Turk. J. Vet. Anim. Sci.
2008; 32(6): 475-482

Research Article

Toxicity of *Heliotropium dolosum*, *Heliotropium circinatum*, and *Senecio vernalis* in Parental Quail and Their Progeny, with Residue Evaluation of Eggs

Yesari ERÖKSÜZ[1,*], Ali Osman ÇERİBAŞI[1], Aydın ÇEVİK[1], Hatice ERÖKSÜZ[1], Fatma TOSUN[2], Uğur TAMER[3]

[1]Department of Pathology, Faculty of Veterinary Medicine, Fırat University, 23119 Elazığ - TURKEY

[2]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara - TURKEY

[3]Department of Analytical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Ankara - TURKEY

Received: 01.04.2008

Abstract: In all, 160 Japanese quail (80 male and 80 female) were divided into 4 groups (3 test groups and 1 control group). The test groups were fed a diet containing aerial parts (leaves, stems, and flowers) of *Senecio vernalis* (SV group), *Heliotropium dolosum* (HD group), or *Heliotropium circinatum* (HC group) at the level of 30% for 6 weeks, and the control group was fed 0% in order to evaluate parental and progenial toxicity, along with the transference of alkaloid residues to their eggs. The pyrrolizidine alkaloid content in the feed was 390 mg/kg in the HD group, 450 mg/kg in the HC group, and 420 mg/kg in the SV group. No clinical signs or death occurred in the test groups; however, egg production and hatchability significantly decreased in all test groups, as compared to the control group. In spite of the occurrence of specific biochemical and histopathological changes in parental quail, no remarkable changes were observed in their progeny on post-hatching days 0, 10, 20, 30, or 40. Gas chromatography and mass spectrometry (GC-MS) analysis of the eggs indicated the presence of 8.66 µg/g of the pyrrolizidine alkaloid europine in the HD group, 19.05 µg/g of europine and 1.46 µg/g of heliotrine in the HC group, and 3.21 µg/g of senecionine in the SV group at the end of study. In conclusion, the results of the present study provide experimental evidence that the alkaloids transferred to the eggs of quail fed high doses of pyrrolizidine alkaloid-containing plant material.

Key Words: Pyrrolizidine alkaloids, quail, egg, residue, transference, pathological findings, biochemical findings

Heliotropium dolosum, *Heliotropium circinatum* ve *Senecio vernalis*'in Ebeveyn ve Yavru Bıldırcınlarda Toksik Etkileri ve Yumurtada Pirolizidine Alkaloit Kalıntıları

Özet: Yüzaltmış adet Japon bıldırcını (80 erkek ve 80 adet dişi) üçü deneme ve bir tanesi de kontrol olmak üzere dört gruba ayrıldı. Deneme gruplarına % 30 oranında bitkisel materyal (H. dolosum, H. circinatum ve S. vernalis) içeren, kontrol grubuna ise bitkisel materyal içermeyen (% 0) izonitrojenik ve izokalorik rasyonlarla 6 hafta süreyle serbest yemleme yapıldı. Rasyonların pirolizidin alkaloid içerikleri HD grubunda 390 mg/kg, HC grubunda 450 mg/kg ve SV grubunda ise 420 mg/kg idi. Deneme süresince test gruplarında ölüm veya kaydadeğer bir klinik bulguya rastlanmamakla birlikte, altı haftalık deneme süresinin sonunda yumurta üretimi ve kuluçkalılık oranı tüm deneme gruplarında kontrollere göre önemli derecede azaldı. Ebeveyn bıldırcınlarda biyokimyasal ve histopatolojik değişimler görülmüş olmasına rağmen, yumurtadan çıkışı takiben 0, 10, 20, 30 ve 40. günlerde biyokimyasal ve histopatolojik olarak incelenen bıldırcın yavrularında önemli sayılabilecek bir bulguya rastlanmadı. Yumurtaların gaz kromatografik ve kütle spektrometkrik yöntemlerle incelenmesi sonucunda HD grubunda 8,66 µg/g europin, HC grubunda 19,05 µg/g europin ve 1,46 µg/g heliotrin ve SV grubunda ise 3,21 µg/g senesionin pirolizidin alkaloitlerinin varlığı saptandı. Sonuç olarak bu çalışmayla bıldırcınlarda yüksek oranlarda pirolozidin alkaloitlerinin tüketimi sonucu alkaloitlerin yumurtaya geçebileceği deneysel olarak ortaya konmuştur.

Anahtar Sözcükler: Pirolizidin alkaloitleri, bıldırcın, yumurta, rezidü, transfer, patolojik bulgular, biyokimyasal bulgular

* E-mail: yeroksuz@firat.edu.tr

Introduction

Pyrrolizidine alkaloids (PAs) are a large group of naturally occurring plant toxins that cause liver damage in livestock and humans. These compounds have been associated with a number of human poisoning cases (1). The major source of dietary exposure to PAs in humans is grains; however, other food sources, including eggs, milk, and honey, are minor dietary contributors (2). Although it is well known that excretion of PAs with food products can cause a potential health hazard, it is not possible to estimate dietary exposure to PAs from these food sources (2). A limited number of studies have been carried out to investigate the transfer of Pas to eggs as a possible route of excretion in quail and hens (3,4). Residual transfer of heliotrope alkaloids (168 mg/kg, 1.2 to 9.7 mg per egg), including europine, heliotrine, and lasiocarpine and their metabolites, was reported in hens that consumed 26 mg/kg of PAs from feed contaminated with *Heliotropium europeum* (3). In a feeding experiment, however, no free alkaloid was found in the eggs of laying hens fed with *Senecio vernalis* added to their diet at the rate of 4.0% (containing 60 mg/kg of alkaloid) for 210 days (4). As quail metabolize small amounts (8.50%) of ingested PAs (5) and are highly resistant to their toxic effects (6), more of these alkaloids might be transferred without massive pathologic changes (6,7); hence, quail might constitute a good experimental model for studying the residual transference of PAs to eggs.

Thus, the present study was designed to investigate the transference of PA residues to eggs, along with the effects of high level intake of dietary PAs in parental quail and their progeny.

Materials and Methods

Animals

The study included 160 Japanese parental quail (80 male and 80 female). The birds were obtained from a local hatchery, and they were raised in floor pens and fed ad libitum with conventional grower and layer diets until they were 74 days old. One day prior to initiation, the quail were randomly assigned into 4 groups, including 1 control and 3 test groups.

Experimental Design

The 160 quail were divided into 4 groups (3 test groups and 1 control group). The test groups were fed isonitrogenic and isocaloric diets containing 30% plant material, including *Heliotropium dolosum* (HD group), *Senecio vernalis* (SV group), and *Heliotropium circinatum* (HC group), and the control (C group) received 0%. Five pen replicates of 8 animals (40 quail per treatment) were arranged as couples in wire cages and fed the diets containing the dried and ground plant material for 6 weeks. The birds were caged as 1 male-1 female couples in stainless steel raised-bottom cages (30 × 20 × 20 cm) throughout the study. PA content of HC and SV feed were supposed to be the same as used in earlier studies (4,8), whereas the PA content and composition were re-determined for HD feed. The alkaloid content of the feed for the test groups was calculated as 390 mg/kg for the HD group, 420 mg/kg for the HC group, and 450 mg/kg for the SV group. The major specific PAs were europine (67.3%), heliotrine (16.3%), and lasiocarpine (8.1%) for the HC plant material, senecionine (66.65%), senecivernine (10.37%), and seneciphylline (8.51) for the SV plant material (4,8), and the major alkaloids in the HD plant material were lasiocarpine (43.97%), europine (25.95%), and heliosupine (18.49%).

All the procedures used in this experiment were approved by the Fırat University Ethics Committee.

Egg and Embryonic Evaluation

The eggs were collected every day at the same time (1000 hours) for 6 weeks and weighed using a digital scale. In all, 1918 eggs from the test and control groups were used for embryonic evaluation. The eggs were candled on the seventh day of incubation to differentiate infertile eggs from embryonic mortality.

Hatching

After being stored at 10-12 °C (70-75% relative humidity) for 7 days, the eggs from all the groups were incubated separately, at 7 day-intervals and 6 time periods: at 7, 14, 21, 28, 35, and 42 days of the experiment. The eggs were fumigated for 20 min with formaldehyde gas in an incubator and were turned automatically every 6 h every day. The incubation temperature was 37.7 °C and relative humidity was 62.0%. The egg groups were transferred to hatching trays and arranged separately on the 16th day of incubation. Unhatched eggs were broken and examined to confirm embryonal mortality.

Hatching Quail

The hatching birds were weighed the day they hatched and fed a standard diet for 10, 20, 30, and 40 days. At the end of these periods, 10 quail per treatment were bled and sacrificed to determine hepatic weight, and pathological and biochemical changes.

Diets

All the diets, which were devoid of any antibiotics or medication, were isonitrogenic (16.0% crude protein) and isocaloric (3.5% crude fat). Water and feed were provided ad libitum. The basal diet contained ground yellow corn (61.1%), soybean meal (17.5%), dehydrated alfalfa (13.9%), molasses (4.5%), and mineral and vitamin mix (3.0%).

Plant Materials

All the plants were collected from Elazığ and its vicinity in 2005. Dried and ground aerial parts of HC, HD, and SV were added to each diet at the rate of 30% as a component of the diets.

Feed Consumption

Feed consumption was determined on a weekly basis. To determine feed consumption of the males and females separately, 5 quail of each sex and group (75 days old) were fed individually for 2 weeks. The mean ratio of feed consumption between the males and females was calculated and applied to the original values.

GC-MS Analysis for PAs in Eggs

At the end of the sixth week, a total of 16 eggs, 4 eggs from each group, were sampled and put into glass tubes after the egg shells were broken. The tubes were stored at –20 °C until GC-MS analysis. PAs concentration in the eggs was measured by a slightly modified version of gas chromatography, as described previously (8). GC-MS was carried out with a Hewlett-Packard model 6890 gas chromatograph combined with a Hewlett-Packard model 5972 A MS detector. The column was HP-5 5.0% phenylmethyl siloxane (50 m 0.32 mm ID, 0.17 µm film thickness). Carrier gas was helium with a 1 ml/min flow rate. Injection temperature was 250 °C. Column temperature was programmed from 120 °C (5 min) to 260 °C (10 min) at the rate of 10 °C/min, splitless. Electron ionization (EI) data were acquired using 70-eV electron energy and the source temperature was 190 °C. Full scan data were adjusted by scanning the first quadrupole from 30 to 550 m/z in 1.0 s. Library search was carried out using the Wiley GC-MS library.

For sample preparation, contents of the eggs were stirred and lyophilized, and were later defatted with petroleum and extracted with methanol using a Soxhlet apparatus. Each extract was evaporated and taken up in 0.5 M H_2SO_4 and then reduced with Zn dust. The mixture was filtered and made alkaline with 25.0% ammonia solution. The homogenate was applied to an Extrelut (Merck, Darmstadt, Germany) column. The column was eluted with CH_2Cl_2. The eluate (CH_2Cl_2 fraction) was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at 40 °C. The residue was dissolved in CH_2Cl_2 for GC-MS determination. The external standard method was used for quantitative measurement. Senecionine was purchased from Fluka (17806, Buchs, Switzerland). The detection limit for the analysis was 0.137 µg/ml.

GC-MS Analysis for *Heliotropium dolosum*: Ten grams of air-dried and powdered aerial plant parts were homogenized twice in 0.5 M H_2SO_4, and each time were left to stand at room temperature for 1 h. Then, the mixture was filtered and divided into 2 equal portions, each of which was processed separately. The first portion was made alkaline with a 25% ammonia solution. The homogenate was applied on an Extrelut (Merck) column. The column was eluted with CH_2Cl_2. The eluate was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at 40 °C. The residue (fraction A) was regarded as free tertiary bases. The second portion was reduced with Zn dust, filtered, and made alkaline with a 25% ammonia solution. The homogenate was applied on an Extrelut (Merck) column. The column was eluted with CH_2Cl_2. The eluate was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure at 40 °C. The residue (fraction B) indicated total alkaloid content (tertiary bases and N-oxides). The PA content of fractions A and B was determined using GC-MS.

Biochemical Studies

Biochemical parameters, including direct bilirubin, total bilirubin, albumin, total protein, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamic transaminase (GGT), were determined using an autoanalyzer (Olympus AU600) at the end of the sixth week in the parental quail and on post-hatching days 10, 20, 30, and 40 in their progeny.

Necropsy and Histopathology

All tissues were grossly examined. Major organ weights and body weights were determined, and relative organ weights were then calculated in parental quail and

hatched quail at the ages of 1, 10, 20, 30, and 40 days. Organs, including the liver, spleen, heart, kidneys, pancreas, and small and large intestines, of all animals were fixed in 10% neutral formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin-eosin (H&E). As required, frozen sections were stained with oil Red O as well as with the van Gieson technique (9).

Statistical Analysis

Average cumulative feed consumption, weight gain, egg production, plant intake, and PA intake were calculated. All these parameters, organ indexes, and biochemical findings were compared between the groups by Duncan's test in order to detect significant differences (10). Fertility, hatchability, and embryonic mortality were compared using the chi-square test (11).

Results

All the birds survived until the end of study. There were no apparent exposure-related clinical signs in the male and female test groups.

Feed, Plant, and Alkaloid Intake

During the 6-week study period, mean cumulative feed consumption in both male and female test groups was significantly less than that of the controls. At the end of the sixth week, the HD group had the lowest feed consumption rate; the feed consumption rate was also lower in the HC and SV groups than in the controls (Table 1). Although there were no significant differences in plant consumption between the test groups, alkaloid consumption was highest in the HC group and lowest in the HD group (Tables 1 and 2).

Table 1. Total feed (g), plant (g/kg), and alkaloid consumption (mg/kg) in the study groups.

Sex	Parameters (0-6 weeks)	GROUPS			
		HD	HC	SV	C
Male	Feed Intake (g)	491.8[c]	600.2[b]	598.9[b]	686.4[a]
	Plant Consumption (g/kg)	862.0[a]	926.0[a]	908.0[a]	0.0[b]
	Alkaloid Consumption (mg/kg)	1120.0[c]	1388.0[a]	1274.0[b]	0.0[d]
Female	Feed Intake (g)	723.1[c]	763.3[b]	789.3[b]	972.1[a]
	Plant Consumption (g/kg)	1236.0[a]	1238.0[a]	1216.0[a]	0.0[b]
	Alkaloid Consumption (mg/kg)	1606.0[c]	1856.0[a]	1702.0[b]	0.0[d]

[a,b,c]$P < 0.001$.

Table 2. Initial and final body weight, changes in body weight, and egg production at the end of 42 days.

Female Groups	Body weight (g)			Total egg production (g)
	Initial	Final	Change	
HD	208.2	146.9[c]	–61.1[c]	115.0[d]
HC	228.8	179.5[b]	–49.3[b]	180.2[c]
SV	234.5	190.7[b]	–43.8[b]	232.1[b]
C	232.1	241.7[a]	9.6[a]	455.1[a]
Male Groups				
HD	198.2	152.7[c]	–45.4[c]	
HC	195.2	171.7[b]	–23.4[b]	
SV	199.3	166.6[b]	–32.66[b]	
C	191.6	214.5[a]	22.9[a]	

[a,b,c]$P < 0.001$.

Body Weight

Final body weight was significantly lower in all the test groups than in the control group (Table 2). The decrease from basal to final weight was most striking in the HD group.

Egg Production

Total egg production was significantly lower in all test groups than in the control group, and differences between the test groups were significant (Table 2). The HD group had the lowest egg production.

Absolute and Relative Organ Weights in Parental Quail

Both the absolute and relative organ weight changes were not statistically significant, except for liver weight in the HD group females (Table 3).

Biochemical Findings in Parental Animals

Serum biochemical changes are shown in Table 4. Significant differences in all the parameters examined were observed between the test and control groups, except for ALP activity in the HD group males.

Hatching Results

Statistical analysis results for fertility rate, hatchability, and number of embryonic deaths are shown in Table 5. With regard to hatchability of fertile eggs, embryonic death, and fertility rate, significant differences were observed between the all the test groups and the control group.

There were no apparent exposure-related clinical signs or teratologic effects observed in the hatched quail of the test group parents.

Body and Hepatic Weights in Progeny

On hatching day, body weight was lower in all the test groups than in the control group (Table 5). On the 10th post-hatching day, the difference was limited to only the HD group. There were no differences between the test groups and control group with regard to absolute (data not shown) and relative hepatic weight (Table 6) on examination days 0, 10, 20, and 40.

Table 3. Absolute and relative organ weights in the study groups.

Sex	Parameter	Organ	HD	HC	SV	C	P <
Male (n = 10)	Absolute weight (g)	Liver	2.5^{b}	2.3^{b}	2.1^{b}	3.6^{a}	0.001
		Lungs	1.2	1.2	1.1	1.3	NS
		Spleen	0.06	0.08	0.08	0.08	NS
		Heart	1.2^{c}	1.4^{b}	1.4^{b}	1.6^{a}	0.001
		Testes	3.2^{c}	4.2^{ab}	4.9^{b}	5.2^{a}	0.001
	Relative weight	Liver	1.7	1.5	1.2	1.6	NS
		Lungs	0.7	0.8	0.6	0.5	NS
		Spleen	0.05	0.05	0.04	0.05	NS
		Heart	0.8	0.9	0.8	0.7	NS
		Testes	2.0	2.8	2.7	2.5	NS
Female (n = 10)	Absolute weight (g)	Liver	2.1^{b}	2.4^{a}	2.3^{ab}	2.2^{ab}	0.05
		Lungs	1.2	1.2	1.3	1.3	NS
		Spleen	0.08	0.1	0.1	0.1	NS
		Heart	1.1	1.2	1.4	1.8	NS
	Relative weight	Liver	2.1^{b}	2.4^{a}	2.3^{ab}	2.3^{ab}	0.05
		Lungs	0.8	0.6	0.8	0.6	NS
		Spleen	0.05	0.05	0.05	0.04	NS
		Heart	0.7	0.7	0.6	0.7	NS

NS: Not significant. Relative weight: Organ weight × 100/body weight.

Table 4. Serum biochemical changes in the study groups.

Sex	Parameters	GROUPS				
		HD	HC	SV	C	P <
Male (n = 10)	AST (U/l)	515.0[a]	425.0[b]	470.3[b]	489.1[b]	0.05
	ALP (U/l)	36.9	29.7	33.3	22.5	NS
	Gamma-GT (U/l)	4.0[a]	4.8 [a]	3.0[b]	1.5[c]	0.001
	Total Billirubin (mg/dl)	4.0[a]	3.8[a]	2.7[b]	2.5[b]	0.05
	Total Protein (mg/ml)	0.9 [b]	0.6[bc]	0.5 [c]	1.9[a]	0.001
	Albumin (mg/ml)	0.7[c]	0.9[b]	1.4[a]	1.7[a]	0.001
	Direct Bilirubin (mg/dl)	0.03 [b]	0.03 [b]	0.1 [b]	0.08 [a]	0.05
Female (n = 10)	AST (U/l)	428.0[a]	353.7[ab]	431.7[a]	343.8[b]	0.05
	ALP (U/l)	27.6[c]	152.3 [ab]	99.1[bc]	205.0[a]	0.001
	Gamma-GT (U/l)	4.3[a]	3.0[ab]	3.2[ab]	1.8[b]	0.05
	Total Bilirubin (mg/dl)	2.3[a]	0.9[b]	0.5[c]	0.6[bc]	0.001
	Total Protein (mg/ml)	0.6[bc]	0.8[b]	0.5[c]	2.3[a]	0.001
	Albumin (mg/ml)	0.7[c]	0.9[b]	1.5[a]	1.7[a]	0.001
	Direct Bilirubin (mg/dl)	0.08[a]	0.03[b]	0.1[b]	0.03[b]	0.05

NS: Not significant.

Table 5. Hatching results in the study groups.

Parameters	HD	HC	SV	C
Total number of eggs	232.0	431.0	511.0	744.0
Fertility rate (%)	82.8[ab]	76.4[b]	84.1[a]	83.6[a]
Hatchability of fertile eggs (%)	68.5[b]	59.3[c]	68.9[b]	77.3[a]
Embryonic death (%)	14.7[ab]	18.7[a]	16.2[a]	10.1[b]

[a,b,c]P < 0.001.

Table 6. Body weight and relative hepatic weight in hatched quail (n = 30) on days 0, 10, 20, and 40.

Groups	Body weight (g) on day			
	1	10	20	40
HD	6.6 [b]	18.0 [b]	47.0	84.1
HC	6.18[c]	20.6 [ab]	47.8	84.0
SV	6.7[b]	19.9[ab]	47.0	89.5
C	7.6 [a]	20.5 [a]	47.0	94.6
Groups	**Relative hepatic weight (g) on day**			
	1	10	20	40
HD	4.0	4.3	3.9	2.6
HC	4.1	4.2	4.0	2.7
SV	3.9	4.3	4.0	2.9
C	4.0	4.2	4.2	2.7

[a,b,c]P < 0.001.

Gross and Histopathological Changes in Parental Quail

There were no remarkable gross changes in the test group animals. The severity and incidence of microscopical lesions were somewhat more pronounced in females than in males, and were most prominent in the HD group (Table 7). The main histological change was periportal or irregular oval cell proliferation in clumps or in cords (Figure 1). Bile duct hyperplasia and fibrotic liver changes, together with bile pigment deposits, were generally seen the HD group (Figure 2). Megalocytosis in hepatocytes was not detected in any of the test groups; however, mild cytomegaly was observed in all test groups. The cytomegalic cells had pale nuclei with marginated chromatin and prominent nucleoli of normal size. Proximal tubules in the kidneys also showed mild cytomegaly in the HD and HC groups.

PAs in Eggs

The only alkaloid present in the eggs of the HD group was europine (8.66 μg/g, 52 μg per egg). Both europine (19.05 μg/g, 15.12 μg per egg) and heliotrine (1.46 μg/g, 9.29 μg per egg) were present in the eggs of the HC group, whereas in the eggs of the SV group senecionine (3.21 μg/g, 22.27 μg per egg) was observed.

PAs in *H. dolosum*

Total PAs and tertiary base content of the aerial parts of *H. dolosum* were 0.13% and 0.06%, respectively. N-oxides corresponded to 53.85% of total alkaloids. Alkaloids in fraction A (free tertiary bases) and fraction B (tertiary bases and N-oxides) were identified as europine (8.37% and 25.95%), heliotrine (1.14% and 4.81%), lasiocarpine (76.31% and 43.97%), echimidine (3.76% and 6.78%), and heliosupine (10.42% and 18.49%).

Pathological Changes in Progeny during the Post-Hatching Period

Neither macroscopic nor microscopic changes were detected in quail hatchlings on post-hatching days 1, 10, 20, and 40.

Biochemical Changes in Quail at Post-Hatching Period

No differences in serum biochemical parameters were observed on post-hatching days 20, 30, and 40 (data not shown).

Discussion

Earlier studies of PAs in quail indicated that quail are highly resistant to the chronic effects of both *Senecio* spp. and *Heliotropium* spp. alkaloids (6,7). The present study is one of the few to examine the transference of PA residues to eggs. Previous chicken and quail studies only addressed the chemical analysis of eggs (3,4) or postembryonic toxicity following oral exposure of the dams (6). Several human case studies have indicated PA fetotoxicity with considerable histological liver damage (1); however, fetotoxicity studies of laboratory animals intravenously injected with 50-80 mg/kg of PAs (senecionine, monocrotaline, lasiocarpine, and heliotrine) indicated that these alkaloids cross the rat placenta and caused minimal microscopic changes in fetuses (12). In the present study senecionine (66.65% of total) in the SV group and europine (67.33% of total) in the HC group (4,8) were the 2 major alkaloids in the plant materials that transferred to eggs; however, lasiocarpine, a major alkaloid (43.97% of the total alkaloid content) in the HD group, could not be detected in the eggs, whereas europine, a minor alkaloid, was present in the eggs. This could be explained by differences in metabolism and chemical characteristics of each specific alkaloid, including reactivity, stability, and solubility. In our previous study laying hens fed a diet containing 4.0% *S. vernalis* consumed 1.55 mg/kg per day without transferring a detectable level of free alkaloids (4). In the present study daily senecionine intake in the SV group was 27.01 mg/kg—17.42-fold higher.

Decreased ALP activity in the laying quail in all the test groups could have been due their molting or low egg production, as suggested by our earlier study on hens fed

Table 7. Liver histopathological changes in the study groups.

Sex	Histopathological findings	GROUPS			
		HD	HC	SV	C
Male	Oval cell proliferation	9/20	8/20	6/20	0/20
	Fibrosis	5/20	0/20	0/20	0/20
	Bile pigment deposition	7/20	0/20	0/20	0/20
	Fatty change	7/20	5/20	6/20	4/20
	Early regenerative nodule	0/20	0/20	0/20	0/20
	Bile duct hyperplasia	3/20	1/20	0/20	0/20
Female	Oval cell proliferation	12/20	8/20	9/20	0/20
	Fibrosis	9/20	5/20	2/20	0/20
	Bile pigment deposition	9/20	4/20	1/20	0/20
	Fatty change	12/20	9/20	11/20	7/20
	Early regenerative nodule	3/20	2/20	0/20	0/20
	Bile duct hyperplasia	10/20	3/20	1/20	0/20

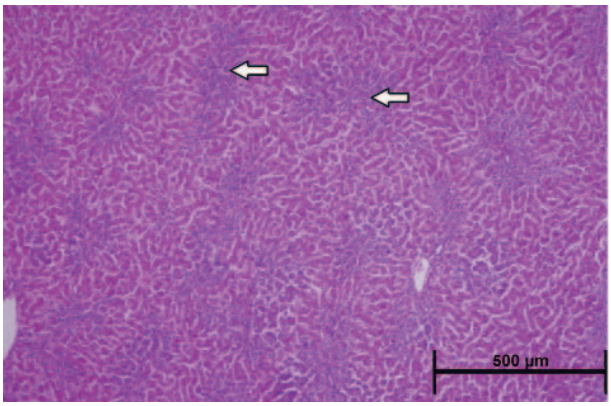

Figure 1. Multifocal oval cell proliferations (arrows) in the liver (HC group female).

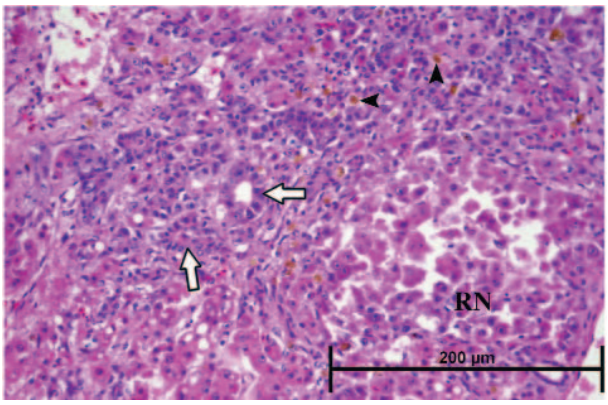

Figure 2. Bile duct proliferation (arrows), bile pigment deposition (arrow heads), and early regenerative nodule (RN) formation (HD group female).

Senecio vernalis (4). Toxic effects in parental animals in the present study were marked by specific pathological and biochemical changes, lower fertility rates, and higher embryonal mortality than in the control group; however toxic effects in the postembryonic period were not detected, in terms of gross, histopathological, and biochemical examinations, and hepatic weight of the progeny.

In conclusion, the results of present study provide experimental evidence of alkaloid transference to the eggs of quail fed diets containing high doses of PAs.

References

1. IPCS (International Programme on Chemical Safety): Environment Health Criteria 80, Pyrrolizidine alkaloids. World Health Organization, Geneva, 1988; 1-20, 81-85.
2. ANFSA (Australia New Zealand Food Authority): Pyrrolizidine Alkaloids in Food. A Toxicological Review and Risk assessment. Technical Series Report 2. Canberra, Australia, 2001; www.foodstandards.gov.au/-srcflies/TR.
3. Edgar, A., Smith, L.W.: Transfer of pyrrolizidine alkaloids into eggs: food safety implications. In: Tu, A.T., Gaffield, W. Eds., Natural and Selected Synthetic Toxins: Biological Implications. American Chemical Society, Washington D.C. 2000; pp. 118-128.
4. Eröksüz, H., Eröksüz, Y., Özer, H., Yaman I., Tosun, F., Akyüz-Kizilay, C., Tamer, U.: Toxicity of *Senecio vernalis* to laying hens and evaluation of residues in eggs. Vet. Hum. Toxicol., 2003; 45: 76-80.
5. Huan, J.Y., Miranda, C.L., Buhler, D.R., Cheeke, P.R.: Species differences in the hepatic microsomal enzyme metabolism of the pyrrolizidine alkaloids. Toxicol. Lett., 1998; 99: 127-137.
6. Buckmaster, G.W., Cheeke, P.R., Arscott, G.H., Dickinson, E.O., Pierson, M.L., Shull, L.R.: Response of Japanese quail to dietary and injected pyrrolizidine (Senecio) alkaloids. J. Anim. Sci., 1977; 45: 1322-1325.
7. Eröksüz, Y., Eröksüz, H., Özer, H., İlhan, N., Çevik, A., Yaman, I., Çeribasi, A.O.: Toxicity of dietary *Heliotropium dolosum* seed to Japanese quail. Vet. Hum. Toxicol., 2002; 44: 264-268.
8. Eröksüz, H., Eröksüz, Y., Özer, H., Çeribasi, A.O., Tosun, F., Tamer, U., Akyüz-Kizilay, C.: Toxicity of dietary *Heliotropium circinatum* to rats. Vet. Hum. Toxicol., 2003; 45: 198-201.
9. Luna, L.G.: Manuel of Histologic Staining Methods of Armed Forces Institute of Pathology. McGraw-Hill Book Company, New-york, 1968; 222, 226..
10. Snedocor, G.W., Cochran, G.: Statistical Methods. 6th edn., Iowa State University Press, Ames, Iowa: 1980; 507-508.
11. Evrim, M., Güneş, H.: Biyometri. İstanbul Üniversitesi Veteriner Fakültesi, Yayın No 31, İstanbul, 1994, 33-36.
12. Bhattacharyya, K.J.: Foetal and neonatal responses to hepatotoxic agents. J. Pathol. Bacteriol., 1965; 90: 151-161.