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ORIGINAL PAPER

Effects of butein on renal ischemia/reperfusion injury: An experimental study

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Summary ***Objectives: Renal ischemia/reperfusion (I/R) injury is a common cause of acute kidney injury. The aim of this study was to investigate the effect of butein on renal I/R injury.***
Materials and methods: Twenty-seven rats were randomly allocated to three groups (n = 9): a sham group, a renal I/R-untreated (control) group, and a renal I/R-butein group. The sham group underwent only opening and closing of the peritoneum. In the control group, an experimental I/R model was created and 1 cc isotonic saline was applied to the peritoneum. In the butein group, the experimental I/R model was created and 1 mg/kg butein was administered intraperitoneally 15 minutes before the beginning of ischemia. The left kidneys of the rats were histopathologically examined for tissue damage caused by I/R.
Results: Histopathological examination of the tissue damage revealed that all kidneys in the sham group were normal. By contrast, 2 in the control group (22.2%) had small focal damaged areas, 1 (11.1%) had < 10% cortical damage, 5 (55.6%) had 10-25% cortical damage, and 1 (11.1%) had 25-75% cortical damage. The butein group had 1 (11.1%) normal kidney, 2 (22.2%) with small focal damaged areas, 4 (44.4%) with < 10% cortical damage, and 2 (22.2%) with 10-25% cortical damage. Tissue damage was significantly lower in the sham group than in the control and butein groups ($p < 0.01$). No statistically significant differences were observed in the histopathology of the control and butein groups ($p > 0.05$).
Conclusions: Intraperitoneal administration of butein had no significant effect on renal tissue injury.

KEY WORDS: Butein; Oxidative stress; Renal ischemia; Reperfusion injury; Rat.

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INTRODUCTION

Ischemia/reperfusion (I/R) injury is a common cause of renal injury arising from a variety of clinical circumstances, including partial nephrectomy, renal transplantation, renal artery angioplasty, hydronephrosis and iatrogenic trauma (1, 2). The pathologic processes underlying this injury are complex and involve inflammation, reactive oxygen radicals, apoptosis and necrosis (3, 4). Tissues that have undergone I/R are subjected to pro-inflammatory processes of cytokine release and the production of *reactive oxygen radicals* (ROR) by neutrophils (5). The production of ROR is considered a key reason for uncontrolled oxidative stress during the reperfusion period (4). Thus, oxidative stress is extremely important in renal I/R injury (6), and targeting its processes is an ideal therapeutic approach.
The importance of I/R injury preventing during nephron sparing surgery is major issue in urology practice. Serum creatinine can increase and glomerular infiltration rate can decrease after nephron sparing surgery even in the patients who have healthy contralateral kidney. To preserve better kidney function leads lowering the risk of development of metabolic or cardiovascular disorders (7). To date, many pharmacological agents such as *N-acetylcysteine* (8), *Allopurinal* (9) or *Mannitol* (10) have been identified to decrease I/R injury after nephron sparing surgery. Another potential compound that can target I/R injury is butein, a polyphenolic compound which has reported biological activities ranging from antioxidant properties (11) to anti-fibrogenic, anti-inflamatory (12). It can also exert a protective effect on ischemia or I/R damage (13).
Against this background, we hypothesized that butein using may reduce I/R injury in rats by its anti-oxidative effects. To the best of our knowledge, there is no information about of the anti-oxidative effects of butein in rat model of renal I/R injury.

MATERIALS AND METHODS

All experimental and surgical procedures were approved by the *Institutional Animal Care and Use Committee of University of Bezmialem* (*Istanbul, Turkey*) (Approval Number/ID: 2016/134).
The procedures complied with the *Guide for the Care and Use of Laboratory* Animals and were conducted according to animal care guidelines (14). A total of 27 male *Wistar-Albino* (WA) rats (8 weeks old, weight 220-250 g) were purchased from the *University of Bezmialem* (*Istanbul,*

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Turkey). The animals were kept in captivity under the same nutritional and environmental conditions. Rats were entrained under a 12:12 h light: dark cycle with stable temperature (21 ± 2°C) and humidity (60 ± 10%). The rats had food and sterile water available ad libitum.

Experimental design

The WA rats were randomly divided into three groups (n = 9 in each group):

1. *Sham group:* After sterile conditions were obtained, a midline laparotomy was performed. The left kidney pedicle was then dissected. No other procedure was performed, and the incision was closed in two layers.
2. *Control group (Renal I/R injury and plasebo group):* After sterile conditions were obtained, a midline laparotomy was performed. Isotonic saline (1 mg/kg) was applied intraperitoneally 15 minutes before the beginning of arterial clamping. The left kidney pedicle was clamped with an artery clamp for 45 minutes. After 45 minutes of left renal ischemia, the occlusion clamp was removed and the incision was closed in two layers.
3. *Renal I/R injury and butein group:* After sterile conditions were obtained, a midline laparotomy was performed. Butein (1 mg/kg) was applied intraperitoneally 15 minutes before the beginning of arterial clamping. The left kidney pedicle was clamped with an artery clamp for 45 minutes. After 45 minutes of left renal ischemia, the occlusion clamp was removed and the incision was closed in two layers.

Butein was sourced from *Farmasina Medical and Chemical Products Company* (*Kayışdağı, Ataşehir, Istanbul*).

Induction of renal I/R injury

The rats were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg, *Ketalar, Eczacıbasi, Turkey*) and xylazine (10 mg/kg). The midline laparotomy and dissection of the left kidney pedicle were then performed as already described, followed by the I/R procedure for the control and butein groups. All procedures were performed under sterile conditions. The rats were sacrificed 24 h after completion of the reperfusion procedure and kidney samples were obtained.

Histological analysis

Renal tissues were extracted from rats in all groups (24 h after surgery), as well as following intraperitoneal injection of butein (1 mg/kg) in the butein group. Kidney tissue was embedded in paraffin and 5 µm tissue sections were taken for *Hematoxylin and Eosin* (H&E) staining. An independent pathologist blinded to the treatment groups, analyzed three different tissue sections from rats in each treatment group, using a Zeiss Axio Imager A2 microscope (*Carl Zeiss AG, Germany*).

The histological evaluations of the renal tissue were graded as follows: 0, normal; 0.5, small focal damage areas; 1, areas of tubular epithelial cell necrosis and desquamation, including < 10% of cortical tubules; 2, similar changes, including 10-25% cortical tubules; 3, similar changes including 25-75% cortical tubules; and 4, similar changes including > 75% cortical tubules. This scoring system is shown in Table 1 (15).

Statistical analysis

Table 1.
Scoring system for renal histopathology.

Score	Histopathological pattern
0	Normal
0.5	Small focal damaged areas
1	< 10% Cortical damaged zone
2	10-25% Cortical damaged zone
3	25-75% Cortical damaged zone
4	> 75% Cortical damaged zone

Statistical analysis was performed using the SPSS 22 software. Before starting to study, we performed power analysis. The power analysis showed that 9 subjects per group would be needed to have a 80% chance of achieving statistical significance at the $p < 0.05$ level.

The Kolmogorov-Smirnov test was performed to determine the normality of data. The results are expressed as mean ± SD. Mann-Whitney U and Kruskal-Wallis tests were performed for comparison of two and three independent samples, respectively. A p value below 0.05 was considered statistically significant.

Results

Histopathological examination of tissue damage revealed that all rats in the sham group had normal kidneys. By contrast, 2 rats in the control group (22.2%) had small focal damaged areas, 1 (11.1%) had < 10% cortical damage, 5 (55.6%) had 10-25% cortical damage, and 1 (11.1%) had 25-75% cortical damage. The butein group had 1 rat (11.1%) with normal kidneys, 2 (22.2%) with small focal damaged areas, 4 (44.4%) with < 10% cortical damage, and 2 (22.2%) with 10-25% cortical damage. Renal cortical damage was significantly lower in the sham group than in the control and butein groups ($p < 0.01$). No statistically significant difference was noted in the histopathology of the control and butein groups ($p > 0.05$).

Histopathologic results for the experimental animals were shown in Table 2. Histological images of the damage according to the scoring system for the rat renal cortex sections after IR injury were shown in Figures 1-4

Table 2.
Histopathologic results for the experimental animals.

	Sham group n (%)	Control group n (%)	Butein group n (%)	Total
Normal	9 (100)	0	1 (11.1)	10 (37.0)
Small focal damaged areas	0	2 (22.2)	2 (22.2)	4 (14.8)
< 10% damaged cortical zone	0	1 (11.1)	4 (44.4)	5 (18.5)
10–25% damaged cortical zone	0	5 (55.6)	2 (22.2)	7 (25.9)
25–75% damaged cortical zone	0	1 (11.1)	0	1 (3.7)
Min-max (median)	0-0 (0)	0.5-3 (2)	0-2 (1)	0-3 (0.5)
p[a]	0.001**			
Sham-Control group[b]	0.001**			
Sham-Butein group[b]	0.001**			
Control-Butein group[b]	0.105			

*[a] Kruskal-Wallis test; [b] Mann-Whitney U test; **p < 0.01.*

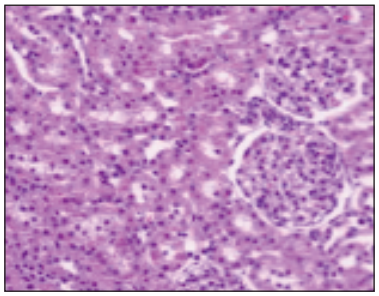


Figure 1.
Normal cortex (Histopathologic score = 0).

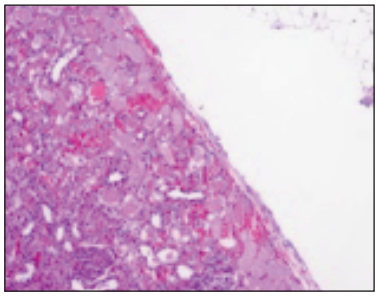


Figure 2.
Cortical focal necrose areas (Histopathologic score = 1).

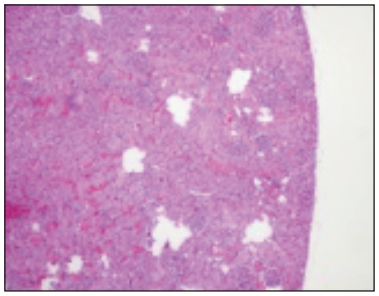


Figure 3.
More common cortical necrose areas (Histopathologic score = 2).

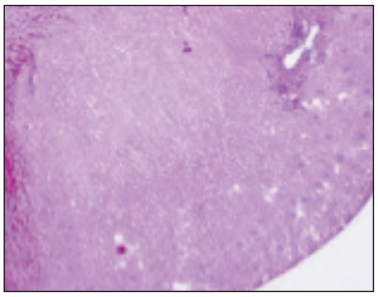
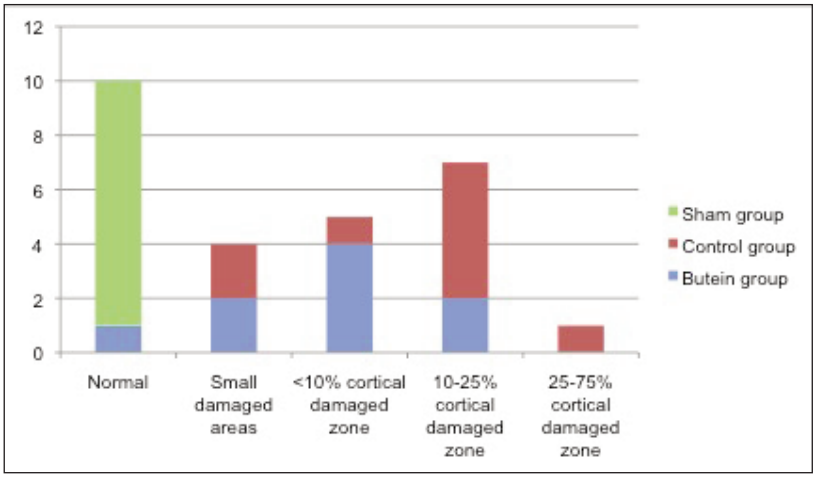


Figure 4.
Extensive cortical necrose areas (Histopathologic score = 3).

Figure 5.
Dissociation of histopathologic tissue damage according to groups.



and dissociation of histopathologic tissue damage according to groups was shown in Figure 5.

Discussion

The complicated pathogenesis of renal I/R injury is due to the broad range of effects on vascular endothelial and tubular epithelial cells (16). These effects are characterized by the cellular accumulation of waste products, imbalances in electrolytes and acid-base levels (17). Renal I/R injury induces an inflammatory response by triggering the immune systems. An infiltration of leukocytes then occurs in the site of inflammation, followed by activation of tubular epithelial cells (18, 19). The influx of neutrophils and macrophages into injured renal tissue results in the secretion of pro-inflammatory cytokines, including TNF-α, HMGB1, IL-6, and IL-1β (20), while the infiltrated cells themselves reduce blood flow in the kidney and disrupt microcirculation (21). Significant increases in pro-inflammatory cytokine levels have been reported following renal I/R injury in rats (22).

In the present study, butein was examined for its potential effects on regulating renal I/R injury. We considered that butein may serve a protective role in the rat model of renal I/R injury model by regulating the level of inflammatory cytokines. Butein is an important herbal polyphenol in traditional Japanese and Korean medicine, where it has been used as an analgesic, antibiotic, antithrombotic, anticancer, and anti-inflammatory medicine (23). It has demonstrated a broad spectrum of activity in both in vivo and in vitro models, including antineoplastic, anti-inflammatory, and antioxidant effects (24), but the mechanism underlying its antiinflammatory properties is unclear.

The present evidence suggests that butein inhibits many enzymes and pro-inflammatory mediators and that it also suppresses macrophage-mediated inflammation. One study performed in macrophage culture showed suppression of lipopolysaccharide-dependent nitrite and PGE2 production by butein and a pronounced anti-inflammatory effect (25). Application of butein to cell cultures resulted in COX-1 and COX-2 inhibition (11). Lee and colleagues suggested the use of butein as a treatment for intestinal inflammation, as butein reduced the expression of IL-8 in intestinal epithelial cell cultures (26). Butein also reduced E-selectin expression in human umbilical vein cells and expression of ICAM-1 and VCAM-1 in endothelial cells (27). In addition, butein inhibited proinflammatory gene activity by inhibiting NF-kBeta activation and inhibited the release of TNF-α, IL-6 and IL-8 (28). Many studies have shown that butein neutralizes and inhibits production of ROR and protects the cells from ROR damage. In living organisms, ROR arise as a result of normal biological metabolism and they can distort the structures of DNA, fats, proteins and carbohydrates. The best known of these oxygen

radicals are the superoxide anion, hydrogen peroxide, and hydroxyl radicals. *Cheng et al.* used CCl4 to cause cellular damage to the liver and then tried to repair this cellular damage using butein and α-tocopherol. Butein showed a similar antioxidant effect to that of α-tocopherol, but butein had the advantage of eliciting an effect at lower doses (11).

I/R-induced damage differs histologically in some respects from chemically induced damage. For example, the arterial circulation in the renal cortex is somewhat suppressed following I/R, due to tubulo-glomerular arteriolar vasoconstriction, cellular swelling, tubular obstruction, and vascular occlusion arising from extravasation of white blood cells and erythrocytes from the external medulla. The proximal tubules affected by I/R are damaged due to warm ischemia during recirculation and this leads to regression of the renal function when circulation returns to its original state. During re-infusion, the tubular lumen diameter increases, while punctures from the proximal tubules block the tubules, creating resistance to fluid ingestion in the handle and proximal tubules.

The result is a decrease in tubular reabsorption due to cellular damage, while capillary expansion increases the intratubular pressure in the external medullary collecting tubes. Subsequently, in addition to a 12% reduction in blood flow, the GFR is also reduced by 90% (29).

The tubular tissue undergoes atrophy, the tubular lumen diameter increases, the tubules undergo hyalinization, and reactive atypia, brushy edge loss, cellular swelling, nucleus deformity, and leukocyte infiltration are observed.

In our study, histopathologic evaluation of the tissue response to butein in the renal I/R rat model was compared with the non-butein control group and the sham group. Histopathologically significant differences were found between the sham group and the other groups, but no statistically significance was noted histopathologically between the I/R rats treated with butein or the saline control ($p > 0.05$).

The current study has two major limitations: Plasma concentrations of creatinine and urea were not measured, and no biochemical analysis of renal tissue was performed.

Conclusions

The intraperitoneal administration of butein to the rat experimental I/R model did not result in a statistically significant effect on renal tissue I/R injury. Nevertheless, this study is important since, to the best of our knowledge, it is the first study in the literature to test butein for effects on renal I/R. Further meaningful results can be obtained in comparative studies of different applications of butein.

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