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Original Article

Effect of Dietary Oyster Extract on the *p*-aminophenol-induced Nephrotoxicity in Rats

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Summary

Recently, chronic kidney disease (CKD) has been estimated to affect about 20 million people in Japan. CKD is associated with a several fold increased risk of arteriosclerosis related disease. Oysters (*Crassostrea gigas*) contain many nutrients and are used widely in Japan. Oyster extract contains major components including minerals, carbohydrates, protein, and amino acids. Various effects on health maintenance and life-style related diseases of oyster extracts have been reported so far. The present study evaluated the preventive effects of oyster extract on the formation of chemically induced nephrotoxicity in rats. Rats fed an AIN-93G modified diet containing oyster extract (1.0 %) After 6 weeks, the rats were injected intraperitoneally with *p*-aminophenol (PAP, 0.5 mmol/kg body weight). Urine was collected before and after injection, and the kidney was removed at 48 h after injection.

Dietary oyster extract caused a decrease in blood urea nitrogen (BUN), return to the normal levels of urine volume, urinary N-acetyl-β-D-glucosaminidase (NAG) activity, and creatinine concentration, and reduction in the severity of tubular basophilic change and nuclear division in tubular epithelium compared with control diets. However, the severity of tubular necrosis and tubular basophilic change in the midzone area and vacuolar degeneration in renal and arcuate arteries were not significantly different. Dietary oyster extract recovered proximal tubular epithelial cell function in PAP-induced nephrotoxicity in rats.

Introduction

Recently in Japan, chronic kidney disease (CKD) has been estimated to affect about 20 million people. CKD is associated with a several fold increased risk of arteriosclerosis related disease such as cardiovascular disease (CVD)[1)]. In addition, kidney injury in diabetes is mediated by several growth factors that are synthesized in higher amounts, e.g., angiotensin II (Ang II), transforming growth factor beta, vascular endothelial growth factor, and connective tissue growth factor [2,3)]. Therefore, it is important to consider strategies to reduce CKD associated with metabolic syndrome because CKD is also a risk factor for the development of CVD, and consequently for the increased morbidity and mortality associated with this disease [4)].

p-aminophenol (PAP) is a well-known metabolite of acetaminophen and phenacetin and a derivative of anilines in industrial use [5−8)]. PAP induces acute tubular necrosis affecting the proximal straight tubule and causes elevated blood urea nitrogen (BUN) concentration within 24 h of administration in rats [9)]. Certain urinary enzyme activities can be used as an index of renal damage [10)].

Oyster (*Crassostrea gigas*) extract contains many nutrients and is used widely in Japan. The major components of oyster extract include minerals, carbohydrates, protein, and amino acids. In particular, many trace nutrients such as zinc are present at high levels. Various nutrient availabilities for health maintenance and life-style related diseases from oyster extract have been reported [11−13)].

The present study examined the effect of oyster extract on the biochemical parameters of urine and serum and renal histopathology on PAP-induced nephrotoxicity in rats.

Material and Methods

1. Materials

Oyster extract was obtained from Japan Clinic Co., Ltd.

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(Kyoto, Japan). *p*-aminophenol hydrochloride (PAP) was purchased from Nacalai Tesque (Kyoto, Japan). All of the diet components were products of Oriental Yeast (Tokyo, Japan).

2. Animals care

All experimental protocols were reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the "Guide for the Care and Use of Experimental Animals" of the Ministry of Education, Culture, Sports, Science and Technology of Japan. Three-week-old male Fischer 344 rats were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). The rats were housed in plastic cages in a temperature-controlled room (22–24℃) under a 12 h light/dark cycle. Rats were assigned to groups (12 rats each) that were fed either of two diets (Control group, Oyster Group) prepared according to the AIN-93G (Control group) and modified diet containing 1 % (w/w) oyster extract (Oyster group) (Table 1)[14]. After 42 days, the rats were divided into groups of six (Control, Control+PAP, Oyster, and Oyster+PAP). Control+PAP and Oyster+PAP groups were injected intraperitoneally with 0.5 mmol/kg body weight of PAP in 0.9 % NaCl. Urine was collected every 24 h for two days prior to and for two days following the injection, urine volume was measured by graduated cylinder before being stored at −80℃ until analysis.

Table 1 Composition of experimental diets (g/kg)

Component	Control	Oyster
	(g/kg diet)	
α-corn starch	132.0	130.7
β-corn starch	397.5	393.5
Casein	200.0	198.0
Sucrose	100.0	99.0
Cellulose powder	50.0	49.5
Mineral mixture*	35.0	34.7
Vitamin mixture*	10.0	9.9
L-cystine	3.0	3.0
Choline bitartrate	2.5	2.5
Soybean oil	70.0	69.3
Oyster extract	-	10.0

Diets were prepared based on the AIN-93G composition.
*AIN-93G formula.

After injection of PAP for 48 h, under ether anesthesia, blood was collected, and serum was harvested by centrifugation at 3,000 *g* for 15 min before being stored at −80℃ until analysis. The kidney was rapidly removed in their entirety and were rinsed, frozen in liquid nitrogen, and stored at −80℃.

3. Biochemical measurements of urine and serum

Urine level of urea creatinine (U-Cre), γ-glutamyltransferase (GTP), and N-acetyl-β-D-glucosaminidase (NAG) activity were assayed using commercial kits ; for U-Cre, Creatinine Test Wako (Wako Pure Chemical Industries) ; γ-GTP, γ-GTP c-Test Wako (Wako Pure Chemical Industries) ; and for NAG, NAG-Test SHIONOGI (Shionogi Pharmaceuticals Ltd., Osaka).

Serum levels of total protein (TP), albumin (Alb), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) were assayed using an Olympus AU 5431 automatic analyzer. Blood urea nitrogen (BUN), creatinine, and uric acid (UA) were assayed using commercial kits ; for BUN, BUN B-Test Wako (Wako Pure Chemical Industries) ; for Cre, Creatinine Test Wako (Wako Pure Chemical Industries) ; and for UA, Uric Acid c-Test Wako (Wako Pure Chemical Industries).

4. Renal histopathology

Kidneys were removed from rats immediately after sacrifice. Kidneys were sectioned longitudinally through the hilum, and blocks were fixed in 10 % neutral-buffered formalin solution. Tissues were embedded in paraffin, sectioned 5 μm thick, and stained with hematoxylin and eosin. Tubular necrosis, tubular basophilic changes, nuclear division, and vacuolar degeneration were observed using a light microscope.

5. Statistical analysis

Data are expressed as means ± SEM of six rats. The significance of differences between means was determined using Tukey-Kramer test. Differences with $P < 0.05$ were considered significant.

Results and Discussion

1. Biochemical alternations in serum of rats fed oyster extract

Table 2 shows the serum biochemical parameters. BUN and Cre were the most significant parameters indicative of renal damage. The PAP injection groups (Control+PAP and Oyster+PAP) showed higher levels of BUN and Cre compared with the non-injection groups. The concentration of BUN tended to be lower in the Oyster+PAP group compared with the Control+PAP group. In contrast, the concentrations of Cre were similar between the Control+PAP and Oyster+PAP groups. The concentration of TP, Alb, GOT, GPT, and UA were not significantly different among the all groups.

Table 2 Biochemical parameters in serum of rats fed the experimental diets

	TP	Alb	GOT	GPT	BUN	Cre	UA
Control	6.4 ± 0.1	4.0 ± 0.1	83.5 ± 4.1	36.5 ± 1.7	14.5 ± 1.5[a]	0[a]	1.8 ± 0.3
Oyster	6.5 ± 0.1	4.1 ± 0.1	83.7 ± 5.9	37.0 ± 2.1	14.0 ± 1.2[a]	0[a]	2.0 ± 0.1
Control+PAP	6.5 ± 0.1	3.9 ± 0.1	81.8 ± 3.9	37.7 ± 4.0	63.7 ± 9.2[b]	1.2 ± 0.7[b]	1.3 ± 0.1
Oyster+PAP	6.4 ± 0.2	3.9 ± 0.1	82.7 ± 6.3	35.5 ± 5.2	54.4 ± 2.9[b]	1.3 ± 0.5[b]	1.5 ± 0.2

Data are means ± SEM (n = 6). Values not sharing a common letter are significantly different at $P < 0.05$.
TP, total protein ; Alb, albumin ; GOT, glutamic oxaloacetic transaminase ; GPT, glutamic pyruvic transaminase ; BUN, Blood urea nitrogen ; Cre, Creatinine ; UA, Uric Acid.

2. Urine volume and biochemical alternations in urine of rats fed oyster extract

Figure 1 shows the changes in urine volume before and after the injection of PAP. Injection of PAP caused a remarkable elevation in the urine volume. The urine volumes in the 2 days after the PAP injection tend to be lower in the Oyster+PAP group compared with the Control+PAP group.

Figure 2 shows the alterations in urinary NAG activity, U-Cre concentration and γ-GTP activity. Administration of PAP caused a remarkable reduction in the U-Cre concentration and elevation of NAG and γ-GTP activities. The rats in the Oyster+PAP group showed a significant return to the normal level of U-Cre and NAG activities compared with rats in the Control+PAP group.

Because serum GOT and GPT of liver origin did not change, elevation of γ-GTP activity in the urine was con-

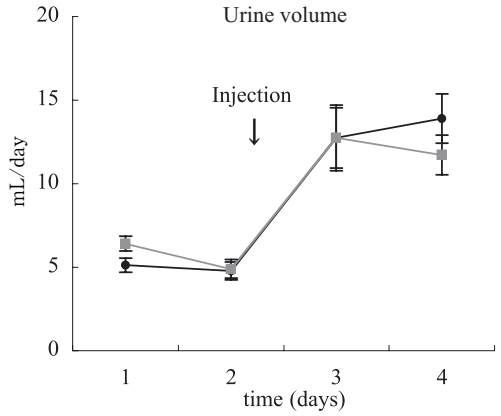
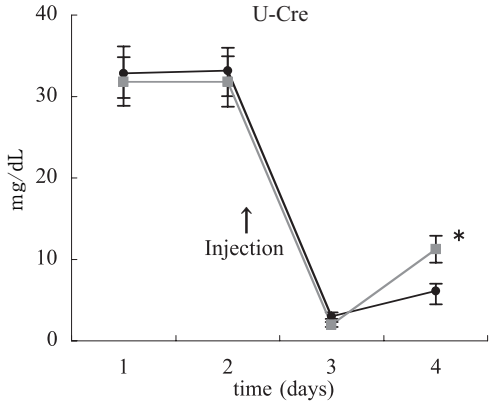
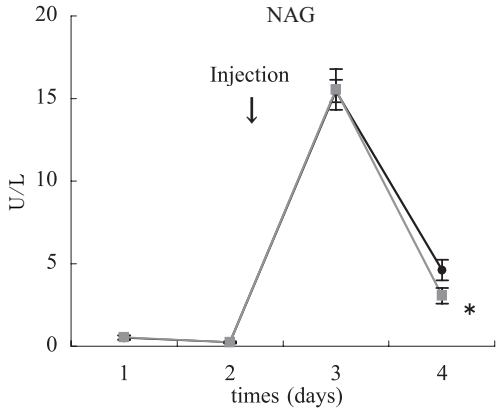


Fig. 1 Effect of oyster extract on urine volume in Control+PAP (●) and Oyster+PAP (■) rats.
Male Fischer 344 rats were injected intraperitoneally with PAP (0.5 mmol/kg body weight) on day 3 at 9 a.m. Urine was collected every 24 h (9 a.m. to 9 a.m.) for 2 days prior to and for 2 days following the injection.
Data are means ± SEM (n = 6). Asterisks shows significant difference at $P < 0.05$.





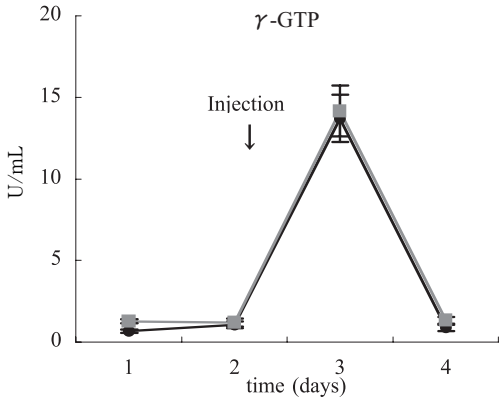


Fig. 2 Alteration of U-Cre concentration, urinary NAG activities and γ-GTP activity in Control+PAP (●) and Oyster+PAP (■) rats.
Experimental conditions are the same as described in Figure. 1.
Data are means ± SEM (n = 6). Asterisk shows significant difference at $P < 0.05$.

sidered to be of kidney origin. The γ-GTP level in the urine did not change in the Control+PAP and Oyster+PAP groups. γ-GTP localizes to the plasma membrane of tubular epithelial cells in the kidney, and NAG is abundant in lysosomes in proximal tubular epithelial cells. Hence, rats in the Oyster+PAP group showed quicker recovery of proximal tubular epithelial cell function compared with rats in the Control+PAP group, but no significant recovery of the plasma membrane of tubular epithelial cells in the kidney.

Cre was filtrated in glomerulus of kidney. Therefore, the rats of the Oyster+PAP group showed quicker recovery of glomerulus function resulting in the rapid recovery of U-Cre concentration to the normal level compared with the rats in the Control+PAP group.

3. Renal histopathology

Figure 3-a, b shows normal renal architecture. Figure 3-c, d shows a typical section from Control+PAP rats at 48 h after injection with an overview and basophilic tubular necrosis/changes, and Figure 3-e, f shows renal sections of Oyster+PAP rats in overview and of tubular necrosis.

Table 3 shows a summary of the histopathological changes seen in renal sections from rats fed the experimental diets and injected with PAP. When the rats were not injected with PAP, the severity of each pathological change was not significantly different between the two groups. When the rats were injected with PAP, the severity of nuclear division and vacuolar degeneration in the tubular epithelium were significant lower in rats fed oyster extract containing diet compared with the control diet. The severity of tubular necrosis and tubular basophilic changes in the midzone area and vacuolar degeneration in the renal and arcuate artery were not significantly different between the Control+PAP and Oyster+PAP groups. Therefore, dietary oyster extract results in a reduction in the severity of pathology changes in the tubular epithelium.

In addition, the dietary oyster extract group showed quicker recovery of proximal tubular epithelial cell and

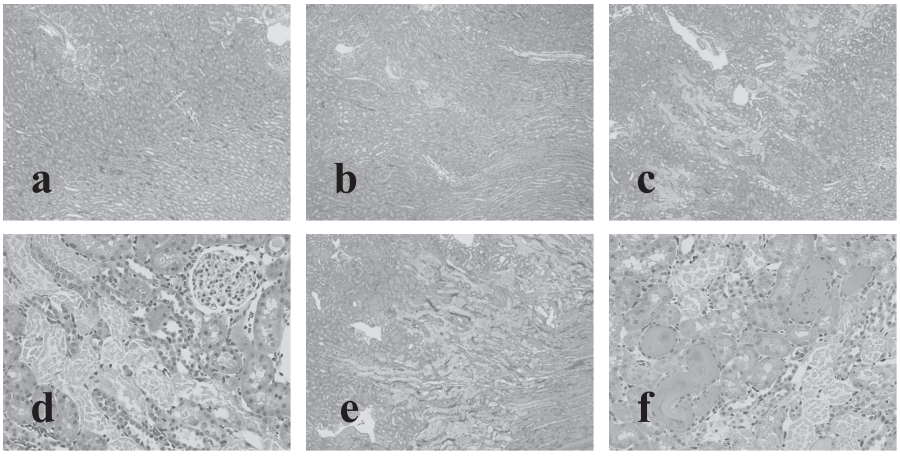


Fig. 3 Microphotographs of rat kidneys following PAP induced kidney damage. Hematoxylin and eosin stained sections of renal cortex from Control (a) and Oyster extract fed (b) rats. A typical section from Control+PAP rats 48 h after injection showing renal sections of overview (c) and tubular necrosis/change basophilic (d). A typical section from Oyster+PAP rats 48 h after injection showing renal sections in overview (e) and tubular necrosis (f).

Table 3 Histopathological changes in renal of rats fed the experimental diets or PAP-induced nephrotoxicity

	Midzone area		Tubular epithelium		Renal and arcuate artery
	Tubular necrosis	Tubular basophilic change	Nuclear division	Vacuolar degeneration	Vacuolar degeneration
Control	0^{a}	0^{a}	0.7 ± 0.3^{a}	0^{a}	0.3 ± 0.2^{a}
Oyster	0^{a}	0^{a}	0.7 ± 0.4^{a}	0^{a}	0.3 ± 0.2^{a}
Control+PAP	3.2 ± 0.5^{b}	2.3 ± 0.3^{b}	2.7 ± 0.3^{b}	2.3 ± 0.5^{b}	0.8 ± 0.3^{b}
Oyster+PAP	2.5 ± 0.8^{b}	2.8 ± 0.7^{b}	1.0 ± 0.5^{a}	1.3 ± 0.2^{c}	0.8 ± 0.4^{b}

Rats were injected intraperitoneally with PAP at 0.5 mmol/kg body weight.
Kidneys were removed 48 h after the injection.
The severity of pathological change was scored on an arbitrary scale of 0 to 4.
0, normal ; 1, slight ; 2, mild ; 3, moderate ; 4, severe.
Data are means ± SEM (n = 6). Values not sharing a common letter are significantly different at $P < 0.05$.

glomerulus function resulting in return of NAG activity, urinary volume serum BUN levels and U-Cre concentration to the normal level compared with dietary control diets. Hence, with the dietary oyster extract there was rapid renal tubular epithelial cell regeneration following nephrotoxicity due to the injection of PAP compared with the control diet.

The major components of the oyster extract were minerals, carbohydrates, protein, and amino acids. In present study, it was not determined which components of the oyster extract affected the quick recovered of proximal tubular epithelial cell function in PAP-induced nephrotoxicity in rats. Therefore, future studies will determine which components of the oyster extract are responsible for the recovery of renal function.

Conclusion

The present study evaluated the effect of oyster extract on nephrotoxicity in rats injected with PAP using several indices; BUN and Cre in serum, urine volume, urinary NAG activity, and Cre concentration and renal histopathological changes. Dietary oyster extract led to decreased serum BUN and induced a return to the normal levels of urine volume, urinary NAG activity, and U-Cre concentration, and the extract reduced the severity of pathological changes in tubular epithelium. Therefore, the oyster extract prevented CKD.

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