Hot Water Extract of *Triticum aestivum* L. (Common Wheat) Ameliorates Renal Injury by Inhibiting Apoptosis in a Rat Model of Ischemia/Reperfusion

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**ABSTRACT**

**Objectives**: Interruption and subsequent restoration of blood flow into the kidney result in renal injury. As an approach to preventing the renal injury, we determined the optimal conditions and the underlying mechanisms by which supernatant of hot water extract of ground *Triticum aestivum* L. (extract) attenuated ischemia/reperfusion (I/R) injury.

**Methods**: One hour after administration of the extract (400 mg/kg) by intraperitoneal injection, renal I/R injury was generated by clamping the left renal artery in rats after surgical removal of the right kidney, followed by reperfusion. The maximal difference between the vehicle-treated and the extract-treated group under ketamine/xylazine or enflurane anesthetization was assessed at varying periods of ischemia (30–45 min) and reperfusion (3–48 hr), based on the renal function assessed with serum creatinine levels, tissue injury with hematoxylin/eosin staining, and apoptosis with terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling staining.

**Results**: Enflurane anesthetization with 40 min of ischemia and 24 hr of reperfusion was identified to be the optimal condition, under which condition serum creatinine levels and tubular damage in the extract-treated group were significantly reduced compared with those in the vehicle-treated group (1.3±0.2 versus 2.7±0.3 mg/dL, *P* < 0.01, and average score 1.8±0.1 versus 3.5±0.3, *P* < 0.01, respectively). These beneficial effects were mediated by inhibition of apoptotic cascades through attenuation of renal tissue malondialdehyde levels, Bax/Bcl-2 ratio and caspase-3 levels.

**Conclusions**: The extract conferred renal protection against ischemia/reperfusion injury in rats by scavenging reactive oxygen species and consequently blocking apoptotic cascades, plausibly augmented by enflurane protection.

**Key words**: *Triticum aestivum* L., water extract, kidney, ischemia/reperfusion, apoptosis

**Running Head**: Triticum Effect on Renal Injury

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### Introduction

Ischemic acute renal failure, a subset of acute renal failure (ARF) (recently also referred to as acute kidney injury)¹, is attributed to the reduction in blood flow to the kidney, leading to increase of creatinine concentration in the blood²,³. In the severe cases of the event such as shock or cardiac surgery, interruption or prolonged reduction of blood flow results in ischemia to the kidney, especially in the outer medulla region, caused by combination of countercurrent oxygen exchange and selective reduction in blood supply⁴, subsequently causing injury to the tubular cells due to depletion of ATP⁵,⁶. Although early restoration of blood flow to the ischemic kidney is one way to prevent the ischemic ARF, it further augments production of the reactive oxygen species (ROS), predisposing more injury to the...
cells already weakened by ischemia. As a result of ischemia followed by reperfusion (ischemia/reperfusion, I/R), the tubular cells become dead by apoptosis as well as necrosis. Thus, one way to prevent ischemic ARF caused by I/R injury is to attenuate tubular cell death by eliminating ROS and consequently blocking apoptotic cascades mediated through reduction of Bax/Bcl-2 ratio, inhibition of procaspase-3 activation, and subsequent attenuation of generation of DNA nicks.

_Triticum aestivum_ L. has been consumed as a staple food for a long time. In addition, the light grains of _Triticum aestivum_ L. has been prescribed for treating palpitations, insomnia and lack of concentration in traditional Chinese medicine. As another indication of _Triticum aestivum_ L., we have reported that hot water extract of milled whole wheat (extract) ameliorated cultured neuronal cell death caused by β-amyloid added into the culture medium, resulting from reduced production of ROS mediated by up-regulation of glutathione, an essential endogenous antioxidant. Furthermore, administration of the extract alleviated hypoperfusion–induced chronic brain injury via inhibition of inflammatory reactions in rats subjected to bilateral ligation of carotid arteries. In the present study, we determined, in connection with our previous studies, whether the extract protected the kidney from I/R injury, accompanied by revealing the underlying mechanisms in relation to ablating ROS, using malondialdehyde as marker of lipid peroxidation, and also in relation to blocking the subsequent apoptotic cascades.

### Materials and Methods

#### 1. Preparation of the extract

The extract was prepared as described previously. Briefly, the grains of _Triticum aestivum_ L., collected at Gurye, Jeonnam province, Korea, were ground in a mill, and ground _Triticum aestivum_ L. was extracted in water at 90–100 °C for 1 hr. The whole extract was centrifuged to get supernatant, and the supernatant was concentrated with vacuum dryer, and then freeze–dried into powder. The extract was analyzed to be composed of 4.9% water, 5.9% ash, 7.9% protein, 0.2% fat, and 81.1% carbohydrate, including 15.4% total dietary fiber (TDF).

#### 2. Animals

Eight–week–old male Sprague Dawley (SD) rats were purchased from Samtaco Inc. (Osan, Korea). Experiments were carried out according to the guidelines for the animal care and use of laboratory animal protocols approved by the Institutional Animal Care and Research Advisory Committee of Catholic University of Daegu. Animals were housed with food and water available _ad libitum_ under diurnal lighting conditions and in a temperature–controlled environment until the day of the experiment.

#### 3. Rat renal ischemia/reperfusion model

Renal I/R injury was generated by clamping of the left renal artery in male SD rats (250–300 g) after surgical removal of the right kidney, as described previously, with some modifications. Rats were anesthetized either with intraperitoneal injections of ketamine (100 mg/kg) and xylazine (5 mg/kg) to identify the optimal period of ischemia, or first with 5% and then 3% inhalant enflurane for maintenance in 70% air and 30% oxygen to investigate whether enflurane provides protection. Unless stated otherwise, the following experimental conditions were used: the body temperature was maintained at 36±0.5 °C throughout the experiments (i.e., up to the end of anesthetization), with a heating pad (Harvard Apparatus, Holliston, MA). After midline incision, the right kidney was surgically removed. Then the remaining left kidney underwent renal artery occlusion by clamping for 40 min (ischemia), followed by recirculation of blood for another 24 hr (reperfusion), after which the rats were sacrificed for further examinations. When rats were anesthetized with ketamine and xylazine, the following experimental conditions were selected: the body temperature, 36±0.5 °C; periods of ischemia, 30, 35, 40 or 45 min; period of reperfusion, 24 hr. When rats were anesthetized with enflurane, the following experimental conditions were selected: the body temperature, 36±0.5 °C; period of ischemia, 40 min; periods of reperfusion, 3, 6, 12, 18, 24 or 48 hr.

#### 4. Extract administration

Rats were randomly assigned to one of three groups: (1) extract–treated group (I/R + extract), (2) vehicle–treated group (I/R), or (3) sham group. In the extract–treated group, rats received the extract (400 mg/kg) dissolved in 0.9% saline by intraperitoneal injection 1 hr prior to occlusion. In the vehicle–treated group, rats received 0.9% saline only by intraperitoneal injection 1 hr prior to occlusion. In the sham group, experimental procedures were the same as those in the vehicle–treated group, except that there was no occlusion by clamping.