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Buckwheat Extract Inhibits Progression of Renal Failure

TAKAKO YOKOZAWA,*,† HYUN YOUNG KIM,† GEN-ICHIRO NONAKA,‡ AND KENICHI KOSUNA‡

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-0194, Japan, and Amino Up Chemical Company, Ltd., 363-32 Shin-Ei, Sapporo 004-0839, Japan

Rats subjected to partial resection of the parenchyma showed reduced radical-scavenging activity in the remaining kidney and increased severity of renal tissue lesions. However, in similarly nephrectomized rats given buckwheat extract, the state of oxidative stress improved by restoring the decreased activities of reactive oxygen species-scavenging enzymes such as superoxide dismutase and catalase. The degree of mesangial proliferation, severity of extratubular lesions such as crescents and adhesions, glomerulosclerosis index, and severity of tubular interstitial lesions also improved. In addition, nephrectomized rats given buckwheat extract showed improvement in renal function, as indicated by decreased serum level of creatinine, with a significant decrease in the level of methylguanidine, a uremic toxin produced from creatinine in the presence of hydroxyl radical.

KEYWORDS: Renal failure; buckwheat; polyphenol; oligomers of catechin and epicatechin

INTRODUCTION

The advent of effective treatment for chronic renal failure was a memorable event in the 20th century. Although advances in techniques such as dialysis and kidney transplantation have played an important role in treatment success, drug therapy, such as erythropoietin therapy for renal anemia, is also showing promise. Agents that inhibit the progression of chronic renal failure are also playing a role in treatment during the preservation phase, with the clinical use of the spherical active carbon Kremezin and antihypertensive drugs in early renal failure (*1*). However, in reality, measures against chronic renal failure are insufficient, and the number of dialysis patients is still increasing rapidly. In Japan, where kidney transplantation remains a rare option, intensive treatment in the preservation phase or earlier is currently the most practical way to treat patients with chronic renal failure. With this in mind, it is important to understand the causes and progression of chronic renal failure and to develop effective methods for preventing or delaying the progression of the disease.

In the process of developing new treatment, we found that antioxidative or radical-scavenging mechanisms appeared to inhibit the progression of renal failure (*2*−*8*). We therefore considered that it would be valuable to develop antioxidants that enhance the body's antioxidative mechanisms. Such agents need to be very safe and inexpensive to produce, because chronic diseases need ongoing treatment. In a previous study (*9*), we found that buckwheat extract contains useful antioxidants and is obtainable in large quantities by a purification process. The activity of the buckwheat components rutin, quercetin, and hyperoside has recently been attracting attention. However, in an earlier study we showed that buckwheat extract was composed mainly of oligomers of the newly found principal components catechin and epicatechin, which exerted a repairing effect in a model of ischemia-reperfusion disorder, a type of circulatory disturbance (*10*). This finding suggests that increased radical scavenging activity can benefit the kidney.

In the present study, we examined the effects of buckwheat extract in nephrectomized rats with chronic renal failure caused by deterioration in antioxidative functions and the presence of oxidative stress.

MATERIALS AND METHODS

Extraction and Structural Confirmation of Buckwheat Components. As described previously (*10*), whole grains of buckwheat (Polygonaceae, *Fagopyrum esculentum* Moench; 1 kg) were ground and subjected to extraction with a 15 times greater volume of water at 100 °C for 1 h. After filtration of the insoluble materials, the extract was concentrated under reduced pressure to afford a residue (50 g), which was applied to a Sephadex LH-20 column. Elution with water yielded nonphenolic compounds such as sugars, proteins, and minerals. Further elution with water containing increasing amounts of methanol (0−60%) gave two fractions, each consisting of flavonoids and flavan-3-ol derivatives (including proanthocyanidins). The flavonoid fraction was separated by chromatography over MCI gel CHP 20P (Mitsubishi Chemical Industries, Ltd.) using water containing increasing proportions of methanol (30−80%) to yield quercetin, rutin, and hyperoside, which were identified by comparisons of high-performance liquid chromatography and proton nuclear magnetic resonance spectra with those of authentic specimens. On the other hand, the flavan-3-ol fraction was chromatographed over Sephadex LH-20 using ethanol to give catechin (0.6 g), epicatechin (0.3 g), and oligomeric proanthocyanidins (ca. 10 g). The oligomeric nature of proanthocyanidins was confirmed by thiolysis with benzylmercaptan in acetic acid as described in previous papers (*11, 12*), affording 4-benzylthioethers of catechin and epicat-

* Author to whom correspondence should be addressed (fax +81-76-434-4656; e-mail yokozawa@ms.toyama-mpu.ac.jp).
† Toyama Medical and Pharmaceutical University.
‡ Amino Up Chemical Co. Ltd.

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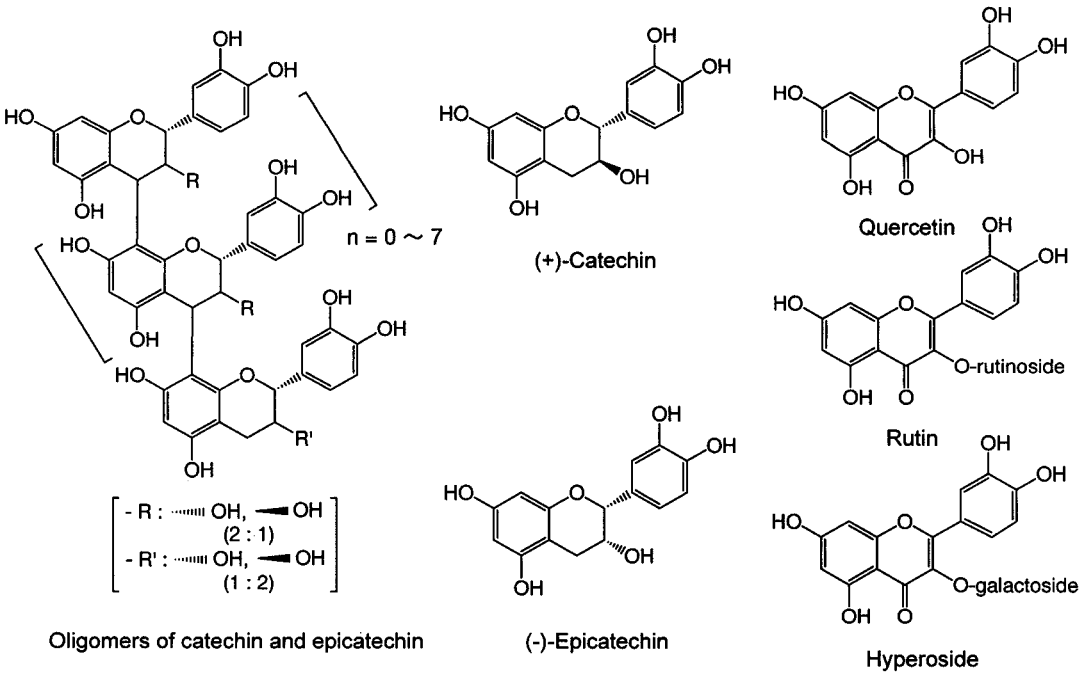

Figure 1. Compounds isolated from whole grains of buckwheat.

echin, as well as small quantities of catechin and epicatechin. The structural formulae isolated from buckwheat extract are given in **Figure 1**.

Animal Experiments. *Animal Preparation.* The "Guidelines for Animal Experimentation" approved by Toyama Medical and Pharmaceutical University were followed in these experiments. Male Wistar rats weighing about 200 g underwent resection of two-thirds of the left kidney and, 10−14 days later, total excision of the right kidney (*13*, *14*). Blood urea nitrogen levels were determined after recovery from the operation, and the rats were then divided into three groups of eight. There were no significant differences in blood urea nitrogen level among the three groups. A further group of eight acted as normal controls. One surgical group and the normal control group were given water; the other two groups were given buckwheat extract orally at a dose of 100 or 200 mg/kg of body weight/day by stomach tube for 90 consecutive days. To ensure that food consumption was similar among the four groups, they were raised on a pair-feeding schedule. Throughout the experimental period, there were no statistically significant differences among the four groups in changes in body weight. After induction of anesthesia by intraperitoneal administration of sodium pentobarbital (50 mg/kg of body weight), blood samples were obtained by cardiac puncture, and the serum was separated immediately by centrifugation (3000 rpm, 15 min, 4 °C) after coagulating for several hours in a cold room at 4 °C. The kidneys were subsequently extirpated from each rat following perfusion of ice-cold physiological saline through the renal artery, and then one part was immersed in formalin for histological findings and the other part was kept at −80 °C until enzyme assay.

Enzyme Assays. The kidney was homogenized with a 4-fold volume of iced physiological saline, and the activity of enzymes in the homogenate was then determined. Superoxide dismutase (SOD) activity was assayed by the nitrous acid method (*15*, *16*), and catalase activity was determined from the decrease in the amount of hydrogen peroxide (H_2O_2) (*17*). Glutathione peroxidase (GSH-Px) activity was determined by colorimetry of 2-nitro-5-thiobenzoic acid, a compound produced by the reaction between glutathione and 5,5′-dithiobis(2-nitrobenzoic acid) (*18*). Protein levels were determined according to the method of Lowry et al. (*19*), using bovine serum albumin as a standard.

Histological Findings. The kidney was fixed in Bouin's fixative, embedded in paraffin, and cut into thin sections. They were stained with hematoxylin−eosin, periodic acid−Schiff, or periodic acid−methenamine silver stain and observed by light microscopy. Mesangial proliferation was rated as normal, slight, moderate, or severe, and the percentage of glomeruli with extracapillary lesions relative to the total number of glomeruli was calculated as the incidence of extracapillary lesions. All glomeruli were observed for glomerular sclerosis, and the proportion of sclerotic lesions in each glomerulus was rated as grades 0−4, using the method of Raij et al. (*20*), where grade 1 represents involvement of up to 25% of the glomerulus and grade 4 represents sclerosis of 75−100% of the glomerulus. The glomerular sclerosis index was obtained by averaging the scores for all glomeruli from each rat. The severity of tubulointerstitial lesions was assessed according to three grades: normal, mild, or severe. Rats from which 50 or fewer glomeruli were obtained were excluded from analysis.

Determination of Guanidino Compounds. The serum was deproteinized by the addition of trichloroacetic acid (final concentration = 10%). After centrifugation at 3000 rpm for 15 min, the supernatant was filtered through a 0.2-mm membrane filter, and the filtrate was analyzed. Creatinine and methylguanidine (MG) were measured in a Japan Spectroscopic liquid chromatograph with a step-gradient system according to the method of Higashidate et al. (*21*). A fluorescence spectrometer, model FP-210 (excitation = 365 nm, emission = 495 nm; Japan Spectroscopic Co., Tokyo, Japan), was used for the detection of creatinine and MG on the column.

Statistics. Data are presented as the mean ± SE. Differences among groups were analyzed by Dunnett's test. Significance was accepted at $p < 0.05$.

RESULTS

Enzyme Activities. Table 1 shows the activity of reactive oxygen species-scavenging enzymes in each group. In rats with induced renal failure given no buckwheat extract, enzyme activities were significantly lower than in normal rats: 48% lower for SOD activity, 35% lower for catalase activity, and 14% lower for GSH-Px activity. The administration of buckwheat extract significantly increased SOD and catalase activities. The SOD activity, which was 9.10 units/mg of protein in the surgical controls, increased significantly to 11.96 units/mg of protein in nephrectomized rats given buckwheat extract at 100 mg/kg of body weight/day. A further increase in the dose to 200 mg produced a further increase in SOD activity. Similarly, the catalase activity in nephrectomized rats given buckwheat extract orally was significant greater at both the 100- and 200-mg dosage levels than in the surgical controls. There were no

Table 1. Activities of Reactive Oxygen Species-Scavenging Enzymes in Kidney Homogenate

group	dose (mg/kg of BW/day)	SOD[a] (units/mg of protein)	catalase[a] (units/mg of protein)	GSH-Px[a] (units/mg of protein)
nephrectomized rats				
control		9.10 ± 0.42^{c}	145.2 ± 9.8^{c}	80.11 ± 4.41^{c}
buckwheat extract	100	11.96 ± 0.51^{ce}	162.2 ± 9.4^{cd}	82.96 ± 3.22^{b}
buckwheat extract	200	14.11 ± 0.68^{ce}	183.3 ± 6.4^{ce}	84.98 ± 4.03^{a}
normal rats		17.55 ± 0.60	224.7 ± 6.3	93.21 ± 4.01

[a] Statistical significance: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs normal rats; [d] $p < 0.05$, [e] $p < 0.001$ vs nephrectomized control rats.

Table 2. Histopathological Evaluation of the Kidney

parameter	control	buckwheat extract-treated group[a] 100 mg	200 mg
degree of mesangial proliferation			
normal	0	0	3
slight	2	5	3
moderate	3	3	2
severe	3	0	0
incidence of extracapillary lesions (%)	30.5 ± 7.7	27.9 ± 7.1	26.9 ± 8.2
glomerular sclerosing index	1.75 ± 0.11	1.53 ± 0.10	1.00 ± 0.24^{a}
severity of tubulointerstitial lesion			
normal	1	2	6
mild	2	4	2
severe	5	2	0

[a] Statistical significance: [a] $p < 0.001$ vs control rats.

significant variations in GSH-Px activity after administration of buckwheat extract.

Histological Findings. As shown in **Table 2**, no rats in the surgical control group exhibited normal proliferation of the mesangium. The proliferation was rated as slight in two, moderate in three, and severe in three. Although the group treated with 100 mg of buckwheat extract also contained no rats exhibiting normal proliferation, slight proliferation was found in as many as five rats, and three rats showed moderate proliferation. In the group treated with 200 mg of buckwheat extract, there were three rats with normal proliferation, three with slight proliferation, and two with moderate proliferation, whereas no rats showed severe proliferation. **Table 2** also shows the percentages of extracapillary lesions present. In comparison with the value of $30.5 \pm 7.7\%$ in the surgical control group, the percentage was reduced, but not significantly so, in the buckwheat extract-treated group. The glomerular sclerosis index was 1.75 ± 0.11 in the surgical control group, and this was reduced to 1.53 ± 0.10 in the 100-mg treatment group, although this decrease was not statistically significant. A further increase in the dose to 200 mg produced a further significant decrease in the level of glomerular sclerosis. The severity of tubulointerstitial lesions was normal in one rat, mild in another two, and severe in the remaining five in the surgical control group, as shown in **Table 2**. In contrast, there were no rats with severe lesions in the group treated with 200 mg of buckwheat extract; a normal state was evident in six rats, and mild lesions were found in two. Under light microscopy, the renal tissue of control rats that had undergone resection of five-sixths of their kidney volume exhibited moderate mesangial proliferation, dilated tubules, interstitial fibrosis, and cellular infiltration. In contrast, nephrectomized rats given 200 mg of buckwheat extract exhibited nearly normal glomerular and tubulointerstitial histology. Typical glomerular morphology is illustrated in **Figure 2**.

Guanidino Compounds. Table 3 shows the effect of oral buckwheat extract on parameters of guanidino compounds. The creatinine level in the surgical control rats was significantly higher than in the normal rats. The level of MG was 4.69 μg/dL in rats that had undergone nephrectomy, whereas MG was not detected in the sera of normal rats. In contrast, oral administration of 100 mg of buckwheat extract caused a significant decrease in the levels of creatinine and MG compared with those in the surgical controls. A further increase in the dose to 200 mg produced a further decrease in levels of these guanidino compounds.

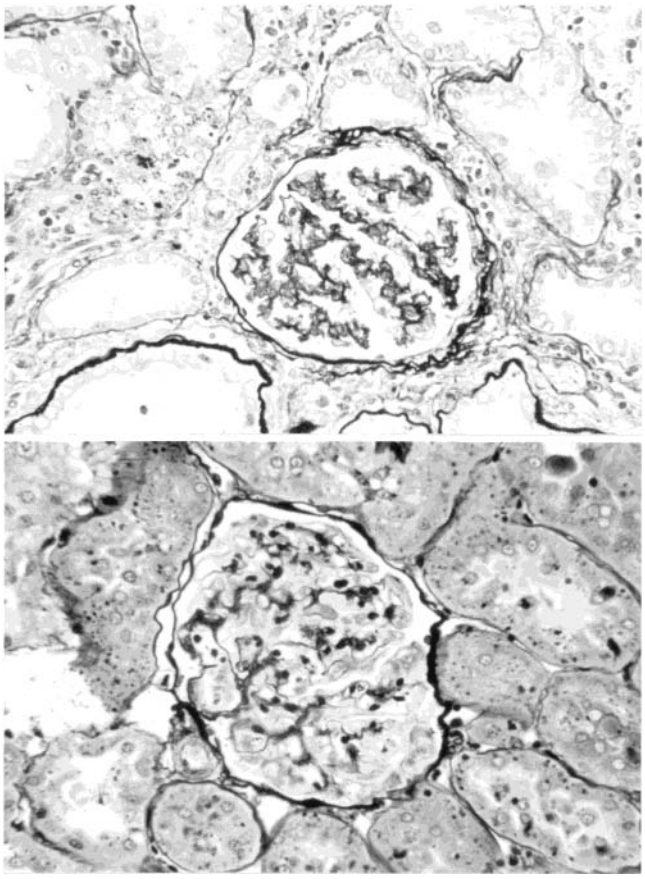

Figure 2. Photomicrographs of the glomeruli obtained from rats in the nephrectomized control (upper panel) and 200 mg of buckwheat extract-treated (lower panel) groups. Magnification: ×50. (Figure is reproduced here at 65% of original size.)

Table 3. Serum Guanidino Compounds

group	dose (mg/kg of BW/day)	Cr[a] (mg/dL)	MG[a] (μg/dL)
nephrectomized rats			
control		2.86 ± 0.11^{a}	4.69 ± 0.11
buckwheat extract	100	2.31 ± 0.10^{ab}	3.39 ± 0.11^{b}
buckwheat extract	200	1.92 ± 0.07^{ab}	2.56 ± 0.11^{b}
normal rats		0.63 ± 0.04	ND

[a] ND, not detectable. Statistical significance: [a] $p < 0.001$ vs normal rats; [b] $p < 0.001$ vs nephrectomized control rats.

DISCUSSION

In experiments using unilaterally nephrectomized rats, decreased antioxidative activity and increased oxidative stress in the remaining kidney were observed by Schrier et al. (*22*), Harris et al. (*23*), and Sanaka et al. (*24*). In the present study, in nephrectomized rats that had lost five-sixths of their functioning kidney tissue, there was a significant decrease in SOD and catalase activities in the remaining tissue, with a decreasing trend in GSH-Px activity, suggesting that the free radical-scavenging system was disturbed. SOD is an enzyme that catalyzes the disproportionation reaction from O_2^- to H_2O_2 and serves as the primary protection system against injuries by active oxygen (*25*). Catalase specifically eliminates H_2O_2, inhibits the production of hydroxyl radical ($^{\bullet}OH$) and OCl^-, and catalyzes the disproportionation reaction to H_2O and O_2 (*26*, *27*). In this regard, catalase is regarded as a more important antioxidative enzyme than SOD. When nephrectomized rats were orally given 100 or 200 mg of buckwheat extract for 90 days, the activities of both SOD and catalase were restored toward normal values, suggesting that buckwheat extract exerted influences on the $O_2^- \rightarrow H_2O_2 \rightarrow H_2O$ system. However, unlike catalase activity, GSH-Px activity was not affected by buckwheat extract, although the two enzymes both eliminate H_2O_2.

Yoshikawa et al. (*28*) reported that catalase works mainly to eliminate high concentrations of H_2O_2, whereas the main role of GSH-Px is to eliminate H_2O_2 when its concentration is low or when catalase activity is inhibited. They stated that catalase works complementarily with GSH-Px in the elimination of H_2O_2 on some occasions, whereas the two enzymes function independently on other occasions. GSH-Px activity depends on the amount of the substrate glutathione present, and variations in this activity are influenced by the activity of enzymes such as glutathione synthetase (an enzyme involved in glutathione biosynthesis), glutathione-*S*-transferase, glutathione reductase (an enzyme involved in the glutathione regeneration system), and glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (two enzymes working in the pentose phosphate pathway, which provides the substrate NADPH) and the amounts of their substrates (*29*). For these reasons, we consider that GSH-Px is not a simple H_2O_2-scavenging enzyme and that glutathione biosynthesis and the oxidation−reduction system have a complex involvement in the elimination H_2O_2. This would explain why the behavior of GSH-Px by buckwheat extract administration is different from the behavior of SOD and catalase, the actions of which are mainly restricted to radical scavenging.

In the nephrectomized rats, there was histological evidence of advanced renal tissue damage: mesangial proliferation was advanced, with progression of extratubular lesions, such as crescents and adhesions, an increased glomerulo-sclerosis index, and advanced tubular interstitial lesions. In contrast, in nephrectomized rats given buckwheat extract, these lesions were less severe, and this lack of severity was more prominent in rats given buckwheat extract at 200 mg/day than in those given 100 mg/day.

It is expected that accumulation of uremic toxins in the body under the condition of renal failure affects the renal tissue directly or indirectly, leading to deterioration of the histology and functioning of the kidney. A vicious cycle may be formed during this process, which consequently leads to end-stage renal disease (*30*). However, in nephrectomized rats given buckwheat extract, the serum creatinine level was reduced, indicating improvement in renal function. In addition, there was a significant decrease in the level of MG, the strongest uremic toxin, which is produced from creatinine increasingly in the presence of $^{\bullet}OH$ (*31*−*33*). This indicates that the radical-scavenging effect of buckwheat extract may have improved the systemic milieu, cutting off the vicious cycle. Thus, we suggest that buckwheat extract helps to inhibit the progression of renal failure. Buckwheat extract also directly eliminates $^{\bullet}OH$, O_2^-, and DPPH. In particular, oligomers of the newly found main components catechin and epicatechin have high radical-scavenging activities, whereas the corresponding activities of catechin, rutin, and quercetin are relatively low (data not shown). Thus, oligomers of catechin and epicatechin seem to be the core radical scavengers in buckwheat extract. However, it remains to be elucidated whether it is the polyphenols or their metabolites in orally administered buckwheat that have this activity.

Recently, attention has been paid to the role of the functional components of foods, as the third function of foodstuffs, in the maintenance of health and prevention of diseases. Hence, methods are now being developed to make good use of these functional food components (*34*). Because the presence of active oxygen and free radicals is related to the occurrence of diseases and aging (*35*, *36*), the use of dietary antioxidants that inhibit oxidative damage in the body has great potential benefits. However, our knowledge of the functions of dietary antioxidants in the body is limited, and further study of such antioxidants is urgently needed. The search for new antioxidants in food is currently underway. We observed that buckwheat extract improved the renal injury induced by ischemia-reperfusion (*10*). In our present study, we also found that administration of buckwheat extract showed improvement in mesangial proliferation, extratubular lesions, and tubular interstitial lesions, restored the decreased activities of reactive oxygen species-scavenging enzymes, and decreased serum levels of creatinine and MG in nephrectomized rats, leading to amelioration of oxidative stress. These findings indicate that buckwheat is beneficial to circulatory disturbances in which free radicals are involved.

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