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Safety and Effects on Lipid Parameters of *Rubus coreanus* and *Atractylodes japonica* in Ovariectomized Rats

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Abstract

Defatted methanol extracts of the medicinal plants, *Rubus coreanus* Miq. (RC) and *Atractylodes japonica* Koidzumi (AJ) were added at the levels of 0.1, 0.5, or 2% (w/w) to high cholesterol diets and fed to ovariectomized Sprague-Dawley female rats, weighing 212.6±1.8 g for four weeks. Weight gains were lower in RC and AJ groups than the control group, but there were no changes in uterus weights. Serum levels of triglyceride decreased by 20~27% in the experimental groups fed 0.1% of each extract (0.1RC and 0.1AJ), compared with that of control (Ovx). Serum cholesterol levels were not changed in the RC groups but increased in the group fed 2% of the AJ extract. Liver levels of cholesterol and triglyceride were reduced in both the RC and AJ groups. Microscopic observation revealed that there were no morphological alterations in liver, lung, heart, spleen and kidney tissues of the experimental groups. Plasma levels of albumin, BUN, creatinine, sodium, potassium and phosphate in the RC and AJ groups were in normal ranges. Serum GOT and GPT activities were, however, higher in the 2.0AJ than Ovx group. These results suggest that the extracts of the *Rubus coreanus* Miq. and *Atractylodes japonica* Koidzumi at dietary levels as low as 0.1% may be utilized as hypotriglyceridemic ingredients for functional foods.

Key words: *Rubus coreanus* Miq., *Atractylodes japonica* Koidzumi, lipid, tissue morphology

INTRODUCTION

Rubus coreanus Miq. (Rosaceae, Rubi Fructus), a type of red raspberry, grows wild in Korea and China and is gathered for its unripe fruit, which is a folk medicine used for treatment of impotence and as a diuretic. The botanical origin of the Rubi Fructus found in the Korean crude drug market is mostly derived from the fruits of *R. crataegifolius* and seems to be sometimes mixed with the fruit of *R. parvifolius. R.* sp. which is closely related to *R. phoenicolasius* (1). The fruits are rich in sugars, organic acids and several vitamins, as well as triterpenoids (2,3), and also include various antioxidants, tannins (4) and phenolic acids (5). Extracts of the fruit have been found to show considerable antioxidant activity in various test systems irregardless of ripeness (6). Kim et al. (7) have reported that the number and size of follicles in rat ovary were increased by the administration of the water extract. Lee et al. (8), on the other hand, have reported that both water and ethanol extracts of the fruit inhibited the growth of various types of cancer cells and stimulated T and B cell activities. However, these physiological effects have not been clearly related to active compounds of fruits of *R.* sp.

The rhizome of *Atractylodes japonica* Koidzumi (Compositae) has been used clinically in oriental medicine for diuretic and analgesic purposes as well as for stomach disorders. Atractylon and its derivatives, with significant hypoglycemic (9) and antihepatotoxic effects (10) were isolated from its rhizomes. Yu and Hwang (11) have reported that *A. lancea* DC, which is similar to *A. japonica* Koidzumi has shown proliferative effects in bone marrow cells. The effect has been attributed to stimulation of the intestinal immune system by active polysaccharide components of the plant (12), but other possibilities can not be excluded. Recently, during our preliminary screening for phytoestrogens from 50 kinds of oriental medicinal herbs, it was revealed that methanol extracts of *A. japonica* and *R. coreanus* have strong proliferative effects on MCF-7 estrogen-dependent human breast cancer cells and osteoblast-like ROS 17/2.8 cells. This result suggests that *A. japonica* and *R. coreanus* may have components with estrogen-like activity (13).

The purpose of the present study was to investigate

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the *in vivo* effects of the methanol extracts of *R. coreanus* and *A. japonica* on lipid metabolism using ovariectomized female rats, and its safety was also examined to determine the safety of its use in functional foods.

MATERIALS AND METHODS

Preparation of methanol extracts from medicinal plants

One hundred grams of ground dried fruit of *R. coreanus* (RC) and rhizome of *A. japonica* (AJ) were extracted three times with 1 L of 80% aqueous methanol at room temperature, filtered, and evaporated under reduced pressure. The concentrated methanol extracts were redissolved with 80% aq. methanol (100 mL) and washed twice with *n*-hexane (200 mL) to remove lipids. Yields of the defatted methanol extracts from RC and AJ were 8.9 and 10.2%, respectively. These extracts were added at the level of 0.1, 0.5 and 2% to the synthetic diets of the ovariectomized female rats as described below.

Animals and diets

Seventy female Sprague-Dawley rats weighing about 180±5 g (8 weeks old) were obtained from the Korean Center for Experimental Animals (Daegu, Korea) and maintained on standard chow in a facility equipped with stainless steel cages that had wire mesh bottoms, maintained a constant temperature (22±1°C) and lighting (light on, 08:00~20:00 h) for 2 weeks, and then were ovariectomized. Average weight of rats at this point were 212.6±1.8 g, the initial weight for the following four weeks of experimental feeding. The surgery was conducted under anesthesia by intraperitoneal injections (2 mL/kg) that consisted of a mixture of pentobarbital (50 mg/mL) and urethane (230 mg/mL). Postsurgery, ovariectomized rats were then randomly allocated to seven groups (each n=10), one control group (Ovx) and three groups of *Rubus coreanus* Miq. (RC) and *Atractylodes japonica* Koidzumi (AJ), depending on the level of the extracts in the diet. The dietary levels of RC and AJ methanol extracts were 0.1, 0.5, or 2% (w/w) and accordingly, the six test groups were designated 0.1RC. 0.5RC, 2.0RC, 0.1AJ, 0.5AJ, and 2.0AJ. The basic diet for the seven groups consisted of the AIN76 composition for rats (14) with the addition of 0.5% cholesterol. Addition of the plant extracts and cholesterol was substituted for equal weights of the starch contents in the respective diets. During the four week feeding period, six experimental groups (each of the three RC and AJ groups) were isocalorically *pair-fed* to Ovx. Food intake and body weight were measured daily and every three days, respectively. The experiment was according to the guidelines for animal experiments provided by Catholic University of Daegu.

Biochemical analysis

After four weeks of the experimental diet the rats were anesthetized with ether, and blood was drawn from abdominal *vena cava* into a BD vacutainer tube containing polymer barrier material. Serum was prepared by centrifugation and used for lipid determination without freezing and later stored at -70°C for measuring other blood indices by an automatic analyzer. The liver was excised after removing the blood. It was blotted dry and quickly frozen in liquid nitrogen for subsequent measurement of lipids. Serum total cholesterol, HDL-cholesterol and triglyceride (TG) were measured by using an enzymatic kit (Shinyang Chemical Co., Seoul, Korea). Lipids from rat liver were extracted by the method of Folch et al. (15) and cholesterol and triglyceride were measured with the aid of the detergent, triton X-100 (16) using an enzymatic kit (Shinyang Chemical Co., Seoul, Korea). Thiobarbituric acid reactive substances (TBARS) in serum and liver were measured by the methods of Yagi (17) and Ohkawa et al. (18), respectively, using 1,1,3,3-tetraethoxyporpane as the standard.

Blood indices and tisuue morphology

Plasma levels of total protein, albumin, BUN, creatinine, sodium (Na) and potassium (K) were measured by an automated analyzer (Cobas Integra 700, Roche, Switzerland). For tissue morphology, small pieces of liver, lung, heart, spleen and kidney tissues were excised and fixed in 2.5% glutarylaldehyde. The fixed tissues were successively dehydrated by ethanol. After staining with hematoxylin-eosin tissue morphology was examined under a light microscope (19).

Statistical analysis

Student's *t*-test was used to compare differences between the Ovx and RC or AJ groups, and differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Body and organ weights

All seven experimental groups started with similar mean body weights of 212.6±6.5 g but at the end of the study, all three RC groups had gained significantly less weight. The AJ groups generally appeared to gain smaller weights too, even though only the 2.0% AJ group showed significance (Table 1). Both RC and AJ groups tended to eat less food, but food efficiency ratios of all groups were parallel to those of their weight gains. It has been reported that animals gain weights after ovariectomy compared with normal or sham-operated ani-

Table 1. Body weights, weight gains and food efficiency ratios of control and experimental groups of ovariectomized rats for four weeks

Group	Initital wt (g)	Final wt (g)	Weight gain (g/day)	Food intake (g/day)	Food efficiency ratio
Ovx	211.0±10.7	312.7±17.8	2.97±0.15	14.60±0.27	0.202±0.007
R. coreanus Miq.					
0.1RC	213.5±12.2	297.9±23.1	2.47±0.25*	13.77±0.57	0.176±0.013*
0.5RC	213.4±11.3	300.1±14.7	2.58±0.22*	13.98±0.57	0.181±0.010*
2.0RC	216.1±8.2	297.2±22.6	2.36±0.17*	14.42±0.44	0.175±0.009*
A. japonica Koidz.					
0.1AJ	210.0±10.7	308.5±13.9	2.89±0.08	14.45±0.23	0.200±0.003
0.5AJ	212.2±10.8	303.5±11.8	2.67±0.12	13.90±0.29	0.192±0.008
2.0AJ	211.7±12.0	300.3±20.9	2.61±0.18*	14.19±0.40	0.182±0.009*

Ovx group was fed a basic diet described in Materials and Methods. Three RC and AJ groups each fed diets containing additional methanol extracts of *R. coreanus* Miq. and *A. japonica* Koidz. at the level of 0.1, 0.5 and 2%, respectively. Data are mean±SD values for groups of 9~10 rats each. Values with asterisk (*) are significantly different from that of Ovx at $p<0.05$.

mals (20). The higher weight gain after the ovariectomy is ascribed mainly to a lack of estrogen action of suppressing lipoprotein lipase (21) and stimulating hormone sensitive lipase (22) in adipose tissue. It is of interest whether substance(s) present in RC or AJ may play similar roles in suppressing weight gains to that of estrogen, since their methanol extracts exhibited estrogenicity in MCF-7 estrogen-dependent human breast cancer cells and osteoblast-like ROS 17/2.8 cells (13). Even if they do, the uterus weights were as small as that of Ovx (Table 2), suggesting that the putative estrogenic activities in the extract were not strong enough to restore the uterus to a normal size of 300~350 mg (20). Therefore, it is not clear how RC groups gained less weight, even though their food intakes were not very different from that of Ovx group. Except for uterus weights, the five other organ weights in the experimental animals were shown to be equal relative to their body weights (Table 2). Weights of liver, heart and spleen were not different among the experimental groups. On the other hand, kidney weights tended to be larger while lung weights were smaller in the RC and AJ groups compared with Ovx.

Cholesterol and triglyceride levels in serum and liver

After four weeks of experimental diets, serum levels of total cholesterol were not different among the groups except for an increase in 2.0AJ group (Fig. 1). Serum levels of HDL-cholesterol (lower part of each bar in the figure) were not changed by diets containing either RC or AJ. This may imply that LDL+VLDL cholesterols were increased in 2.0AJ group compared with Ovx. On the other hand, serum levels of triglyceride were reduced in all of the RC and AJ groups. Liver levels of cholesterol and triglyceride of the experimental groups (Fig. 2) were somewhat similar to those seen in the serum, but cholesterol concentrations in the liver of the 2.0AJ group were not increased, and decreases in liver triglyceride levels were only significant in the 2.0RC and 2.0AJ groups.

From these results, it is apparent that RC and AJ methanol extracts had a more pronounced effect on serum and liver triglyceride levels than on cholesterol levels. Further study is needed on the mechanisms of

Table 2. Organ weights of control and experimental groups of ovariectomized rats for four weeks (g/100 g bw)

Group	Uterus (mg/rat)	Liver	Heart	Lung	Kidney	Spleen
Ovx	94±50	2.90±0.23	0.371±0.038	0.508±0.021	0.499±0.008	0.242±0.028
R. coreanus Miq.						
0.1RC	90±17	2.97±0.26	0.361±0.014	0.484±0.024	0.518±0.039	0.226±0.034
0.5RC	95±30	3.07±0.38	0.362±0.008	0.476±0.030	0.518±0.015	0.251±0.034
2.0RC	109±28	3.05±0.56	0.376±0.008	0.459±0.034	0.487±0.015	0.252±0.016
A. japonica Koidz.						
0.1AJ	87±21	3.28±0.46	0.364±0.021	0.456±0.027	0.527±0.016	0.260±0.021
0.5AJ	76±16	3.06±0.28	0.356±0.041	0.467±0.020	0.511±0.018	0.226±0.049
2.0AJ	94±13	3.38±0.52	0.362±0.036	0.479±0.033	0.539±0.025	0.227±0.014

Conditions are the same as described in Table 1.
Data are mean±SD values for groups of 9~10 rats each.

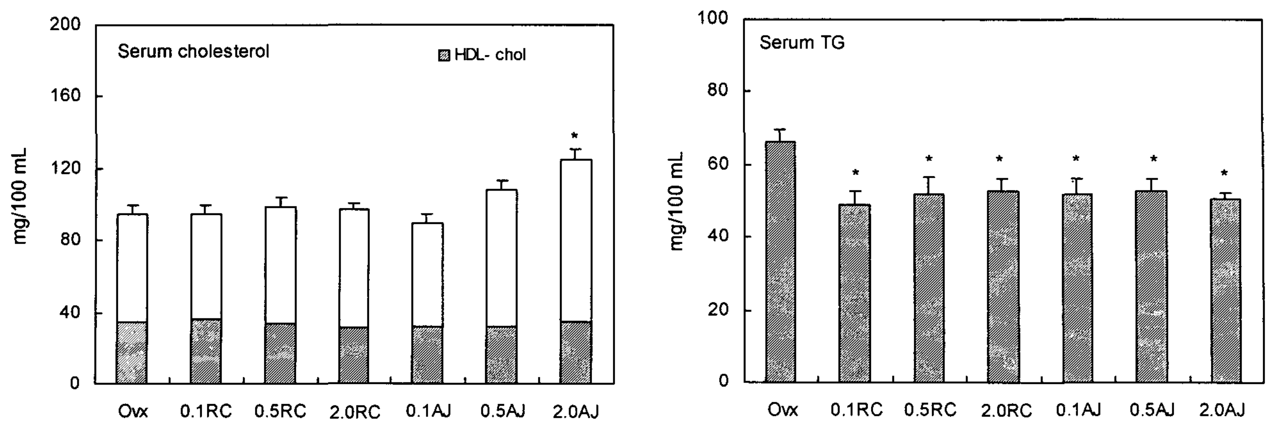


Fig. 1. Serum levels of cholesterol and triglyceride in control and experimental groups of ovariectomized rats for four weeks. Ovx group was fed a basic diet described in Materials and Methods. Three RC and AJ groups fed diets containing additional methanol extracts of *R. coreanus* Miq. and *A. japonica* Koidz. at the level of 0.1, 0.5 and 2.0% each. Values with asterisk (*) are significantly different from that of Ovx at $p<0.05$.

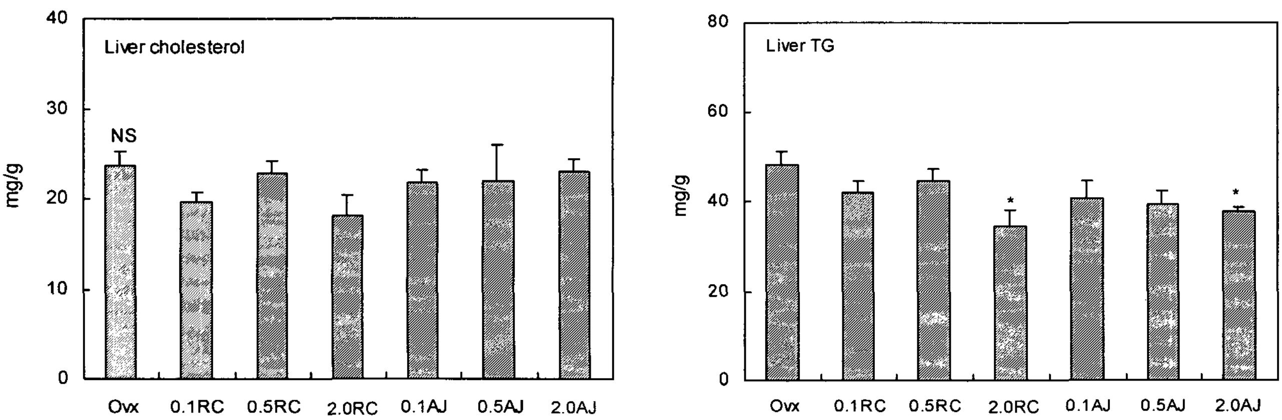


Fig. 2. Liver levels of cholesterol and triglyceride in control and experimental groups of ovariectomized rats for four weeks. Conditions are the same as described in Fig. 1. Values with asterisk (*) are significantly different from that of Ovx at $p<0.05$.

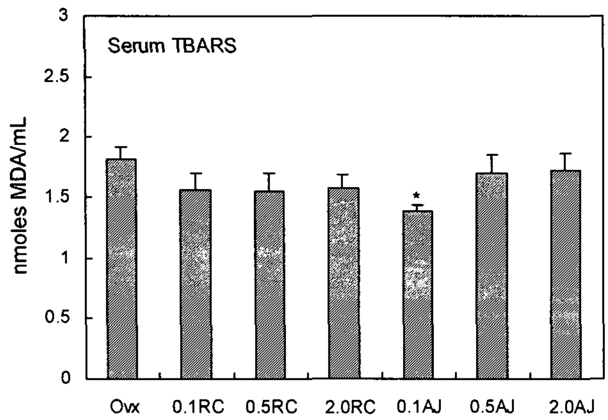
action of the active components in RC and AJ methanol extracts.

TBARS in serum and liver

Concentrations of thiobarbituric acid reactive substances (TBARS), indicative of lipid peroxidation in serum and liver are shown in Fig. 3. TBARS were not very different among groups, but were significantly lower in the 0.1AJ group than those of Ovx. TBARS contents of Liver were, however, generally lower in all RC and AJ groups than those of Ovx with significance reductions in 0.1RC and all of AJ groups. Judging from the contents of vitamins and polyphenols (2-5) in RC and AJ and from the result of another study (6), it was predictable that RC and AJ groups in the present study would have reduced levels of TBARS in the serum as well as in the liver. The lack of change in the serum levels may be due to well maintained homeostasis in the blood. The discrepancy from the result of another study (6) could be ascribed to differences in the test system, i.e. *in vitro* vs *in vivo* in the present study. Antioxidant activity of AJ appeared to be related to its radical scavenging and therefore, antihepatotoxic effects were shown in rat hepatocytes (10). The functional substances from RC and AJ need to be isolated and further tested for antioxidant activity *in vivo* in future studies.

Blood indices, organ weights, and tissue morphology

Blood indices used for common vital signs were measured in rats fed RC and AJ methanol extracts and compared with the Ovx group. As shown in Table 3, levels of albumin, blood urea nitrogen (BUN), creatinine, Na and K were not different among control and experimental groups. Serum GOT and GPT levels were not changed the groups fed 0.5% RC or AJ, but GOT and GPT levels were significantly increased in the 2.0AJ group, while only the GPT level was increased in the 2.0RC group compared with the Ovx group, although the elevated levels were within the normal range reported for rats (23). There were no significant morphorogical alterations in kidney, spleen, heart, lung and liver tissues by feeding RC and AJ methanol extracts (Fig. 4).



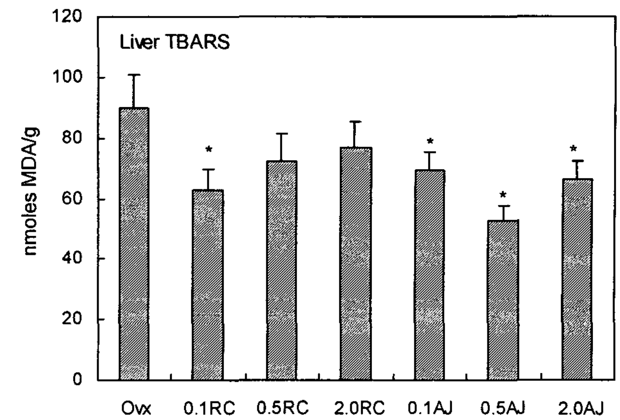


Fig. 3. Serum and liver levels of TBARS in control and experimental groups of ovariectomized rats for four weeks. Conditions are the same as described in Fig. 1. Values with asterisk (*) are significantly different from that of Ovx at $p<0.05$.

Table 3. Serum levels of total protein, albumin, BUN, creatinine, Na, K, GOT and GPT of control and experimental groups of ovariectomized rats for four weeks

Group	Total protein (g/dL)	Albumin (g/dL)	BUN (mg/dL)	Creatinine (mg/dL)	Na (mEq/L)	K (mEq/L)	GOT (Units/mL)	GPT (Units/mL)
Ovx	6.80±0.25	2.95±0.11	13.10±0.71	0.78±0.03	145±1.8	4.98±0.88	63.1±5.7	22.1±3.6
R. coreanus Miq.								
0.1RC	6.87±0.12	2.92±0.05	13.53±0.52	0.74±0.02	145±0.4	5.39±0.38	73.6±9.1	21.7±2.4
0.5RC	6.95±0.16	3.04±0.10	12.98±0.65	0.76±0.03	146±0.8	4.93±0.14	61.2±6.6	23.6±3.4
2.0RC	6.60±0.16	2.80±0.06	13.07±1.12	0.80±0.02	144±0.5	5.12±0.16	73.9±14.1	34.1±5.4*
A. japonica Koidz.								
0.1AJ	6.90±0.22	2.94±0.07	13.20±0.64	0.83±0.03	145±0.4	5.03±0.32	77.7±12.5	31.6±8.4
0.5AJ	6.92±0.17	2.99±0.05	12.79±0.67	0.77±0.02	145±0.5	5.20±0.30	63.2±6.4	23.9±4.2
2.0AJ	6.95±0.08	3.00±0.03	12.99±0.34	0.75±0.02	145±0.5	5.61±0.49	87.2±12.5*	37.9±4.4*

Conditions are the same as described in Table 1.
Data are mean±SD values for groups of 9~10 rats each.
Values with asterisk (*) are significantly different from that of Ovx at $p<0.05$.

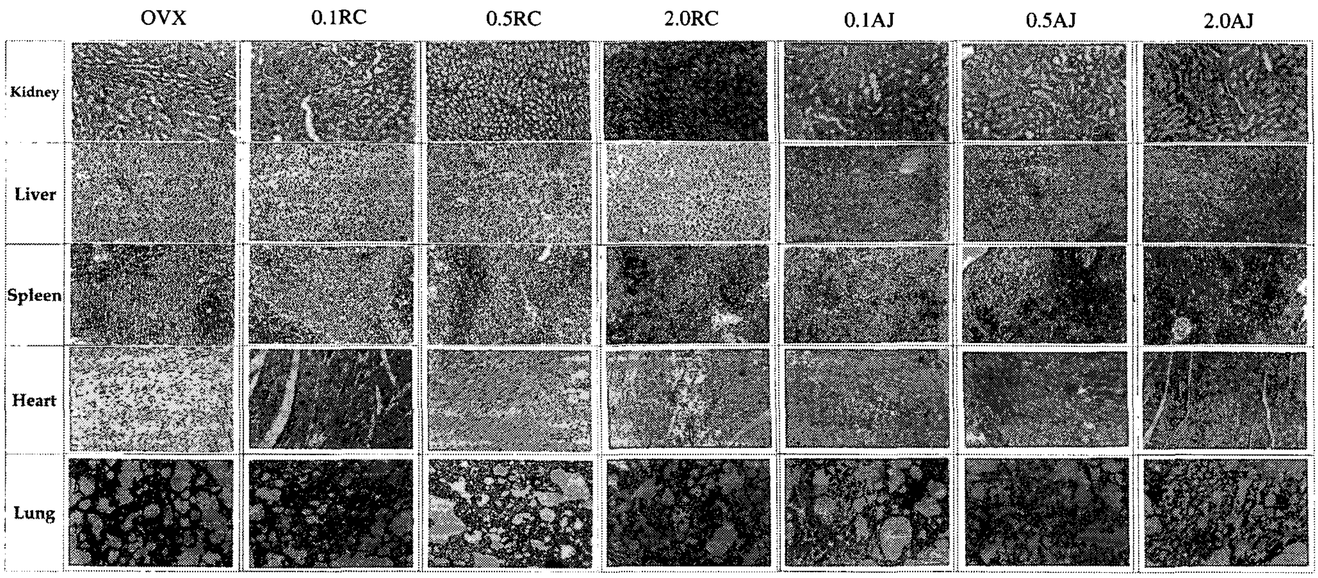


Fig. 4. Light micrographs of kidney, liver, spleen, heart and lung of ovariectomized rats fed control and experimental diets for four weeks. Conditions are the same as described in Fig. 1.

CONCLUSION

The present results show that *R. coreanus* and *A. japonica* have hypotriglyceridemic effects. The effect is regarded as meaningful to Koreans since hypertriglyceridemia is prevalent compared to that observed in Western people. The effect also appears to be related to low weight gain. Although the methanol extracts of either *R. coreanus* or *A. japonica* did not improve serum cholesterol status, they may be able to effectively reduce tissue or serum lipid peroxidation, which can lead to serious degenerative diseases. It is of significance that the effects were clearly observed at safe levels, as low as or lower than 0.1 or 0.5% of the extracts. Otherwise, serum cholesterol and GOT levels were increased by 2% AJ. Therefore, *R. coreanus* extract is believed to have the greater potential as a functional foods ingredient in terms of safety and efficacy.

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