione peroxidase; <sup>3</sup>Superoxide dismutase; <sup>4</sup>Total antioxidant capability.Means in a column without common superscripts differ significantly ( $P < 0.05$ ).**Plasma enzymes activities and blood parameters**

Results related to the effect of different levels of enalapril on several plasma enzymes (AST, ALT, ALP and CK) activities are presented in Table 5. These data indicated that enalapril at the high levels (30 and 60 ppm) significantly reduced

AST, ALP and CK activities in plasma ( $P < 0.05$ ). Table 6 shows that 60 ppm of enalapril in drinking water significantly increased HDL in plasma ( $P < 0.05$ ). The other blood parameters were not significantly affected by treatments.

**Table 5.** Effects of different levels of enalapril on the serum activities of CK<sup>1</sup>, ALP<sup>2</sup>, AST<sup>3</sup> and ALT<sup>4</sup> of broiler chickens

Enalapril (ppm)	CK <sup>1</sup> (IU/L)	ALP <sup>2</sup> (IU/L)	AST <sup>3</sup> (IU/L)	ALT <sup>4</sup> (IU/L)
0	4020 <sup>a</sup>	2420 <sup>a</sup>	271.5 <sup>a</sup>	4.75
15	3995 <sup>a</sup>	2380 <sup>a</sup>	258.7 <sup>ab</sup>	4.50
30	2800 <sup>b</sup>	2150 <sup>b</sup>	241.5 <sup>b</sup>	4.01
60	2792 <sup>b</sup>	1980 <sup>b</sup>	215.5 <sup>c</sup>	3.95
SEM	95	87	15	0.89
P-value	0.025	0.001	0.001	0.191

<sup>1</sup>Cratine kinase; <sup>2</sup>Alkaline phosphatase; <sup>3</sup>Aspartate aminotransferase; <sup>4</sup>Alanine aminotransferase.Means in a column without common superscripts differ significantly ( $P < 0.05$ ).**Table 6.** The effect of enalapril on blood parameters of broiler chickens

Enalapril (ppm)	Protein (mg/dL)	Glucose (mg/dL)	Hemoglobin (g/dL)	Hematocrit (%)	WBC (10 <sup>3</sup> /μL)	RBC (10 <sup>6</sup> /μL)	Cholesterol (mg/dL)	HDL (mg/dL)	Triglyceride (mg/dL)
0	3.80	229.25	7.20	29.22	153.02	2.17	120.25	47.35 <sup>b</sup>	83.25
15	4.35	230.13	7.24	29.14	159.19	2.35	110.45	51.25 <sup>b</sup>	42.00
30	3.90	229.50	7.90	30.25	161.15	2.51	117.00	53.00 <sup>ab</sup>	31.25
60	4.20	221.90	8.25	28.19	158.10	2.60	115.86	62.50 <sup>a</sup>	30.50
SEM	0.43	15.50	0.43	2.85	3.50	0.13	8.50	3.43	5.50
P-value	0.321	0.470	0.290	0.270	0.141	0.413	0.210	0.010	0.108

Means in a column without common superscripts differ significantly ( $P < 0.05$ ).

## Discussion

### Growth performance and mortality due to ascites

In the present study, enalapril administration in the broiler, improved growth performance while reduced right ventricle hypertrophy and mortality due to ascites (Table 2 and Table 3). RV / TV is an indicator of the earlier experience of the heart to increased pulmonary arterial pressures (Geng *et al.*, 2004). These results agree with de Cavanagh *et al.* (2001), who found, 20 mg enalapril/L drinking water in rats under oxidative stress, significantly increased body weight, antioxidant defenses and reduced hypertrophy in the heart. Enalapril administration could increase antioxidant status and caused increasing plasma nitric oxide (NO) levels in animals. NO was possibly derived from the potentiation of bradykinin by persistent using enalapril treatment. Increased NO level caused increasing the potential for endothelial vasodilatation and subsequently, prevents high pulmonary arterial pressure and reduced ascites (Baluchnejadmojarad *et al.*, 2004).

Endothelium protective effect of enalapril is associated with hydrogen peroxide scavenging action. Further studies supported the idea that enalapril might offer some protection to smooth muscle cells in vascular endothelial cells by improving antioxidant status in abdominal aortas (Baluchnejadmojarad *et al.*, 2004; Kim *et al.*, 2013). So that, probably, these effects of enalapril cause improvement in digestion and absorption of nutrients, as, many researchers have reported that enalapril possesses an antioxidant effect (Benzie and Tomlinson, 1998; Kim *et al.*, 2013). The mechanism of this antioxidative effect of antihypertensive drug including enalapril is unclear (Kim *et al.*, 2013).

In this study, antihypertensive drug (enalapril) significantly increased the SOD activity, while reduced the GPx activity in plasma. It is believed that some of the antihypertensive drugs including enalapril via antioxidant effects can increase SOD activity and contemporaneously MDA level in rats (Cabell *et al.*, 1997; de Cavanagh *et al.*, 2001). Enalapril via antioxidative effects can reduce erythrocyte osmotic fragility (EOF) and pulmonary hypertension. Enalapril might decrease EOF via two probable mechanisms; either by altering the lipid profile of the cell membrane or exerting antioxidant activity. Increase in membrane cholesterol alters membrane surface area and

decreases membrane fluidity (de Cavanagh *et al.*, 2001). Thus, a decreased amount of membrane cholesterol permits higher deformability and less EOF. The results of this experiment strongly suggested that enalapril had additive effects on improving cardiopulmonary performance and reducing pulmonary hypertension, and these may have been mediated by reductions in oxidative stress, reduced MDA in plasma, (Table 4) and subsequently, increased availability of nitric oxide and attempt to promote vasodilatation of reducing pulmonary hypertension.

### Antioxidant enzymes and MDA concentration in plasma

MDA concentration is an important index for lipid peroxidation and oxidative damage caused by ROS in the cell (Igbal *et al.*, 2002). These results agree with several researchers that reported enalapril markedly reduced vascular O<sub>2</sub>- production in cultured aortic smooth muscle cells (Baluchnejadmojarad *et al.*, 2004) and MDA in plasma and liver in the rat (Kim *et al.*, 2013). It is believed that capability of enalapril to scavenge the free radical is better than several well-established antioxidants, such as ascorbate, glutathione (GSH) and cysteine (Kedziora-Kornatowska *et al.*, 2000; Kim *et al.*, 2003). Data from present study, suggested that enalapril supplementation in drinking water had a consistent and significant effect in decreasing MDA in serum relative to the control, while the decreases in birds fed higher levels of enalapril (30 and 60 PPM) were significant, which this reduced MDA could be related to increased T-AOC resource of cells. T-AOC in the cells and plasma contributes to the balance of active oxygen, and T-AOC is a potent parameter reflecting the status of all the antioxidants in serum and body fluids (Rajani *et al.*, 2011).

### Plasma enzymes activities and blood parameters

Our study showed that ALT, AST, ALP and CK plasma levels of birds under induced ascites were higher and enalapril could significantly reduce AST, ALP and CK activity. Arab *et al.* (2006) reported that in birds under induced ascites and oxidative stress, the plasma levels of ALT, AST and LDH were higher. These researchers suggested that tissue oxidation results in the injury of organs, including lung, heart and liver. Probably, enalapril could protect the cell from free radical via antioxidant effects

and consequently reduce those enzymes in plasma. The study showed that administration of enalapril in drinking water significantly increased HDL in plasma. Increasing HDL could be attributed to the fact that enalapril may reduce the stress of low temperature by reducing released serum corticosterone and consequently corticosterone may affect lipid metabolism (Mohammed, 2010).

### Conclusion

In summary, enalapril was effective in reducing RV / TV, mortality due to ascites, and plasma MDA concentration in broiler chickens under cold-induced ascites. Body weight gain and feed conversion ratio also improved at 30 and 60 ppm levels of enalapril. Enalapril may have balancing effects on cardiopulmonary performance by increasing nitric oxide bioavailability via antioxidant effects through a greater substrate for nitric oxide syntheses, and probably by reducing the losses of nitric oxide associated with oxidative stress and reducing endothelial damage by free radicals.

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تاثیرات انالاپریل بر عملکرد رشد، تلفات آسیتی، وضعیت آنتی‌اکسیدانی و فراسنجه‌های خون در جوجه‌های گوشتی تحت آسیت القایی به روش سرما

Fathi M, Haydari M & Tanha T

گروه علوم دامی، دانشکده کشاورزی، دانشگاه پیام نور، تهران، ایران

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چکیده

تعداد ۶۰۰ قطعه جوجه خروس گوشتی یک‌روزه سویه راس ۳۰۸، به چهار گروه آزمایشی با ۵ تکرار حاوی ۳۰ پرنده، شامل؛ شاهد (بدون انالاپریل)، ۱۵، ۳۰ و ۶۰ پی‌پی‌ام، انالاپریل در آب آشامیدنی، تقسیم شدند. از روز ۲۱ تا ۴۹، همه پرندگان جهت القای آسیت، در معرض هوای سرد قرار گرفتند. تلفات برای تعیین مرگ و تشخیص آسیت بررسی شدند. در آخر آزمایش (روز ۴۹)، ۲ جوجه از هر تکرار به صورت تصادفی انتخاب و کشتار شد. افزایش وزن بدن، خوراک مصرفی و ضریب تبدیل خوراک محاسبه شد. پروتئین کل، گلوکز، گلیکول قرمز، گلیکول سفید، تری‌گلیسرید، لیپوپروتئین با دانسیته بالا، فعالیت آنزیم‌های آلکالین فسفاتاز، اسپاراتات آمینوترانسفراز، آلانین آمینوترانسفراز و کراتین کیناز و همچنین فراسنجه‌های وضعیت آنتی‌اکسیدان شامل؛ کل ظرفیت آنتی‌اکسیدان، فعالیت آنزیم‌های سوپراکسیددیسموتاز و گلوتاتیون پراکسیداز و سطح مالون‌دی‌آلدئید در پلاسما اندازه‌گیری شدند. نتایج نشان داد، در روز ۴۹، سطح ۳۰ و ۶۰ پی‌پی‌ام انالاپریل، به‌طور معنی‌داری، سبب بهبود ضریب تبدیل خوراک و افزایش وزن حاصله شدند. در مقایسه با سایر گروه‌ها، این سطوح انالاپریل (۳۰ و ۶۰ پی‌پی‌ام)، سبب کاهش مالون‌دی‌آلدئید و فعالیت گلوتاتیون پراکسیداز شد، در حالی‌که، همان زمان، فعالیت آنزیم سوپراکسیددیسموتاز و کل ظرفیت آنتی‌اکسیدان پلاسما را افزایش داد. علاوه بر این، سطوح ۳۰ و ۶۰ پی‌پی‌ام انالاپریل، به‌طور معنی‌داری سبب کاهش فعالیت آنزیم‌های اسپاراتات آمینوترانسفراز، آلکالین فسفاتاز و کراتین کیناز در پلاسما شد. تلفات ناشی از آسیت و شاخص نسبت وزن بطن راست به کل بطن در گروه‌های سطوح ۳۰ و ۶۰ پی‌پی‌ام انالاپریل، به‌طور معنی‌داری پایین بود. همچنین، در مقایسه با گروه شاهد، انالاپریل، سبب افزایش لیپوپروتئین با دانسیته بالا در پلاسما شد. نتیجه‌گیری این‌که، انالاپریل توانست سبب بهبود عملکرد رشد و کاهش تلفات در جوجه‌های گوشتی شود.

کلمات کلیدی

جوجه گوشتی  
آسیت  
انالاپریل  
فراسنجه‌های خون  
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نویسنده مسئول

Mokhtar Fathi  
fathi\_mokhtar@yahoo.com

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