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THE PROTECTIVE AND THERAPEUTIC EFFECTS OF ARTICHOKE EXTRACT ON MULTIPLE ORGAN FAILURE INDUCED BY EXPERIMENTAL SEPSIS IN RATS

Ayse Nur Hazar-Yavuz[1]; Muhammet Emin Cam[1,2,3]; Ayse Eda Acar[1]; Saadet Alan[4], Levent Kabasakal[1]

[1] Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Turkey
[2] Department of Mechanical Engineering, University College London, London, UK
[3] Center of Nanotechnology and Biomaterials Research, Marmara University, Istanbul, Turkey
[4] Department of Pathology, Faculty of Medicine, Inonu University, Malatya, Turkey
ayse.hazar@marmara.edu.tr

INTRODUCTION

Sepsis is a clinical or microbiologically documented infection with systemic inflammatory response syndrome. Septic shock is a non-normalized hypotension despite proper and adequate fluid replacement, sepsis in the tissues and organs with perfusion disorder. Multiple organ failure syndrome defines multiple organ dysfunction which requires support and intervention to maintain homeostasis. It is usually seen in the later stages of sepsis and septic shock and has a high mortality rate [1].

Despite a better understanding of the pathogenesis and developments in the field of antimicrobial therapy, sepsis is still an important condition in the intensive care unit. Multiple organ failure is one of the most important factors affecting mortality and morbidity. With the experimental and clinical studies, the pathogenesis of sepsis is better understood, new supportive therapies are developed and it is aimed to decrease the mortality and morbidity rates with these treatment methods [2].

Artichoke (*Cynara scolymus L.*), a plant rich in natural antioxidants, commonly grown in Mediterrenean countries; not only as a nutrient, but since the 4th century with therapeutic efficacy, alternative medicine is also known as a plant in the range of consumption [3]. In the studies carried out on artichoke until now, hepatoprotective, coloretic, cholagogue, hypolipidemic, antibacterial, antioxidant, diuretic and anticarcinogenic activity has been seen and the health protective potential of the artichoke extract has been suggested to come from the antioxidant effect power [4].

The main phytochemicals of artichokes are sesquiterpene lactones, cinnamon and sinapicrine, which are found in the densely green leaves of the leaf rather than the head. The therapeutic activity of artichoke leaf extract is attributed to luteoline, which is a flavon, and to cinnamon, which is the most important phytochemical of it. Sinarine stimulates bile formation by acting on the liver, lowers cholesterol and triglyceride levels in blood; increases liver blood flow, liver regeneration, production and secretion of bile [5].

Flowers and leaves of artichokes consumed as vegetables in our country and is used in appetite, diuretic, bile and liver diseases in the form of 2-3% infusions in folk medicine [6]. To date, according to traditional use or recent literature, artichoke leaf extract has been identified and used as a plant with antidispeptic and choleretic, cholesterol lowering and hepatoprotective activity. Artichoke, which became a phenomenon in phytotherapy at the end of experimental and clinical studies, is a phytochemical suitable for the treatment of gastrointestinal, metabolic and cardiovascular diseases [7].

The aim of our study is to investigate the potential preventive and therapeutic efficacy of artichoke extract against multiple organ failure in the sepsis-induced rats by cecal ligation and perforation because of the increase of incidence of sepsis caused by many risk factors such as increased immune system diseases,

development of resistance to antibiotics by the advancement of medicine and technology, surgical interventions, infections in the community or hospital, burns, and trauma.

MATERIALS AND METHODS

Materials

Artichoke Extract 500® (Source Naturals) standardize *C. scolymus* was prepared suspension in 0.5% carboxymethyl cellulose for administration orally via gavage once daily at 500 mg/kg.

Animals

All animal experiments were carried out with the approval of Marmara University Animal Experiments Local Ethics Committee (permission number: 29.2015.mar). Adult female and male Sprague-Dawley rats (200-250 g) (n=8 in each group) were obtained from Marmara University The Experimental Animal Implementation and Research Center. The rats were housed under controlled temperature (20-22), in humidity (40-60%) and light (12 h/12 h light/dark regime)-regulated rooms. Rats were randomly divided into 5 groups:

- Vehicle-control group (C),
- Sham operation+treatment group (Sham+T),
- Sepsis group (S),
- Pre-treatment+Sepsis group (T+S),
- Sepsis+treatment group (S+T).

Experimental Design

Sepsis was applied by cecal ligation and puncture (CLP) as previous studies [8] in S, T+S and S+T groups. *C. scolymus* treatment started 2 days ago inducing sepsis and continued for 5 days in Sham+T and T+S groups. S+T are treated with *C. scolymus* for only 2 days after CLP operation. All of rats were decapitated 2 days after inducing sepsis and ileum tissue samples which taken after decapitation was homogenized and then centrifuged for Enzyme-Linked Immunosorbent Assay (ELISA) analyses.

Biochemical Analysis

ELISA procedure was applied in accordance with the manufacturer's instructions. Levels of caspases-3 which one of effector caspases playing role in the process of apoptosis [9], myeloperoxidase (MPO) which is an indicator of tissue neutrophil infiltration [10] and nuclear factor kappa-beta (NF-κB) which comprised a family of transcription factors as regulators of pro-inflammatory mediators [11] investigated on liver, ileum and kidney tissues of rats with ELISA. Alanine transaminase (ALT), Aspartate aminotransferase (AST), and creatinine, on rat serum with ELISA.

Histopathological Analysis

The liver, ileum and kidney tissues of animals were harvested and fixed in 10% buffered formalin for 24 h, routinely processed and embedded in paraffin. Tissued sections were cut on glass slides at a thickness of 4 mm and stained with hematoxylin and eosin (H&E). The slides were examined under a light microscope and they have been photographed.

Statistical Analysis

The results of the tests were analysed by one-way ANOVA followed by the Tukey method as a post-test and represented as Mean±S.E.M. P values <0.05 were considered significant. Data analysis was performed GraphPad Prism 6.5 software (San Diego, USA).

RESULTS AND DISCUSSION

Effects of C. scolymus on serum parameters

ALT and AST are the parameters used to determine liver tissue damage and the levels of this damage. The level of ALT and AST increased significantly in the S group while both treatment groups balanced this increase. The decrease in both AST and ALT levels was higher in T+S group than in S+T group. (Figure 1 and Figure 2)

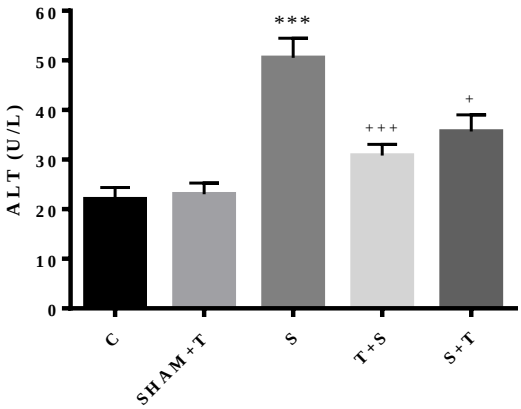


Figure 1: Serum ALT levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. ALT: alanine transaminase, C: control group, SHAM+T: Sham operation+treatment group,

S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

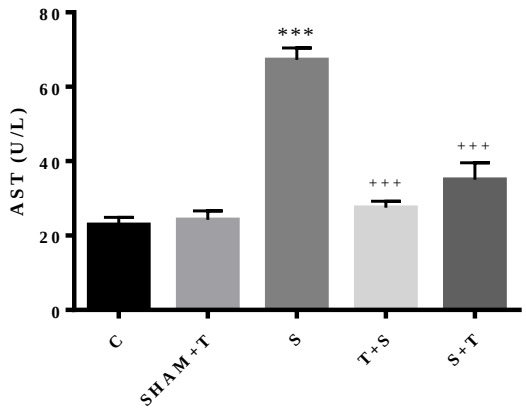


Figure 2: Serum AST levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. AST: aspartate aminotransferase, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

Creatinine was used as an indicator renal damage. While creatinine level increased in sepsis group, treatment groups decreased slightly this increase, but not significantly. (Figure 3)

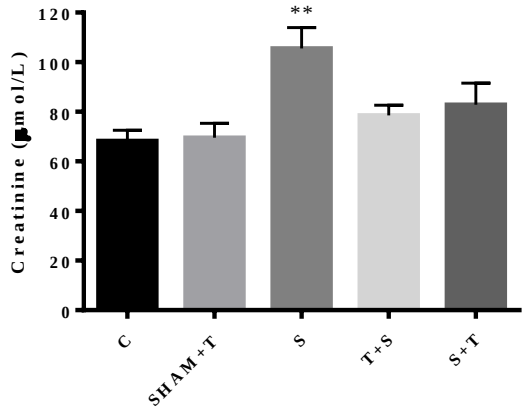


Figure 3: Serum creatinine levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group. C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

Cytokine release is known to be significantly affected by development of sepsis. Therefore, in our study, the effects of treatment on inflammation were evaluated to be used pro-inflammatory (IL-1β, IL-6 and TNF-α) and anti-inflammatory (IL-10 and adiponectin) cytokines. According to results, the pro-inflammatory release increased in the sepsis group, while treatments compensated this increase. T+S group decreased the increase in IL-1β levels more than S+T group. Data are not shown.

Effects of *C. scolymus* on Caspase-3 Levels in Liver, Ileum and Kidney

The caspase-3, one of the effector caspases involved in the apoptosis process, was studied in liver, ileum and kidney tissues. Caspase-3 increased in S group, but this increase was balanced by treatment groups according to the results obtained from all three tissues. (Figure 4, 5, and 6)

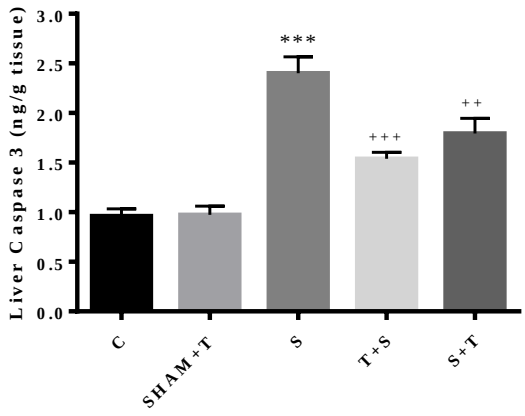


Figure 4: Liver caspase-3 levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

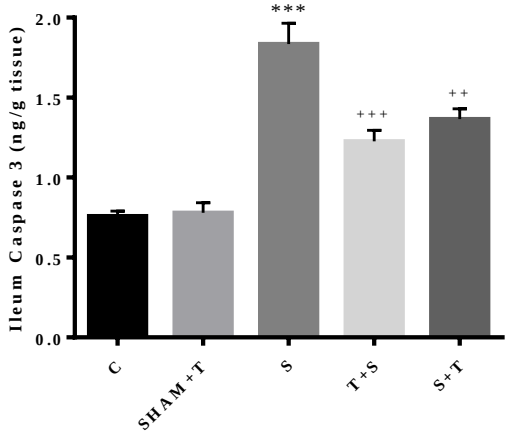


Figure 5: Ileum caspase-3 levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

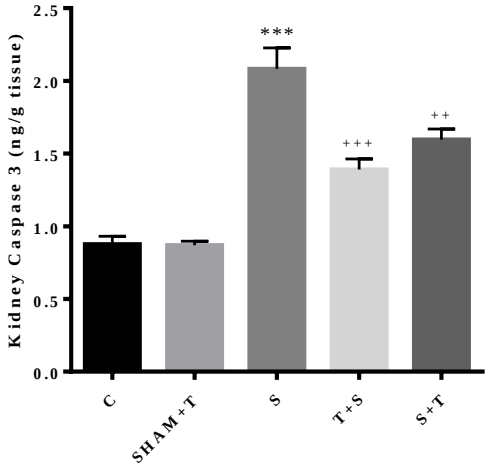


Figure 6: Kidney caspase-3 levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

Effects of *C. scolymus* on MPO Levels in Liver, Ileum and Kidney

The enzyme MPO is a neutrophil-derived enzyme and has apoptotic involved in the apoptosis process. MPO level in liver, ileum and kidney tissues increased in sepsis group, treatment groups decreased this increase. (Figure 7, 8, and 9)

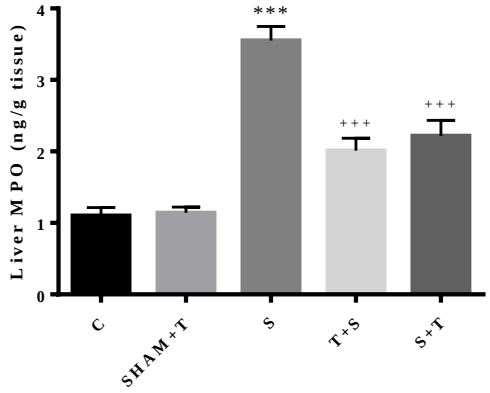


Figure 7: Liver MPO levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. MPO: myeloperoxidase, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

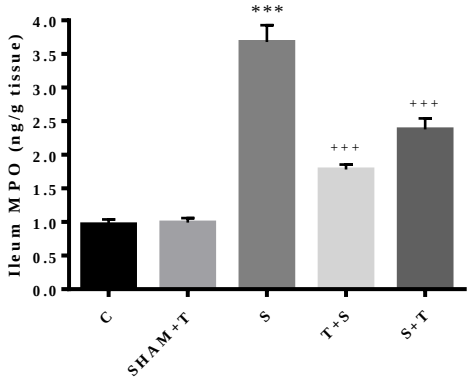


Figure 8: Ileum MPO levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. MPO: myeloperoxidase, C: control group, SHAM+T: Sham operation+treatment group,

S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

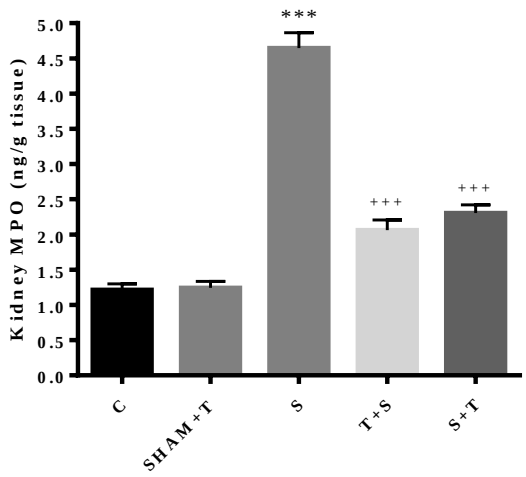


Figure 9: Kidney MPO levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. MPO: myeloperoxidase, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

Effects of *C. scolymus* on NF-κB Levels in Liver, Ileum and Kidney

It is known that activated NF-κB contributes to the inflammation in sepsis by increasing the production of various pro-inflammatory cytokines and nitric oxide. NF-κB level increased in S group, whereas treatment groups decreased it on liver, ileum and kidney tissues. (Figure 10, 11, and 12)

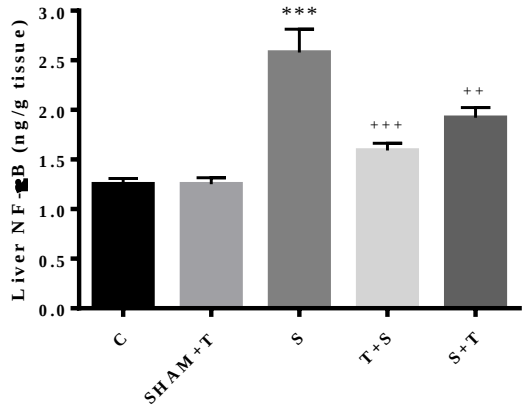


Figure 10: Liver NF-κB levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. NF-κB: nuclear factor kappa-B, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

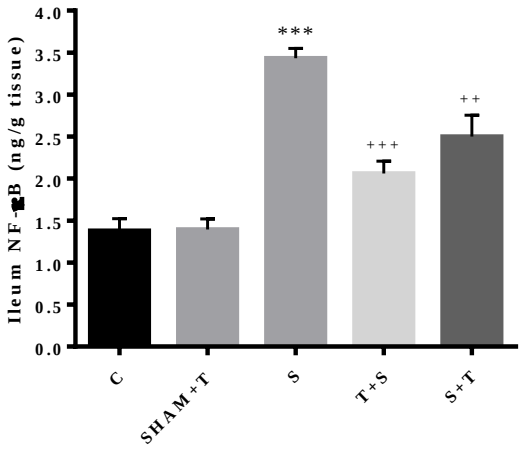


Figure 11: Ileum NF-κB levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. NF-κB: nuclear factor kappa-B, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

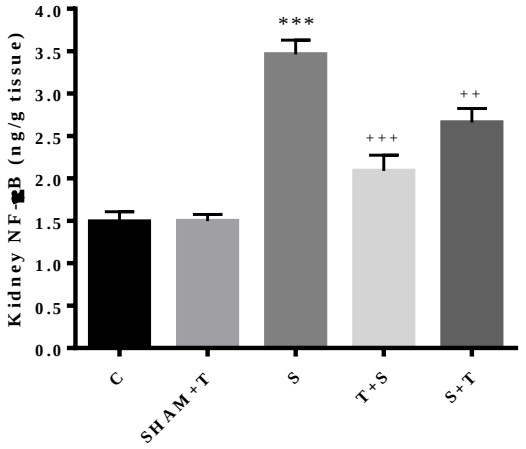


Figure 12: Kidney NF-κB levels. Values are expressed in Mean ± S.E.M. (n=8). One-way ANOVA with Tukey post test was performed using

GraphPad Prism version 6.00 for Windows. Values are represented statistically when *p<0.05, **p<0.01, ***p<0.001 in comparison with control group and $^{+}$p<0.05, $^{++}$p<0.01, $^{+++}$p<0.001 in comparison with sepsis group. NF-κB: nuclear factor kappa-B, C: control group, SHAM+T: Sham operation+treatment group, S: sepsis group, T+S: pre-treatment+sepsis group, S+T: sepsis+treatment group.

Effects of *C. scolymus* on Histopathologic Differences Liver, Ileum and Kidney

Sinusoidal enlargement, congestion, vacuolization on hepatocyte and atrophy were histopathologically observed in S group in liver. Single cell necrosis and focal inflammatory cell infiltration were seen in focal areas. There was a statistically significant difference between S and treatment groups in terms of sinusoidal dilatation (p<0.01) and vacuolization on hepatocytes (p<0.05). Sinusoidal dilatation and vacuolation of hepatocytes were significantly different between treatment groups (p<0.05).

In sepsis group, mixed inflammatory infiltrate containing villus length shortening, edema and neutrophil leukocytes were observed in ileum mucosa. There was a statistically significant difference in inflammation and villous damage between S and treatment groups (p<0.05). There was a significant difference in villus damage between treatment groups (p<0.05), there was no significant difference in inflammation (p>0.1).

Histopathologically, vacuolization was more prominent in the proximal tubules of kidney in S group. There was statistically significant difference between sepsis group and S+T group in vacuolization on proximal tubules (p<0.05).

CONCLUSION

The level of serum ALT, AST, creatinine, IL-1β, IL-6, lL-10, TNF-α and adiponectin; caspase-3, MPO and NF-κB in liver, ileum and kidney tissues severely increased in sepsis group compared to control group. This results demonstrate that the CLP model was successfully induced.

According to our results, *C. scolymus* has protective and therapeutic activities in CLP induced sepsis via antiapoptotic, antioxidant and anti-inflammatory effects on rat liver, ileum and kidney tissues; and also enhanced with ALT, AST, creatinine, IL-1β, IL-6, lL-10, TNF-α and adiponectin on serum.

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