Peppermint and pennyroyal essential oil effect on performance, rumen microbial population and some blood parameters of sheep

Mohamadi, R.[1], Rahchamani, R.[2*], Ghanbari, F.[2], Farivar, F.[2]

[1]*Graduated Student of Animal Physiology, College of Agriculture and Natural Resources, University of Gonbad Kavous, Gonbad Kavous, Iran*

[2]*Department of Animal Sciences, College of Agriculture and Natural Resources, University of Gonbad Kavous, Gonbad Kavous, Iran*

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Correspondence
Rahchamani, R.
Department of Animal Sciences, College of Agriculture and Natural Resources, University of Gonbad Kavous, Gonbad Kavous, Iran
Tel: +98(17) 33226700
Fax: +98(17) 33226700
Email: r_rahchamani@yahoo.com

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Abstract:

BACKGROUND: The use of antibiotics as feed additive in animal feeds due to the appearance of residues in milk and meat and their effects on human health is restricted. Two essential oils with high potential for use in ruminant diet are *Mentha piperita* (peppermint) and *Mentha pulegium* (pennyroyal) essential oil. **OBJECTIVES:** This study was conducted to investigate the effects of essential oils of peppermint and pennyroyal on performance, ruminal microbial population and some blood parameters of sheep. **METHODS:** For this purpose, 9 Dallagh sheep were used in a change-over design experiment in three 21-d periods (14 days as adaptation and 7 days for sample collection). Experimental treatments were 1) basal diet without additive (control), 2) basal diet + 110 mg/d *Mentha piperita* essential oil and 3) basal diet +110 mg/d *Mentha pulegium* essential oil. Sheep were kept in individual cages and had free access to food and water. Rumen fluid was collected before, 4 h and 8 h after morning feeding and a blood sample was obtained 3 h after morning feeding at the last day of each period. **RESULTS:** Essential oils had no effect on performance, blood parameters, pH, ammonia, protozoa, and total viable bacterial count of rumen. Coliforms of rumen fluid significantly decreased at 4 h and increased 8 h after morning feeding following peppermint and pennyroyal supplementation, respectively ($p<0.05$). Acid lactic bacteria count was significantly higher at before and 4 h after morning feeding in pennyroyal treatment compared with other treatments. **CONCLUSIONS:** Although essential oils of *Mentha piperita* and *Mentha pulegium* had some effects on rumen microbial population they had no significant effects on performance and blood metabolites of Dallagh sheep.

Introduction

The use of antibiotics as feed additive in animal feeds due to the appearance of residues in milk and meat and their effects on human health is restricted (Ouwehand, 2010; Vakili, 2013; Anantasook, 2013). In the last few years there has been increasing

attention to the potential of phytogenic and herbal products as alternative for feed antibiotics and growth promoters in ruminant nutrition (Wallace, 2004; Steiner, 2009; Giannenas, 2011).

Essential oils (EO) are a blend of secondary metabolites that have antimicrobial effect on bacteria, fungi and protozoa. EO are aromatic compounds present in many plants that are generally extracted by steam and/or water distillation and common chemically composed terpenoids including monoterpenes, sesquiterpenes and diterpenes. EO may also include a variety of low-molecular aliphatic hydrocarbons, acids, alcohols, aldehydes, lactones, nitrogen and sulfur containing compounds, coumarins and phenylpropanoides (Dorman and Deans, 2000; Wanapat, 2013). Most of these EOs are safe and can be used as feed additive according to antibacterial, antifungal and antioxidant properties (Cawan, 1999). These compounds have been shown to favorably manipulate ruminal fermentation and improve nutrient utilization in ruminants (Greathead, 2003).

Two EOs with high potential for use in ruminant diet are *Mentha piperita* (peppermint) and *Mentha pulegium* (pennyroyal). Peppermint is a perennial plant in lamiaceae family and the principal components of the peppermint oil are menthol (31.53%), 1, 8 cineole (10.67%), menthon (8.2%), menthyl acetate (3.54%), β-caryophyldene (2.8%) and piperitone (0.77%) (Rafii and Shahverdi, 2007). The flowering aerial parts of Pennyroyal have been traditionally used for its antimicrobial properties in the treatment of common cold, sinusitis, bronchitis, cholera, food poisoning and tuberculosis. Most of the plant parts contain compounds with antilatulent, carminative, expectorant, diuretic, antitussive and menstruation agent. *Mentha piperita* and *Mentha pulegium* possess antimicrobial, antioxidant, cytotoxic and abortifacient properties (Ghalamkari et al., 2012; Nobakht, 2011).

However, most researches conducted on EO have been laboratory based (i.e., in vitro). There are very limited in vivo studies that have evaluated effectiveness of these plants EO in sheep. Therefore the aim of present study was to determine the effects of supplementation to the diet of sheep with these herbs on performance, blood metabolites and ruminal fermentation.

Material and Methods

Animals and dietary treatments: Nine Adult male Dallagh sheep with an average initial BW of 32±3 kg were used in a change-over design. The experiment consisted of three 21-day periods (14 days as adaptation and 7 days as sampling) and there were 3 treatments, each consisting of 9 replicates. At the start of the experiment sheep were weighted and randomly assigned to 1 of 3 treatments: 1) basal diet with no additive (control), 2) basal diet supplemented with 100mg/day peppermint EO and 3) basal diet supplemented with 110mg/day pennyroyal EO.

Essential oils: Essential oils were purchased from Barij Essence Pharmaceutical Company (Kashan- Iran). Major compounds of peppermint EO were carvone (55.87%), limonene (23.88%), menthol (3.38%), pinene (2.34%) and pennyroyal EOs were piperiton (33.60%), pippeirtenone (28.71%), α-terpineol (5.52%), eucalyptol (5.01%), piperitenone oxide (3.94%), menthone (3.39%) and α-pinene (3.03%).

Diets and Animal Management: The

basal diet was composed of 30% roughage and 70% concentrate, and the ingredients and nutrient composition of the basal diet (DM basis) is given in Table 1.

Diets were fed as total mixed rations (TMR) and formulated according to NRC (1996) recommendations. The experimental diets were offered twice (08:00 and 18:00 h) daily for ad libitum intake (10% refusals) with one-half of the daily feed allotment offered at each feeding. The essential oils were mixed with a small amount of concentrate and once daily top dressed onto the TMR before feeding to ensure that sheep would consume the full amount offered. Amounts of feed offered and refused were recorded daily for each animal throughout the trial. Body weight was measured at the beginning and the end of each period. The sheep were housed in individual stalls on mattresses bedded with straw in pens (2m× 1.5m) equipped with a water bowl. The pens were located in a covered barn. Each animal had free access to water at all times.

Measurements, sample collection and analysis: Average daily weight gain was calculated throughout the trail. Feed conversion ratio was calculated as the ratio between feed intake and weight gain.

Ruminal fluid was collected from animals at the last day of each period before morning feeding, and 4 h and 8 h after morning feeding via the esophagus using a 60 ml gavage syringe and a lubricated rubber tube. The first 20 ml of ruminal fluid content was discarded to ensure they were not polluted with saliva and then squeezed through four layers of cheesecloth. Rumen pH was measured immediately with a portable pH meter (Metrohm 691, Switzerland).

A 5-ml portion of the strained ruminal fluid was mixed with 5 ml of 0.2 N HCL. All samples were stored at -20oC for subsequent analyses. The ruminal fluid concentration of NH3-N was determined using spectrophotometry by the phenol hypochlorite method of Broderick and Kang (1980). Serial 10-fold dilutions of strained ruminal fluid were prepared and used as inoculum on plate count agar (PCA), Modified Rogosa and Sharp Agar (MRSA) and Violent Red Bile Agar (VRBA) medium for total viable bacteria, acid lactic bacteria and coliforms count respectively (Ghoorchi et al., 2009).

Blood samples were collected at non-heparinized tube from the jugular vein at the end of each period (3 h after the morning feeding), samples were centrifuged (Denley BS400, England) at 10,000 rpm for 10 min and collected serum was frozen at -20^{o}C until analysis. Serum concentration of glucose, total protein and triglyceride were determined using the commercial kits of Darmanfaraz Company (Isfahan, Iran) by spectrophotometry.

A 1-ml portion of the strained ruminal fluid was preserved using 9 ml of MFS (methyl green- formaldehyde- saline) solution for enumeration of protozoa. Protozoa samples were stored at room temperature in the dark until counting. Protozoa were enumerated microscopically in a Neubauer cytometer at ×40 magnification. Each sample was counted twice and if the average of the duplicates differed by more than 10%, the counts were repeated (Cedrola et al., 2015).

Statistical analysis: All data were statistically analyzed according to a randomized complete block design using the general linear model (GLM) procedure of SPSS software (version18). Significant differences between means of treatments were assessed by the tukey test, and the differences among treatments were declared significant

at $p<0.05$. Initial weight of sheep was used as covariate for weight gain data analysis.

Results

Dry matter intake and performance data are presented in Table 2. Initial and final BW did not differ among the experimental treatments and consequently, daily weight gain was not affected by treatments. The mean DM intakes ranged from 0.29 to 0.35 kg/d and were similar for sheep fed basal diet and those consuming diets supplemented with additives. Similarly, feed conversion ratio, expressed as feed: gain ratio (DMI to average daily gain ratio), was similar among treatments.

Mean of the blood metabolites are shown in Table 3. Concentrations of serum glucose, total protein and triglyceride were not affected by additive treatments.

Table 4 represents the effect of experimental treatments on rumen fluid pH, NH3-N and protozoa. Rumen fluid pH was not affected by treatments at before morning feeding, 4 h and 8 h after morning feeding. There was no significant difference in ruminal NH3-N concentration among treatments. No significant influence of experimental diet on rumen protozoa at before morning feeding, 4h and 8 h after morning feeding was observed.

Table 5 summarizes data obtained on the effects of experimental diets on rumen microbial population. Total viable bacterial count had no significant difference before feeding, 4 h and 8 h after feeding. Acid lactic bacteria were influenced by treatments. Before morning feeding and 4 h after morning feeding acid lactic bacteria count significantly increased in pennyroyal treatment compared with control and peppermint treatment. But at 8 h after feeding

Table 1. Ingredient and nutrient composition of basal diet (DM basis). [1] Contained: 21 g/kg Mg, 0.3 g/kg Zn, 2.2 g/kg Mn, 3 g/kg Fe, 0.3 g/kg Cu, 0.001 g/kg Se, 0.1 g/kg Co, 0.12 g/kg I, 195 g/Kg Ca, 80 g/Kg P, 600 IU/g of vitamin A, 200 IU/g of vitamin D, and 2.5 IU/g of vitamin E.

Ingredient	%	Nutrient composition	%
Barley grain, ground	38.34	Dry matter	64.5
Corn silage	25	Crud protein	14
Wheat straw	5	NDF	28.8
Wheat bran	15	Metabolizable energy (Mcal/Kg)	2.76
Soybean meal	15		
salt	0.36		
Mineral-vitamin premix[1]	1		
Pearl powder	0.3		

Table 2. Effect of experimental treatments on performance. * Treatments include: CON: basal diet; PEP: basal diet supplemented with peppermint oil (110 mg/sheep daily); PEN: basal diet supplemented with pennyroyal oil (110 mg/sheep daily).* Standard error of means.

Item	Treatments*			SEM*	p- value
	CON	PEP	PEN		
Initial weight (kg)	33.60	30.37	32.90	2.69	0.94
Final weight (kg)	46.90	54.70	45.67	2.57	0.33
Average daily gain (kg)	0.29	0.32	0.35	0.01	0.34
Dry matter intake (g)	1594	1623	1564	23.94	0.23
Feed conversion ratio	5.38	4.94	4.36	0.03	0.68

Table 3. Effect of experimental treatments on blood metabolites. * Treatments include: CON: basal diet; PEP: basal diet supplemented with peppermint oil (110 mg/sheep daily); PEN: basal diet supplemented with pennyroyal oil (110 mg/sheep daily).* Standard error of means.

Item	Treatments*			SEM*	p-value
	CON	PEP	PEN		
Glucose (mg dl^{-1})	85.47	89.84	76.62	6.43	0.35
Total protein (g dl^{-1})	6.87	6.04	7.52	1.12	0.65
Triglyceride (mg dl^{-1})	86.34	114.16	73.60	15.86	0.20

Table 4. Effect of experimental treatments on pH, NH3-N and protozoa of rumen fluid. * Treatments include: CON: basal diet; PEP: basal diet supplemented with peppermint oil (110 mg/sheep daily); PEN: basal diet supplemented with pennyroyal oil (110 mg/sheep daily). * Standard error of means.

Item	Time	Treatment*			SEM*	p-value
		CON	PEP	PEN		
pH	Before feeding	6.76	6.96	6.93	0.09	0.25
	4 h after feeding	6.35	6.57	6.33	0.09	0.15
	8 h after feeding	6.44	6.29	6.45	0.10	0.48
NH3-N	Before feeding	5.67	5.74	5.74	0.065	0.626
(mg dl^{-1})	4 h after feeding	5.70	5.74	5.69	0.064	0.822
	8 h after feeding	5.67	5.79	5.80	0.060	0.227
Protozoa	Before feeding	6.55	5.55	6.43	0.81	0.64
($\times 10^5$ ml^{-1})	4 h after feeding	6.88	7.68	5.60	0.75	0.16
	8 h after feeding	6.35	7.98	7.83	0.95	0.42

Table 5. Effect of experimental treatments on rumen bacterial population (log cfu/ml). * Treatments include: CON: basal diet; PEP: basal diet supplemented with peppermint oil (110 mg/sheep daily); PEN: basal diet supplemented with pennyroyal oil (110 mg/sheep daily). [a-b] Means within the same row without common letters differ significantly ($p<0.05$). * Standard error of means.

Item	Time	Treatments*			SEM*	p-value
		CON	PEP	PEN		
Total viable bacteria	Before feeding	6.72	6.79	6.84	0.03	0.06
	4 h after feeding	6.78	6.83	6.76	0.06	0.06
	8 h after feeding	6.67	6.76	6.79	0.03	0.09
Acid lactic bacteria	Before feeding	5.19[a]	5.11[a]	5.39[b]	0.04	0.00
	4 h after feeding	5.19[a]	5.02[a]	5.29[b]	0.03	0.00
	8 h after feeding	5.18	5.21	5.30	0.04	0.18
coliforms	Before feeding	3.59	3.44	3.67	0.06	0.04
	4 h after feeding	3.62[a]	3.45[b]	3.72[a]	0.04	0.02
	8 h after feeding	3.51[a]	3.42[a]	3.73[b]	0.05	0.00

there was no significant difference among treatments. Coliforms count was not influenced by treatments before morning feeding but at 4 h after morning feeding in peppermint treatment was significantly lower than control group. Pennyroyal supplementation significantly increased coliforms at 8 h after morning meal.

Discussion

Feed intake and performance: There is little information on the effects of peppermint and pennyroyal EO on DMI and performance in sheep. The findings of present study for DMI was in line with previous researches in growing lambs (Chaves et al., 2008a), sheep (Distel et al., 2007; Nolte and Provenza, 1992) and cattle (Benchaar et al., 2007; Beauchemin and Mcginn, 2006) who found no effect of EO on DMI. In contrast with our results, Demir et al. (2003) reported that pennyroyal EO increased nutrient absorption, growing and weight gain. Also, Lewis et al. (2003) found that pennyroyal EO and canola oil improved daily weight gain in male broiler chicken because of antimicrobial and positive effect on intestinal micro flora. In some researches low levels of EO improved ruminal fermentation and growing performance (Borek, 2001). Our

result confirmed the report which showed that *Leucaena leucocephalaon* and *Salix babylonica* had no effect on weight gain in growing lamb (Salem et al., 2011). The effects of EO on DMI might vary with EO source, type of diet, diet interaction or adaptation of rumen microbial population to EO (Yang et al., 2010a; b; Geraci et al., 2012). Furthermore, it has been reported that it can be affected by a number of dietary or management factors such as body weight, animal growth stage, specific physical and chemical characteristics of diet or rumen fermentation metabolites (Allen, 2000; Yang et al., 2010b).

Blood metabolites: These results are in agreement with some studies that declared peppermint EO feeding did not affect blood metabolites significantly (Hosoda et al., 2006; Ahmadi Naghadehi et al., 2014). Plasma triglyceride concentration is lipid transfer index and triglycerides are lipids that conserve energy in animal tissues (Hatfield et al., 1998). In our study serum triglyceride was not affected by treatments, which was similar to some researches (Hosoda et al., 2006; Ahmadi Naghadehi et al., 2014). The result of total serum protein in this study was in agreement with Ahmadi Naghadehi et al. (2014) who reported that 100 and 200 mg peppermint EO did not significantly changed total serum protein of sheep. It has been reported that concentration of some blood metabolites such as triglycerides can be influenced by EO supplementation via changing of feed intake (Yang et al., 2010b) and no change in blood metabolites in present study may be contributed to lack of DMI alteration by EO.

Rumen pH, NH3-N and protozoa: Rumen pH is a resultant of produced volatile fatty acids (acetate, propionate and lactate), ammonia, rumen buffers and saliva (Vansoet, 1994) and an index for fermentation. In some previous studies it was shown that VFA production decreased and rumen pH increased following EO feeding because of inhibitory effect of phenolic compounds on rumen flora (Benchaar et al., 2007). 100 and 200 mg peppermint EO feeding did not affect rumen pH in sheep (Ahmadi Naghdahi et al., 2014). In agreement with our result peppermint feeding at 5% DM (Hodoso et al., 2006) had no effect on rumen pH in steers. In contrast with our study, peppermint feeding (200 g/day) decreased rumen pH and protozoa count in steers (Ando et al., 2003). Variable effects of EO on rumen pH can result from different diets and levels of EO in studies as when EO was added to diets with higher roughage/concentrate ratio, rumen pH increased (Meyer et al., 2009).

Similarly, supplementation with 0.2g/kg (DM basis) of carvacrol and cinnamaldehyde did not alter ruminal ammonia in growing lamb (Chaves et al., 2008b). In contrast, Hosoda et al. (2006) reported that peppermint feeding at 5% of the diet (DM basis) significantly increased rumen ammonia in Holstein steers. Ruminal ammonia concentration has been reflected in the balance of nitrogen and energy supplies into the rumen, which is associated with microbial activity (McDonald et al., 2002). Also, this is in disagreement with the in vitro results of Newbold et al. (2004), who observed a decrease in ruminal ammonia when rumen contents of cows or sheep supplemented with EO and acid hydrolyzed protein were incubated for 24-48 h in strained ruminal fluid (1 or 110 mg/d, respectively).

EO dose is an important factor for rumen effects (Fraser et al., 2007). Busquet et al.

(2005) showed in vivo that 200 g peppermint powder decreased rumen protozoa. In a study it was reported that peppermint decreased rumen protozoa and ammonia nitrogen (Bach et al., 2005). Our result was in agreement with some researchers (Newbold et al., 2004; Benchaar et al., 2007) who reported that EO feeding had no effect on rumen protozoa in sheep. Similarly, Djouvino et al. (1997) reported that 450 g/d peppermint did not influence protozoa count in sheep.

Rumen bacteria: Rumen bacteria are influenced by diet changes, feed additives, feeding program and physical status. These factors can alter or destroy appearance of new rumen bacteria. The antimicrobial activity of plant extracts is attributed to a number of secondary metabolites, such as saponines, terpenoides (such as carvacrol, carvone, thymol, and terpinen-4-ol) and phenylpropanoides (such as cinnamaldehyde, eugenol, or anethol) compounds, present in the essential oil fraction of many plants (Busquete et al., 2005). Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell (Brenes and Roura, 2010). In a study a mixed EO did not change rumen microbial population (Benchaar et al., 2007). Also, Wallas et al. (2002) observed that 100 mg mixed EO had no effect on rumen bacteria in sheep. Also, it was reported that EO inhibited rumen bacteria (Mcintosh et al., 2003). Factors such as EO type, diet property, species, and geographical situation affect variability and bacterial count including lactic acid bacteria of rumen (Dehority et al., 2003). Our result was in disagreement with a study that showed clove and garlic EO did not affected rumen coliforms (Ghoorchi et al., 2009).

Conclusions: The results of this study showed that although pennyroyal EO increased coliform and acid lactic bacteria of rumen, pennyroyal and peppermint EO had no effect on performance, blood metabolites, rumen pH, NH3-N and protozoa count. More in vivo studies are required to investigate the effects of different supplementations levels on rumen microbial fermentation and blood metabolites to improve nutrient utilization and growth performance in sheep.

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مجله طب دامی ایران، ۱۳۹۶، دوره ۱۱، شماره ۱، ۸۴-۷۵

اثر روغن اسانسی نعناع و پونه بر عملکرد، جمعیت میکروبی شکمبه و برخی فراسنجه‌های خونی گوسفند

روناک محمدی[۱] رضا راه‌چمنی[۲*] فرزاد قنبری[۲] فریبا فریور[۲]

۱) دانش آموخته فیزیولوژی دام، دانشکده کشاورزی و منابع طبیعی دانشگاه گنبدکاووس، گنبد کاووس، ایران
۲) گروه علوم دامی، دانشکده کشاورزی و منابع طبیعی دانشگاه گنبد کاووس، گنبد کاووس، ایران

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چکیده

زمینه مطالعه: استفاده از آنتی بیوتیک‌ها به‌عنوان افزودنی غذایی در دام‌ها به علت باقیماندن آنتی بیوتیک در شیر، گوشت و اثرات آن در سلامت انسان‌ها محدود شده است. روغن اسانسی نعناع و پونه پتانسیل بالایی برای استفاده در جیره نشخوارکنندگان دارند. **هدف:** این مطالعه به‌منظور بررسی اثرات روغن‌های اسانسی نعناع و پونه بر عملکرد، جمعیت میکروبی شکمبه و برخی فراسنجه‌های خونی گوسفند انجام شد. **روش کار:** بدین منظور، از ۹ رأس گوسفند نر نژاد دالاق در قالب طرح چرخشی در ۳ دوره ۲۱ روزه شامل ۱۴ روز به‌عنوان دوره عادت‌پذیری و ۷ روز نمونه‌برداری استفاده شد. تیمارهای آزمایشی شامل تیمار۱ (شاهد): جیره پایه (بدون اسانس نعناع و پونه)، تیمار۲: جیره پایه +mg ۱۱۰ در روز اسانس نعناع و تیمار۳: جیره پایه +mg ۱۱۰ در روز اسانس پونه بودند و گوسفندان در قفس‌های انفرادی بطور آزاد به آب و غذا دسترسی داشتند. به‌منظور تعیین جمعیت میکروبی، pH و ازت آمونیاکی، نمونه‌های مایع شکمبه قبل از خوراک‌دهی صبح، ۴ و ۸ ساعت بعد از خوراک‌دهی صبح جمع‌آوری شدند. خون‌گیری در پایان هر دوره از سیاهرگ گردنی صورت گرفت. **نتایج:** روغن‌های اسانسی نعناع و پونه تأثیر معنی‌داری بر عملکرد، متابولیت‌های خونی، pH، ازت آمونیاکی، تعداد پروتوزوآ و شمارش کلی باکتری‌های مایع شکمبه نداشتند. تعداد کلی‌فرم‌های شکمبه ۴ ساعت بعد خوراک‌دهی صبح در تیمار نعناع کاهش معنی‌دار و ۸ ساعت بعد خوراک‌دهی در تیمار پونه افزایش معنی‌داری نسبت به تیمار شاهد داشت ($p<۰/۰۵$). تعداد باکتری‌های تولید کننده اسیدلاکتیک در زمان قبل و ۴ ساعت بعد از خوراک‌دهی صبح در تیمار پونه بیشتر از تیمارهای شاهد و نعناع بود. **نتیجه‌گیری‌نهایی:** در مجموع، روغن‌های اسانسی نعناع و پونه هر چند بر جمعیت میکروبی شکمبه موثر بودند ولی تاثیری بر عملکرد و فراسنجه‌های خونی گوسفندان دالاق نداشتند.

واژه های کلیدی: متابولیت‌های خونی، روغن اسانسی نعناع، روغن اسانسی پونه، جمعیت میکروبی، گوسفند

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*) نویسنده مسؤول: تلفن: ۳۳۲۲۶۷۰۰ (۱۷)۹۸+ نمابر: ۳۳۲۲۶۷۰۰ (۱۷)۹۸+ Email: r_rahchamani@yahoo.com