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The Effects of Feeding Pinus Pinea Seeds on Some Blood Values in Male New Zealand White Rabbits

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Abstract: A trial involving 16 male New Zealand white rabbits was conducted to determine whether there is any effect of Pinus Pinea seed supplementation on hematological parameters and organs. Rabbits (6-8 months old) were divided into two groups of 8 rabbits. Rabbits in the control group received 10 cc tap water for 49 days. Rabbits in the treatment group received 1 g kg^{-1} of Pinus Pinea seeds in 10 cc of tap water for 49 days. All treatments were given by oral gavage. At the end of the experiment, 10 cc of blood was withdrawn from the ear arteries of each rabbits and sacrificed. No significant differences were detected in mean hemoglobin, hematocrit, red blood cell count, white blood cell count, plasma protein, mean corpuscular hemoglobin concentration, percent neutrophil, eosinophil, basophil, lymphocyte and monocyte of rabbits in control and treatment groups. Overall blood parameters remained within the physiological range in both groups. Liver, kidney, lung, heart and body weights between control and treatment groups did not differ. Moreover, no apparent changes in liver, kidney, liver, testis and brain were detected by gross post mortem and histopatological examination to suggest toxic effect of oral use of Pinus Pinea seeds for 49 days. In conclusion, 49 days of Pinus Pinea seed supplementation did not cause any negative effects on the parameters tested in this study.

Key words: Pinus Pinea seed, rabbit, blood parameters, liver enzymes, hemoglobin, lymphocyte

INTRODUCTION

Pinus Pinea tree is a of Pinaceae family which is grown widely in Eurpoe, North America and also many countries found sides to Aegean and Mediterrnean Seas. Pinus Pinea seeds are frequently added to meat, fish and vegetable dishes. Pine nuts are also used in chocolates and desserts such as baklava (Ozguven and Vursavas, 2005). Pine nut is rich in energy (628 kcal) and consists essential nutrition ingredients such as proteins (11.6 g/100 g), carbonhydrates (19.3/100 g) and fatty acids (61 g/100 g) (Brufau *et al*., 2006). When we compared to total protein ingredients of pine nut to egg, pine nut is richer in tryptophan and histidine (Sabate, 1999). Moreover, the most nutritive structure in the Pinus Pinea seed is fatty acid (61%). This fatty acid composition is 85% unsaturated fatty acid and 15% saturated fatty acid. The most common unsaturated fatty acids are lineloic acid (48.4%), oleic acid (25.5%) and the biggest component is pineloic acid (14.9%) in the Pinus Pinea seed (Asset *et al*., 1999).

It has been previously shown that Pinus Pinea seed reduces blood-chlosterol level (Lee *et al*., 2004; Sabate, 2003; Asset *et al*., 1999). Due to high amount of fatty acid composition of Pinus Pinea seed and other nuts can reduce risk for obesity and also it can regulate the body weight (Lorda *et al*., 2003; Sabate, 2003; Hughes *et al*., 2008). On the other hand due to the small rate of lisin/arginin can reduce the risk of hypercholesterolemia and artherosclerosis (Brufau *et al*., 2006). Although, there are number of studies about hypercholesteromic, anticanserojenic, antioxidant properties of Pinus Pinea seed, there is no study about the toxicological properties of Pinus Pinea seed. Thus the purpose of this study was to determine the effects of Pinus Pinea seed on blood parameters and some liver enzymes of male New Zealand white rabbits.

MATERIALS AND METHODS

Animals and diets: This study was approved by the ethics committee of Suleyman Demirel University. About 6-8 month old male New Zealand white rabbits (n = 17) weighing 2.6 and 3.1 kg were used in the study. One rabbit in treatment group showed allergic reactions to the Pinus Pinea seed and excluded from the study. Thus, total of 16 rabbits were included in the study. Rabbits were housed individually in galvanized cages (50×50×50 cm) and held

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28 days for adaptation prior to experiment. The animals were kept standard laboratory conditions (12 h dark: 12 h light and 24±4°C) during the entire experimental period. The rabbits were fed standard commercial rabbit pellets (Ekinciler Food Company, Burdur, Turkey; 88% dry matter, 9% ash, 16%crude protein, 15% crude fiber and 2600 kcal kg^{-1} of metabolizable energy). Body weights were recorded weekly. Water and feed were provided *ad libitum* and feed intake was measured daily.

Preparation of Pinus Pinea seeds: Pinus Pinea seeds were collected from Kozak/Izmir, Turkey. Pinus Pinea seed-water combinations were prepared daily. Seeds were chopped in a mixer. Oral dose for an individual rabbit prepared separately according to the weekly body weights; 1 g kg^{-1} of crashed Pinus Pinea seeds were suspended in 10 cc of tap water then this mixture was used for oral gavages.

Experiment and sampling: Rabbits were divided into two groups of eight rabbits each. Rabbits in control group received 10 cc of tap water daily for 7 weeks. Rabbits in treatment group were exposed to same amount of Pinus Pinea seed mixture prepared daily.

At the end of the experiment, blood samples from each rabbits were collected from the ear artery. One day after the blood sample collection, the rabbits were euthanized. After opening the abdominal cavity of each animal, the liver, kidney, lung, brain, heart and testicles were excised.

The weights of the organs were recorded. Total erythrocyte and leukocyte were counted with haemocytometers (Merk, 1974). Plasma protein values were measured by a refractometer (Atago, SPR-N, Japan). Percent hemoglobin and hematocrit, concentrations were estimated by cynanmethemoglobin and microhematocrit methods, respectively (Merk, 1974). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentrations (MCHC) were calculated from the values of hematocrit (%) and hemoglobin (Jain, 1986).

Percent leukocyte distribution were determined from the blood smears that were stained with May Grunwald-Giemsa stain. *In vitro* enzymatic colorimetric method was used for the determination of glucose (Glucose GOD-PAP, Biolabo Sa, Maizy, France), triglyceride (TR-F400 CH, Chema Diagnostica, Via Pellegrini Jesi, Italy) and cholesterol (CT-F400 CH, Chema Diagnostica, Via Pellegrini Jesi, Italy) in serum. Alanine aminotransferase (ALT; kinetic enzymatic method without pyridoxal activation), serum alkaline phosphatase (ALP; optimized colorimetric method) and serum aspartate aminotransferase (AST; kinetic enzymatic method without pyridoxal activation) concentrations were measured by auto analyzer (The Roche Modular PP Auto Analyser, Roche Diagnostics, Mannheim, Germany).

Histopathology: After sacrifice, left kidney, left lung, brain, heart, left testicle and a 1 cm wide strip of the left median lobe of the liver was removed and placed into buffered formalin for histological examinations. All tissue samples were routinely processed into paraffin; 5 μm thick sections were stained with hematoxylin and eosin (H and E). The slides were coded and examined in a single-blind fashion by a pathologist.

Statistical analyses: Results are presented as mean±SE. Proc GLM procedure was used to analyze weekly body weight changes and feed intake. All other data were analyzed by Proc t-test procedure. All statistical analyses were carried out using SAS statistical package. The minimum level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Pinus Pinea seeds are consumed frequently due to their nutritive value in Turkey and other Mediterranean countries. The seeds can be consumed raw or roasted. They are used in a variety of traditional dishes. Because of the lack of information about the toxicological properties of Pinus Pinea seeds, it was evaluated any possible toxicological risk of consuming pine nuts for 49 day period. Overall, rabbits in both experimental groups showed no unusual behavior. No toxicological signs or death related to Pinus Pinea seed consumption were observed during the experiment.

Only one rabbit showed acute allergic reaction to Pinus Pinea seed. Time to onset of allergic reaction after oral gavage was 6.5 min. Allergic symptoms in the respiratory tract (wheezing, throat tightness, repetitive coughing and dyspne) were apparent. There was no change of body weight gain and average food intake between control and treated groups (Fig. 1 and 2). The body weight changes and feed intake of the groups followed a similar pattern indicating that pine nut treatments did not adversely affect the utilization of nutrients and metabolism of the animals. The hematological parameters are in Table 1. All parameters evaluated were within the normal values reported for New Zealand white Rabbits (Hein and Hartmann, 2003) and there were no significant differences between control and rabbits treated with Pinus Pinea seeds. Results in the hematological parameters suggested that oral treatment

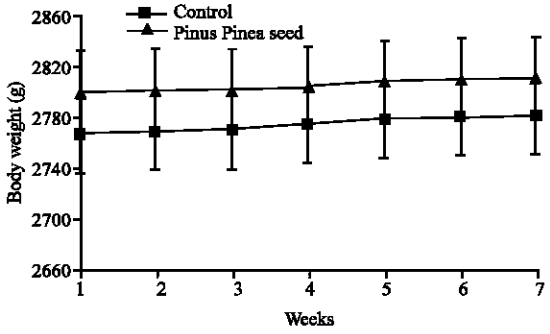

Fig. 1: Body weight changes of male New Zealand white rabbits during the experiment

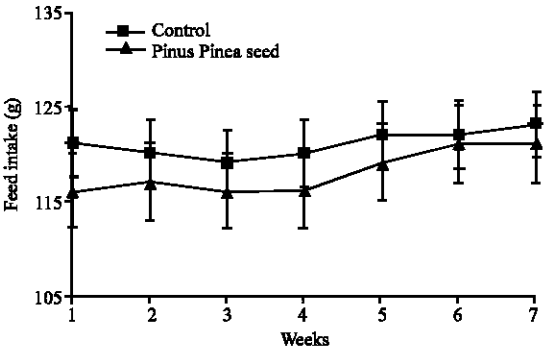

Fig. 2: Average weekly feed intake of male New Zealand white rabbits during the experiment

with Pinus Pinea seeds has no toxic effects on the hematopoiesis and leucopoiesis. Moreover, plasma concentrations of glucose and triglycerides were not affected by treatment. However, Pinus Pinea seed addition to diet tended to decrease cholesterol concentrations in plasma (Table 2).

ALP increases in numerous organs that show cellular damage. Increase in serum ALP levels usually implies a toxic effect on the liver (Ceron *et al*., 1995) whereas an increase in AST levels suggests damage in liver heart or muscle (Boyd, 1983). In the current study, Pinus Pinea seed treatment caused no significant alterations on serum ALT, AST and ALP levels and they were within the normal ranges for rabbits (Hein and Hartmann, 2003). Therefore, results indicated that the treatment has no significant adverse effect in treated rabbits. The weights of the organs were similar and there were no significant differences in organ weights between control and treatment groups. On gross pathological examination of the rabbits in both groups were performed after sacrifice. No treatment related changes were found in treated animals at necropsy. Gross examination revealed no absolute increase in organ weights tested in the current study (Table 3). No pathological lesions were observed in rabbits in different treatment groups. Histopathological parameters (liver, kidney, lung, spleen, heart and brain) also were tested to evaluate whether pine nut treatment resulted any organ toxicity. Including pine nut in the diet did not cause any adverse toxicological effects. No microscopic changes were apparent in any of the organs tested. The histopathological examination of the organs tested in the control and treated groups also showed no differences suggested that extract at dose tested did not result in any adverse toxicological effects on these organs.

Table 1: Effects of Pinus Pinea seed treatments on hematological parameters in New Zealand white male rabbits after oral gavages for 49 days (mean± standard error)

	Treatments		
Parameters	Control	Pinus Pinea seed	p<
Hemoglobin (g dL^{-1})	13.8±0.310	13.5±0.250	0.51
Hematocrit (%)	41.0±1.590	40.4±0.870	0.76
RBC ($\times 10^6$ μL^{-1})[1]	5.76±0.24	5.70±0.41	0.81
MCV (fL)[2]	71.1±2.800	70.4±1.400	0.79
MCH (pg)[3]	24.0±0.700	23.8±0.600	0.80
MCHC (%)[4]	33.8±1.180	33.5±0.800	0.79
WBC($\times 10^3$ μL^{-1})[5]	6.7±0.990	6.5±0.350	0.89
Plasma protein (g dL^{-1})	6.5±0.200	6.3±0.120	0.44
Differential Leucocytes (%)			
Lymphocyte	57.2±4.170	53.2±2.920	0.41
Pseudoeosinophil	34.5±4.010	38.5±3.660	0.42
Eosinophil	3.0±0.890	2.0±0.400	0.42
Basophil	0.6±0.330	0.5±0.290	0.73
Monocyte	4.7±1.050	5.8±0.850	0.48

[1]RBC = Red Blood Cell, [2]MCV = Mean Corpuscular Volume, [3]MCH= Mean Corpuscular Hemoglobin, [4]MCHC = Mean Corpuscular Hemoglobin Concentration, [5]WBC = White Blood Cell, [6]ALT = Alanine Aminotransferase, [7]AST = Aspartate aminotransferase, [8]ALP = Alkaline phosphatase

Table 2: Effects of Pinus Pinea seed treatments on biochemical parameters in New Zealand white male rabbits after oral gavages for 49 days (mean±standard error)

	Treatments		
Parameters	Control	Pinus Pinea seed	p<
ALT (IU L^{-1})[1]	37.75±2.65	37.83±1.790	0.97
AST (IU L^{-1})[2]	21.75±3.56	24.50±7.930	0.73
ALP (IU L^{-1})[3]	73.37±2.27	76.00±2.780	0.39
Glucose (mg dL^{-1})	104.85±9.07	105.62±11.33	0.95
Triglyceride (mg dL^{-1})	47.28±4.73	45.60±6.750	0.83
Total Cholesterol (mg dL^{-1})	24.14±1.39	19.54±2.240	0.09

[1]ALT = Alanine aminotransferase, [2]AST = Aspartate aminotransferase, [3]ALP = Alkaline phosphatase

Table 3: Effects of Pinus Pinea seed treatments on average feed intake and body and organ weights in New Zealand white male rabbits after oral gavages for 49 days (mean±standard error)

	Treatments		
Parameters	Control	Pinus Pinea see	p<
Body wseight (g)	2774±82	2805±92	0.52
Feed intake (g)	121.14±4.07	118.14±3.84	0.48
Liver weight (g)	70.98±3.58	75.07±3.08	0.22
Kidney weight (pair, g)	13.90±0.20	14.49±0.60	0.32
Lung weight (g)	11.11±0.20	11.40±0.20	0.78
Spleen (g)	0.93±0.03	0.91±0.03	0.57
Heart weight (g)	6.70±0.19	6.75±0.41	0.80
Brain (g)	8.72±0.19	8.85±0.20	0.65
Testis weight (pair, g)	7.33±0.17	7.63±0.14	0.24

CONCLUSION

In conclusion, findings of the current study suggested that Pinus Pinea seeds have no toxic effect in male New Zealand white rabbits for the 7 week study period. However, Pinus Pinea seeds should be consumed carefully since they have potential allergic properties.

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