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Full Length Research Paper

The effects of the seeds of *Galeopsis ladanum* on fattening performance in quails and occurrence of rhabdomyolysis in rats

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This study was conducted to investigate the effect of *Galeopsis ladanum* seeds on rhabdomyolysis occurrence in rats as an animal model and fattening performance of quails. Control group was fed with basal diet without *G. ladanum* seeds. Diets of treatment groups contained 2 or 4% of *G. ladanum* seeds, respectively. All animals, quails and rats were fed individually. At the end of the study, 2 and 4% of *G. ladanum* seeds in the diets did not have reverse effect on body weight, weight gain and feed intake in quails. Besides, treatment group one had a better feed conversion ratio at the end of the third week ($p<0.05$) and up to 28 days of age ($p<0.05$). At the end of the feeding period, quails were slaughtered and their drumsticks were fed to the rats either raw or grilled. After consumption of the meat, their serum and organs were subjected to biochemical and pathologic investigations. Although, no pathological finding was observed in the tissue samples, serum myoglobin and enzyme levels were found to be higher than that in the control group ($p<0.01$). Although, no pathological finding was observed in the tissue samples prepared from rats and quails for histopathological examination, the increase in serum myoglobin and enzyme levels may be due to subcellular changes in the target tissues of toxic substance.

Key words: *Galeopsis ladanum*, performance, rhabdomyolysis, quail.

INTRODUCTION

Bird migration is the regular seasonal journey of many bird species and has created one of the most important fall migratory route in Turkey. Quails migrate from Caucasia to Africa twice in a year. Bird hunting is a common practice in Turkey as well as a famous hobby all over the world. Common quails are known to be toxic when eaten during the time of migrations (Bartram and Boland, 2001). Toxicity of birds was long ignored and up to now only a few examples are known. Except quail, some fish (burbot, eel and pike) and wild mushrooms (*Tricholoma equestre*) are reported as the causes of rhabdomyolysis (Tsironi et al., 2004; Buchholz et al., 2000). Wide varieties of potentially toxic seeds of quail crops for human consumption include *Ballota* sp., *Galeopsis* sp., *Hyoscyamus* sp., *Lathyrus* sp., *Lolium* sp., *Stachys* sp., *Hyoscyamus* sp., *Aconitum* sp., *Veratrum* sp., and *Conium* sp. Quails are reported to be toxic during migration period and the poisoning is expressed as coturnism (Lewis et al., 1987; Bartram and Boland, 2001; Tsironi et al., 2004).

Quails can eat *Galeopsis ladanum* seeds that grow widely in the Black Sea region. Vegetation period of the plant lasts up to late in the fall. Therefore, quail can easily find the *G. ladanum* seeds around migration period of fall.

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Rhabdomyolysis is the rapid breakdown of skeletal muscle due to injury to muscle tissue. Muscle destruction leads to the release of the breakdown products of damaged muscle cells into the blood, such as myoglobin that is harmful to kidney and may lead to acute kidney failure. For human, physical symptoms include muscular weakness, swelling, pain, cramping and darkened urine. Rhabdomyolysis is described with elevated serum creatine kinase level on account of skeletal muscle injury (Blanco et al., 2002).

Coturnism has been recognized since ancient times and discussed even in the Book of Numbers (Bartram and Boland, 2001). Although it is rare and often misdiagnosed, it is seen during the migration period of quail. Especially, hunters and their families who eat quail that consumed *G. ladanum* seeds exhibit symptoms of rhabdomyolysis (Aparicio et al., 1999, Kennedy and Grivetti, 1980). Since quail migration occurs in fall, all cases of quail poisoning have been reported in this time of the year.

Yet, there is no sufficient information about coturnism in human who hunts and eats quail feeding on *Galeopsis* seeds that grows all over the Black Sea Region of Turkey located on the way of quail migration route. It is reported that after eating quail, the patients were admitted with some combination of symptoms including muscle tenderness, extremity pain, nausea and vomiting. Despite insufficient information on coturnism and though it is a rarely seen among toxicological syndrome causes of rhabdomyolysis, speculative admissions to hospitals occur because of it.

This study was carried out to determine the effect of *G. ladanum* seeds consumed by quails on rhabdomyolysis occurrence in rats (as human model) and its fattening performance on quails.

MATERIALS AND METHODS

Animal material and diets

A total of 21 ten-day old quails (C*oturnix coturnix japonica*) were randomly distributed to the control and treatment groups, each containing seven and total of 42 adult rats (spraque dawley), and each weighed approximately 200 g, distributed to the groups, containing 14 each. Birds kept in individual cages, each measuring 40 x 28 x 20 cm, and sawdust was used as litter. Rats were kept in metabolic cages. Control group was fed with control diet (not including *G. ladanum*), treatment groups were fed with the diets containing 2 and 4% *G. ladanum* seeds, respectively (Table 2). The seeds were collected from the environment of Samsun Province. The diets of rats consisted of only quail meat for occurrence of rhabdomyolysis. After the quails were slaughtered at the end of the study, rats were fed with 15 g drumstick either raw or grilled. In order to confirm whether the heat denaturalizes toxic substances in meat, the meat was given both grilled and raw. The rats were starved for 48 h prior to the quail meal in order to ensure that they consumed all the meal quickly and would display clinical symptoms. 6 h later after the meal, all rats were slaughtered, and then serum and organs were taken for biochemical analysis and pathological investigations.

Feed analysis

In order to determine the nutrient contents of feed stuffs and *Galeopsis* seeds, analytic methods of AOAC (1984) was used, and to calculate the metabolizable energy levels of diets, the formula recommended by TSE (1991) was used. Qualitative analysis of toxic substances in *Galeopsis* seeds were identified by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS).

Myoglobin and enzyme levels

Serum myoglobin level was determined by CLIA (Chemiluminescence Immuno Assay/Immunology Analyser, Vitros ECiQ, Ortho Clinical Diagnostics, Germany) with commercial kit (Ortho-Clinical Diagnostics, Johnson & Johnson, UK) and enzyme levels were determined by Spectrophotometric/Chemistry Analyzer (Abbott Diagnostics Architect C8000, US) with commercial kit (Abbott, C8000).

Determination of toxic substances

Qualitative analysis of toxic substances in the seeds was performed using gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS).

Determination of occurrence of rhabdomyolysis in rats

Rats and quails were euthanized under deep anaesthesia for histopathological examination. After a systematic necropsy was carried out on the animals, the tissue samples (muscle, liver, heart, spleen and kidney) were fixed in 10% buffered formalin solutions. Paraffin blocks prepared from tissues were cut at a thickness of 4 µm and stained with hematoxylin-eosin.

Determination of quail performance

Quails were fed individually, and feed consumption and live weight gains were determined by weekly weighing.

Determination of slaughtering and carcass characteristics

Quails were not fed 12 h before slaughtering. After slaughtering, carcasses were weighed to determine warm carcass weight and then refrigerated at +4°C for 24 h to determine the cold carcass weight. Hot and cold carcass yields were calculated.

Statistical analysis

Data were analyzed for analysis of variance (ANOVA) using the Least Square Method of the SAS GLM procedure (SAS, 2007) and differences in means were determined with Duncan's multiple-range treatment. All results were summarized as mean ± standard error of means (SEM).

RESULTS AND DISCUSSION

Nutrient contents of *G. ladanum* seeds and the composition of quail diets are shown in Tables 1 and 2, respectively. The mean live weights, live weight gains,

Table 1. Composition of *G. ladanum* plant seed (%).

Nutrient composition	Percentage
Dry matter	93.50
Ash	5.02
Crude protein	18.00
Crude fat	42.00
Crude fiber	8.31
Nitrogen free extract	20.17
Metabolizable energy (kcal/kg)	4573.50
Macro elements	
Ca	0.90
P	0.12
Micro elements (ppm)	
Cu	22.50
Fe	5.53
Mn	22.50
Na	522.50
Zn	171.25
Cr	2.50
K	90.00
Mg	83.70
Co	12.50

feed intakes and feed conversion ratio of the groups are shown in Table 3. The mean carcass weights, carcass yields and organ weights of the groups are shown in Tables 4 and 5, respectively. The mean serum myoglobin and enzyme levels of rats are shown in Table 6. No pathological finding was observed in the tissue samples during optical microscopic examinations of samples prepared from rats and quails for histopathological examination. Qualitative analysis of toxic substances in the seeds was performed using gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). The GC/MS analysis revealed fractions of pentanoic acid, phenylethanol, phenol 4-ethanyl-2 methoxy and phenethanol.

The average live weights of control and treatment groups 1 and 2 were 210.77, 222.78 and 216.53 g, respectively, and there was no statistical difference between groups. At the end of the feeding period, average feed intake of groups were 556.3, 573.5 and 592.3; live weight gain were 176.4, 189.3 and 182.4; and feed conversion ratio were 3.1, 3.0 and 3.2, respectively (0 to 4 weeks). There was no statistical difference between groups in the case of feed intake, live weight gain and the feed conversion ratio (Table 3). No statistical significant difference was found between the groups with regard to the carcass weights and carcass yields (Table 4). There was no statistical significant difference between the groups with regard to organ weights of heart (1.79, 1.97 and 2.01), liver (4.72, 4.99 and 4.83) and gizzard (3.55, 4.01 and 3.91), respectively (Table 5).

One of the world’s most important migration routes passes over The Black Sea Region of Turkey. Generally, quails find *Galeopsis* plant as seeds are formed in a period of vegetation when migrating to Turkey in fall. They become exhausted when they reach the north cost of Turkey. Therefore, since they need urgent energy, quails cannot select the seeds available.

Quail poisoning (coturnism) is an acute dietary toxicological syndrome that may develop after consumption of migratory quails in fall, especially by bird hunters and children.

Clinically, creatine kinase (CK), lactate dehydrogenase (LDH), alanine transaminase (AST) and aspartate aminotransferase (ALT) are assayed in blood tests as a marker of rhabdomyolysis. Elevation of these enzymes is an indication of damage to muscle. It is therefore, indicative of rhabdomyolysis.

The manner of consumption of meat (raw or grilled) was found to have no significant effect on the serum myoglobin and enzyme levels. For rats that ate raw meat, the serum creatine kinase levels in the control group and treatment groups one and two were found to be 638.7, 1276.1 and 1448.2 U/L. In comparison with the control group, serum creatine kinase levels were found to be significantly higher ($p<0.01$) in rats that fed on quail in treatment groups one and two. The results of this study concurs with the results of the study carried out by Harold Conn (2001) in which the creatine kinase levels in the tissue of rats that fed on quail which had consumed *G. ladanum* seeds for one or three days were found to be statistically higher than the control group, which had consumed standard rat food. No negative effect was observed in the consumption of feed in the treatment group, serum creatine kinase levels were found to be significantly higher ($p<0.01$) in rats that fed on quail in treatment groups one and two. The results of this study concurs with the results of the study carried out by Harold Conn (2001) in which the creatine kinase levels in the tissue of rats that fed on quail which had consumed *G. ladanum* seeds for one or three days were found to be statistically higher than the control group, which had consumed standard rat food. No negative effect was observed in the consumption of feed in the treatment groups in which *Galeopsis* seeds were added to the rations in small amounts. Due to the fact that *G. ladanum* seeds have significant oil content with regard to their nutritional composition and contain a high level of energy, there was no negative effect on performance data when it was added in small amounts (2% and 4%) to the quail rations. However, further research could be carried out with higher levels in the quail rations in order to determine the minimum levels that would cause changes in the rat enzyme levels and histopathology.

Table 2. Ingredients and nutrient composition of diets fed to quails (%).

Item	Control	Treatment group 1	Treatment group 2
Ingredients			
Corn	55.60	54.50	53.40
Galeopsis ladanum	-	2.00	4.00
Soybean meal	34.00	34.00	34.00
Fish meal	5.90	5.50	5.10
Oil	2.00	1.50	1.00
Limestone	1.30	1.30	1.20
Dicalcium phosphate	0.50	0.50	0.60
Salt	0.30	0.30	0.30
Vitamin premix	0.25	0.25	0.25
Mineral premix	0.15	0.15	0.15
Nutrient composition			
Dry matter (%)	88.51	88.54	88.57
Crude protein (%)	23.69	23.69	23.69
Ca (%)	0.79	0.80	0.79
P (%)	0.59	0.59	0.60
Metabolizable energy (kcal/kg)	2983	2982	2982

Table 3. Body weight gain means and standard error of means, feed intake and feed:gain ratio by groups ($\bar{X} \pm S_{\bar{X}}$).

Week	Control	Treatment group 1	Treatment group 2	p
Feed intake (g/bird, as-fed basis)				
1	90.5 ± 2.44	90.6 ± 2.28	95.8 ± 2.49	NS
2	133.4 ± 5.35	137.8 ± 4.62	140.2 ± 4.73	NS
3	157.8 ± 4.10	155.1 ± 4.80	155.0 ± 4.37	NS
4	174.5 ± 9.46	189.9 ± 8.12	201.1 ± 9.56	NS
0-4	556.3 ± 18.13	573.5 ± 17.10	592.3 ± 19.87	NS
Body weight gain (g/bird)				
1	43.4 ± 1.99	46.0 ±2.27	46.8 ± 2.39	NS
2	55.1 ± 2.84	57.2 ± 2.86	55.7 ± 3.20	NS
3	39.2 ± 1.22	42.1 ± 2.49	37.4 ± 1.29	NS
4	38.6 ± 2.60	43.8 ± 2.90	42.3 ± 2.56	NS
0-4	176.4 ± 7.23	189.3 ± 8.94	182.4 ± 7.47	NS
Feed:gain ratio				
1	2.1 ± 0.06	1.9 ± 0.05	2.0 ± 0.06	NS
2	2.4 ± 0.03	2.4 ± 0.05	2.5 ± 0.06	NS
3	4.0 ± 0.12[ab]	3.7 ± 0.13[b]	4.1 ± 0.10[a]	*
4	4.5 ± 0.12	4.3 ± 0.11	4.7 ± 0.20	NS
0-4	3.1 ± 0.05[ab]	3.0 ± 0.06[b]	3.2 ± 0.03[a]	*

*p<0.05; NS, non significant; [a] and [b], different letters within the same rows indicate differences among groups.

Table 4. Carcass weight (g) and yield carcass means and standard error of means (%) by groups ($\overline{X} \pm S_{\overline{X}}$).

Carcass characteristic	Number	Control	Treatment group	
			1	2
Body weight	7	210.77 ± 8.15	222.78 ± 9.70	216.53 ± 7.91
Hot carcass weight	7	134.18 ± 7.31	144.75 ± 7.16	141.46 ± 6.39
Cold carcass weight	7	128.04 ± 7.27	138.07 ± 6.95	134.31 ± 6.03
Hot carcass yield	7	0.63 ± 0.01	0.65 ± 0.01	0.65 ± 0.01

Table 5. The organ weights means and standard error of means by groups (g) ($\overline{X} \pm S_{\overline{X}}$).

Organ weight	Number	Control	Treatment group		p
			1	2	
Liver weight	7	4.72 ± 0.74	4.99 ± 0.50	4.83 ± 0.47	NS
Spleen weight	7	0.08 ± 0.01[b]	0.171 ± 0.03[a]	0.10 ± 0.01[b]	*
Heart weight	7	1.79 ± 0.13	1.97 ± 0.12	2.01 ± 0.08	NS
Gizzard weight	7	3.55 ± 0.36	4.01 ± 0.26	3.91 ± 0.30	NS

NS, Non significant *: p<0.05; [a] and [b], different letters within the same rows indicate differences among groups.

Table 6. Means and standard error of means for the serum myoglobin and enzyme levels of rats ($\overline{X} \pm S_{\overline{X}}$).

Parameter	Control	Treatment group			p
		1	2	3	
Raw meat					
CK (U/L)	638.7 ± 32.10[c]	1276.1 ± 86.03[b]	1448.2 ± 102.39[ab]	1603.0 ± 56.00[a]	**
LDH (U/L)	1017.4 ± 101.20[b]	1387.7 ± 89.47[ab]	1471.5 ± 122.26[a]	1534.0 ± 150.00[a]	*
AST (U/L)	146.0 ± 11.22[c]	236.0 ± 16.21[b]	294.2 ± 18.78[ab]	314.0 ± 30.00[a]	**
ALT(U/L)	59.1 ± 8.70[b]	54.8 ± 3.34[b]	85.7 ± 3.69[a]	74.5 ± 0.50[ab]	**
Myoglobin (ng/ml)	180.8 ± 46.15[b]	583.1 ± 36.07[a]	633.4 ± 73.28[a]	604.2 ± 65.40[a]	**
Grill meat					
CK (U/L)	665.2 ±47.51[c]	1294.1 ± 44.20[b]	1504.1 ± 93.62[ab]	1585.5 ± 121.50[a]	**
LDH (U/L)	1043.7 ± 100.79[b]	1438.0 ± 102.76[a]	1598.7 ± 105.79[a]	1579.5 ± 231.50[a]	**
AST (U/L)	142.4 ± 14.57[c]	233.0 ± 22.70[b]	320.4 ± 16.45[a]	304.0 ± 1.00[a]	**
ALT(U/L)	54.8 ± 4.98[b]	61.8 ± 7.95[b]	92.5 ± 5.52[a]	73.0 ± 10.00[ab]	**
Myoglobin (ng/ml)	196.5 ± 42.1[b]	567.1 ± 59.62[a]	668.2 ± 75.86[a]	591.6 ± 56.70[a]	**

*, p<0.05; **, p<0.01; [a, b, c], Different letters within the same row indicate differences among groups. CK, Creatine kinase; LDH, lactate dehydrogenase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Giannopoulos et al. (2006) reported that a 12 year old child was brought to the hospital with complaints of muscle pain, lethargy, cramps and difficulty walking, all of which started within 5 h after consuming quail and that the laboratory treatments results were CPK 17 870 U/L (NR 30 to 170 U/L), LDH 1140 U/L (90 to 120 U/L), AST 585 U/L (NR 5 to 50 U/L) and ALT 328 U/L (NR 10 to 55 U/L). A review of the results shows that CPK increased approximately 100 folds, while LDH, AST and ALT increased by 10 folds. These figures are not comparable with the increase in serum enzyme levels in the rats which were chosen as the animal subject used for the determination of the occurrence of rhabdomyolysis, which is probably due to the differences in the metabolic and detoxification mechanisms between rats and humans.

The syndrome of acute rhabdomyolysis develops when damage to striated muscles occurs. Muscle damage is usually attributed to toxic, ischemic, infectious, inflammatory or metabolic insults, as well as to direct muscle injury. A rarely reported cause of this syndrome is food poisoning due to the consumption of the European migratory quail (coturnism) in human. It was reported in the case of a 60 year-old male who was admitted to the hospital due to acute rhabdomyolysis occurring a few hours after quail consumption (Papanikolaou et al., 2001).

Coturnism has been known since ancient times and was first described in the Bible. It has been observed in the Mediterranean region (Aparicio et al., 1999). Clinical manifestations include pain in the muscles previously exerted during physical activity, muscular cramps and autonomic dysfunction occurring shortly (1.5 to 10 h) after the consumption of quail and usually last up to one to two days. Laboratory tests reveal increased levels in serum muscle enzymes, which usually return to normal values within one and two weeks, and myoglobinuria, which is responsible for the reddish-brown discoloration of urine (Papadimitriou et al., 1996). Four cases of coturnism were reported to be caused by quail consumption; and the patients were admitted with some combination of symptoms including muscle tenderness, extremity pain, nausea and vomiting. They were treated with vigorous isotonic crystalloid hydration and urine alkalinization (Korkmaz et al., 1970).

Tsironi et al. (2004) reported that the most important alkaloid of hemlock, red hempnettle and *G. ladanum* was coniine. In smaller doses, it produces neurotoxic effects, acute rhabdomyolysis and acute renal failure. They were not re-examined in the present study because coniine in seeds of *G. ladanum* was determined in a previous study, but the other fractions existing in plant were determined. Toxic substances in the *G. ladanum* seeds were identified with GC and GC/MS. The GC/MS analysis revealed fractions of pentanoic acid, phenylethanol, phenol 4-ethanyl-2 methoxy and phenethanol. New researches are needed for the examination of these substances.

Although, no pathological finding was observed in the tissue samples prepared from rats and quail for histopathological examination, the increase in serum myoglobin and enzyme levels may be due to subcellular changes in the target tissues of toxic substance.

In conclusion, no negative effect was observed on live weight, live weight gain and consumption of feed when *G. ladanum* plant seed was added to quail rations at the level of 2 and 4%. Although, no pathological finding was observed in the tissue samples, the increase in serum enzyme levels may be due to subcellular changes that may have occurred in the tissue. In addition, the differences between rats and humans with regard to metabolic and detoxification mechanisms may be a critical factor. In order for the muscle breakdown to be more pronounced in the histological examination, *G. ladanum* plant seeds could be added to the rations at higher levels.

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