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研究室で撮影した本人のスナップ写真、及び発表論文のコピーを添付

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研究テーマ フリーラジカル由来腎障害モデルを用いた地楡の検討

2．本年度の研究業績

(1) 学会・研究会等においての口頭発表　㊒・無　（学会名・内容）

第16回和漢医薬学会大会

フリーラジカル由来腎障害モデルを用いた地楡の検討

地楡は、バラ科植物ワレモコウ Sanguisorbae officinalis L. の根である。中国では止血、収斂薬として用いられている。本研究では、フリーラジカルが関与する腎障害モデルを用い、地楡の影響を検討した。その結果、地楡エキスは O_2^-、NOなどのフリーラジカル消去作用を介して、腎機能障害の改善に寄与していることが示唆された。

(2) 学会誌等に発表した論文　㊒・無　（雑誌名・論文名）

1) Protective effect of Sanguisorbae Radix against apoptosis and function of renal tissues subjected to ischemia-reperfusion.
Journal of Traditional Medicines 16, 97-101, 1999.

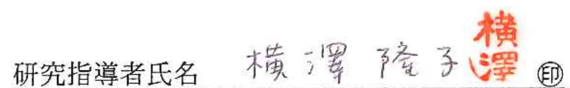
2) Beneficial Effects of Sanguisorbae Radix in Renal Dysfunction Caused by Endotoxin in Vivo.
Biol. Pharm. Bull. 22 (12), 1327-1330 (1999).

３．今後の研究計画

いままで、in vitro と in vivo の実験系において、フリーラジカル惹起する腎障害モデルを用い、地楡エキスの役割を検討していた。今後、臨床的な腎病態に対して、地楡の腎機能の改善作用を調べ、臨床において腎疾患の予防、ならびに治療薬の開発を目ざして実験を続けるつもりです。

４．研究指導者の意見

陳翠萍さんは1997年に来日以来、フリーラジカルの腎疾患に及ぼす影響と和漢薬作用について精力的に研究を行ない、すでに10編以上の原著論文を発表している。また貴財団より供与された研究費によって、日本ではほとんど知られていないが、中国では止血等によく用いられている地楡の新しい作用を実験的に証明した。彼女の真摯な態度こそ将来、日中両国のかけ橋となる研究者になるものと確信している。



研究指導者氏名　横澤隆子　㊞

５．研究報告

別紙形式を参考に、報告本文4000字以上で報告して下さい（枚数自由・ワープロ使用）

タイトル・要旨等は日本語で、KEY WORDS以下は日本語或いは英語で記入して下さい。

研究成果の発表予定がある場合は発表原稿・抄録集等を添付して下さい。

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腎虚血－再灌流、リポポリサッカライド(LPS)による腎機能の障害における地楡の役割

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要　旨

地楡は、*Sanguisorba officinalis* L. ウレモエウ及びその変種の根である。中国では止血、収斂薬として用いられている。私たちの研究グラフは in vitro の実験系で、DPPH、O_2^-、NO などのフリーラジカルに対し、地楡エキスが強い消去活性を示すことを明らかにしてきた。一方、フリーラジカルお主に活性酸素、NO なは腎疾患の発症、進展に密接に関与していることが報告され、特に最近、NO と O_2^- が反応して産生した peroxinitrite は強い細胞毒性としてを有することが注目されている。

本研究では、O_2^-、NO が関与する腎障害モデルを調製作成し、地楡の影響を検討した。まず、腎虚血60分間、再灌流24時間施したラットでは、血清中の尿素窒素、Cr レベルが著しく上昇していたが、地楡エキスを前もって30日間経口投与した群ではこれら値が著しく低下していた。また、虚血－再灌流を施した腎臓では、明瞭な DNA 断片化をラダーを認め、DNA 断片率は正常群より有意に高値を示していたが、地楡エキス処理群では、DNA 断片化を軽減していた。次に、LPS (5 mg/kg 体重) を注射したラット血清中の尿素窒素、Cr レベルも増加し、NOx も著しく上昇していた。これに対し、地楡エキス投与群では有意に低下していた。また、腎組織中の inducible NO synthase 活性も地楡エキス投与群では抑制していた。

これら結果より、地楡エキスは、O_2^-、NO 消去作用を介して、腎機能の障害の改善に寄与していることが示された。

Keyword: Sanguisorbae Radix, O_2^-, NO, renal function.

[Purpose]

Reactive free radicals are important mediators in initiating and aggravating renal damage in acute renal failure[1]. The most related free radical species are superoxide, hydrogen peroxide, hydroxyl radical and nitric oxide. Recently, a

more cytotoxic radical peroxynitrite which is formed by the reaction between nitric oxide and superoxide has been suggested as a major factor in mediating the tissue injury of oxidative stress[2].

Antioxidants and scavengers of free radicals have been considered as therapeutical interventions on prevention and alleviation of organ damage. Synthetic antioxidants demonstrated the efficiency, but they have a limitation in clinical application because of their toxicity, side effect or less selectivity[3,4]. Seeking the natural antioxidant and free radical-scavengers has drawn increasing interest in recent years.

During the process of systematic screening of crude drugs with free radical-scavenging effect, we found that Sanguisorbae Radix extract, which contains a large amount of tannins as its major constituents, has a strong inhibitory effect against O_2^- and NO generation in vitro[5]. We asked whether or not it has the same effect in vivo and thus exerts protection to organs against free radical injury. We hence conducted systimatic studies in several animal models.

[Material and Method]

Preparation of extract from Sanguisorbae Radix. The roots of Sanguisorbae Radix (Sanguisorba officinalis L.), grown in China and supplied by Uchida Wakan-yaku Co., Ltd., Tokyo, Japan, were finely powdered and extracted with distilled water at 100℃ for 1 h (roots : water=1 : 10, w/v). After removal of the insolubles by filtration, the filtrate was concentrated under reduced pressure and then lyophilized to yield a brown residue. The yield of the extract was 17.4% by weight of the original material.

Ischemia-reperfused experiment

(1) Animal and Treatment. Male LWH:Wistar rats with a body weight of 125-130 g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). They were kept in wire-bottomed cages under a conventional lighting regimen with a dark night. The room temperature (about 25℃) and humidity (about 60%) were controlled automatically. Laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan;

comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water were given ad libitum. Following several days of adaptation, the animals were divided into 4 groups - a normal, a control, and two treatment groups - avoiding any intergroup difference in body weight. The normal and control groups were given water, while the others were given Sanguisorbae Radix extract orally at a dose of 100 or 200 mg/kg body weight/day for 30 consecutive days. After induction of anesthesia by intraperitoneal administration of sodium pentobarbital 50 mg/kg body weight, bilateral flank incisions were made and the renal arteries were exposed. Bilateral renal artery occlusion was then carried out for 60 min using a nontraumatic vascular clamp. Following release of the occlusion, the abdomen was sutured, and the animal was returned to the cage. At 24 h after reperfusion, blood samples were obtained by cardiac puncture under anesthesia, and the serum was separated immediately by centrifugation. The kidneys were subsequently extirpated from each rat following renal perfusion through the renal artery with ice-cold physiological saline. The tissues were quickly frozen and kept at -80℃ until analysis. Five rats were used for each experimental group.

(2) Analysis of DNA fragmentation. According to the method of Katoh,[6] the kidney was homogenized and lysed in a cold lysis buffer (10 mM Tris-HCl, 5 mM disodium EDTA, and 0.5% Triton X-100, pH 8.0) for 10 min at 4℃. The DNA was sequentially extracted two times with half volumes of phenol/ chloroform and incubated at 55℃ for 10 min. After centrifugation at 3,000 rpm for 20 min, the upper layer was incubated with 2 μl proteinase K (20 mg/ml) at 37℃ for 60 min followed by incubation with 2 μl ribonuclease (20 mg/ml) at 37℃ for 60 min. The DNA was precipitated by adding 0.1 volume of 10 M ammonium acetate and 2.5 volumes of 100% ethanol and maintained at -20℃ overnight. DNA was collected by centrifugation at 15,000 x g for 20 min, air-dried, and resuspended in TE buffer (10 mM Tris-HCl, 5 mM EDTA, pH 7.4). The resulting DNA preparations were electrophoresed through a 2% agarose gel containing ethidium bromide. Equal quantities of DNA (according to determination of the optical density at 260 nm) were loaded in each lane, and 1-kb and 50-bp multimers were employed as molecular weight standards. DNA fragmentation was visualized and photographed under ultraviolet

illumination. Semiquantitative densitometric analysis of DNA fragmentation was conducted using a BIO-RAD Model GS-670 Imaging Densitometer with Molecular Analyst/PC Software.

(3) Determination of blood constituents. Urea nitrogen and creatinine (Cr) were determined using the commercial reagents BUN Kainos and CRE-EN Kainos (Kainos Laboratories, Tokyo, Japan).

LPS-challanged experiment

(1) Animals and Treatments. Male LWH:Wistar rats with a body weight of 125-130 g were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). They were kept in wire-bottomed cages and exposed to a conventional dark/light cycle. The room temperature (approximately 25℃) and humidity (approximately 60%) were controlled automatically. Laboratory pellet chow (Clea Japan Inc., Tokyo, Japan; comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water were given ad libitum. Following several days of adaptation, the animals were divided into four groups - a normal, a control, and two treatment groups - avoiding any intergroup difference in body weight. The normal and control groups were given water, while the others were given Sanguisorbae Radix extract orally, at a dose of 50 or 100 mg/kg body weight/d, for 30 consecutive days. For the LPS shock experiments, rats were given intravenous LPS, 5 mg/kg body weight. At 6 h after the LPS challenge, the rats were anesthetized by intraperitoneal administration of sodium pentobarbital (30 mg/kg body weight). In separate experiments, 2 groups of rats received a bolus injection plus a continuous infusion of vehicle (saline, 0.6 ml/kg/h) or aminoguanidine (5 mg/kg intravenous bolus loading dose, followed by a continuous infusion of 5 mg/kg/h in 0.6 ml/kg/h saline for 4 h) at 2 h after injection of LPS, according to the method described by Wu et al.[7] Blood samples were obtained by cardiac puncture, and the serum was separated immediately by centrifugation. The kidneys were subsequently removed from each rat following renal perfusion through the renal artery with ice-cold physiological saline. The tissues were quickly frozen and kept at -80℃ until analysis. Six rats were used for each experimental group.

(2) Determination of Urea Nitrogen and Creatinine (Cr) in Serum. Serum

urea nitrogen and Cr were determined using the commercial reagents BUN Kainos and CRE-EN Kainos (Kainos Laboratories, Tokyo, Japan).

(3) Determination of Nitrite and Nitrate in Serum. Nitrite and nitrate levels were measured primarily following the method of Misko et al.[8] Briefly, serum was filtered through an Ultrafree-MC microcentrifuge filter unit (Millipore, Bedford, MA) for 1 h at 14000 rpm to remove hemoglobin released by cell lysis. As nitrite in serum is mostly oxidized to nitrate by reaction with the iron-heme center of hemoglobin, the resulting nitrate was first reduced to nitrite by incubation with nitrate reductase and measured by a microplate assay method based on the Griess reaction.[9]

(4) Measurement of iNOS Activity. Using the methodology of both Vaziri et al.[10] and Suh et al.,[11] kidney homogenates (20% w/v) were prepared in 40 mM cold Tris-buffer (pH 8.0), containing 5 μg/ml pepstatin A, 1 μg/ml chymostatin, 5 μg/ml aprotinin and 100 μM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 12000 x g for 5 min at 4℃ to remove tissue debris. The supernatant was used for the determination of enzyme activity and protein content. iNOS activity was measured using a slight modification of the method of Suh et al.[11] The reaction mixture consisted of 10 μl supernatant in 20 mM Tris-HCl (pH 7.9), containing 4 μM FAD, 4 μM tetrahydrobiopterin, 3 mM DTT, 2 mM L-arginine and 2 mM NADPH. The mixture was incubated at 37℃ for 180 min in 96-well plates. Residual NADPH was oxidized enzymatically with 10 units/ml LDH and 5 mM sodium pyruvate in a final volume of 130 μl, with incubation for a further 5 min at 37℃, and the Griess assay was performed as above.[9] Protein was determined by the micro-biuret method,[12] with bovine serum albumin as a standard.

Statistics Results are presented as means ± S.E. The data were analyzed for statistical significance using Dunnett's method. Differences at $p<0.05$ were considered statistically significant.

[Results]

Ischemia-reperfused experiment

(1) DNA fragmentation. As shown in Fig. 1, kidney DNA was fragmented

into lower-molecular-weight molecules after ischemia-reperfusion. Analysis of the agarose gel electrophoresis pattern revealed a ladder, which was absent in kidney not subjected to ischemia-reperfusion, indicating that oxidative stress induced apoptosis. However, it was confirmed in terms of the electrophoresis pattern and semiquantitative densitometry that oral administration of 100 mg of Sanguisorbae Radix extract decreased the DNA fragmentation significantly compared with that in ischemia-reperfused control kidney. A further increase in the dose to 200 mg produced a further decrease in the DNA fragmentation rate (Table I).

(2) Blood urea nitrogen and Cr. Table II shows the effect of Sanguisorbae Radix extract on parameters of blood constituents after administration of an oral dose. The blood urea nitrogen and Cr levels in ischemia-reperfused control rats were increased significantly in comparison with normal rats. In contrast, the blood urea nitrogen level in rats given Sanguisorbae Radix extract decreased from 138.4 to 82.1 mg/dl at the 100-mg level (a 41% change, $p < 0.001$) and from 138.4 to 54.9 mg/dl at the 200-mg level (a 60% change, $p < 0.001$). Similarly, the Cr level in rats given Sanguisorbae Radix extract showed a significant decrease at both the 100- and 200-mg dosage levels as compared with that in the control rats, as shown in Table II.

LPS-treated experiment

(1) Urea Nitrogen and Cr in Serum. As shown in Table 3, the serum urea nitrogen and Cr levels in LPS-treated control rats were increased significantly in comparison with those in normal rats, reflecting uremia. In contrast, the urea nitrogen level in rats given Sanguisorbae Radix extract for 30 d decreased from 38.1 to 33.8 mg/dl at the 50-mg level (an 11% change, $p < 0.05$) and from 38.1 to 31.6 mg/dl at the 100-mg level (a 17% change, $p < 0.001$). Similarly, the Cr level in rats given Sanguisorbae Radix extract orally for 30 d was significantly decreased at both the 50- and 100-mg dose levels compared with that in the control rats. Moreover, the increased urea nitrogen and Cr levels in LPS-treated control rats were decreased by infusion of aminoguanidine.

(2) Nitrite and Nitrate in Serum. The serum nitrite and nitrate in the LPS-treated rats increased to 6.50 μM, as shown in Table 4. Administration of

Sanguisorbae Radix extract at a dose of 50 mg/kg of body weight/d for 30 d resulted in a significant reduction in the serum nitrite/nitrate levels from 6.50 to 4.39 μM. A further increase in the dose of Sanguisorbae Radix to 100 mg produced a further reduction in the nitrite/nitrate levels. Infusion of aminoguanidine, not saline, significantly reduced the increased nitrite and nitrate level caused by LPS. The level in rats given aminoguanidine was 49% of the control value.

(3) iNOS Activity in Kidney. As shown in Table 4, LPS-treated rats showed a significant increase in iNOS activity in their kidneys (from 1.94 to 3.67 pmol/mg protein/min). However, in the kidneys pretreated with 50 mg/kg body weight/d Sanguisorbae Radix extract, iNOS activity was suppressed significantly to 2.69 pmol/mg protein/min. This suppression became more marked as the dose of Sanguisorbae Radix extract increased, the iNOS activity (2.58 pmol/mg protein/min) in rats given 100 mg being comparable with that in the untreated control group (3.67 pmol/mg protein/min). A significant reduction in iNOS activity was also observed in LPS-treated rats given aminoguanidine.

[Discussion]

Cells die by either apoptosis or necrosis. Cell death by necrosis occurs when cells are exposed to severe injurious conditions. In contrast, apoptosis occurs in a number of physiological processes such as embryogenesis, metamorphosis,[13] cytotoxic T cell-mediated killing of target cells,[14] and death of autoreactive thymocytes,[15] which play an important role in maintaining a normal balance in cell growth, development and elimination. However, recent studies have shown that multiple cytotoxic stimuli causing necrosis can also initiate apoptosis when cells are exposed to the same noxious agents at lower concentration. Apoptosis has been shown to contribute to extensive cell loss in many pathological states including ischemic renal failure.[16] Indeed, the involvement of apoptosis in ischemia-reperfusion injury was also clearly demonstrated in the present study. When rats were subjected to a 60-min period of complete ischemia, followed by 24 h of reperfusion, a ladder pattern

of low-molecular-weight DNA was detected by agarose gel electrophoresis. These fragments displayed a typical DNA ladder pattern indicative of apoptosis at intervals of about 180 bp, a feature that was absent in tissues not subjected to ischemia-reperfusion, indicating that oxidation stress induces apoptosis. In contrast, rats given Sanguisorbae Radix extract orally at a dose of 100 or 200 mg/kg body weight/day for 30 consecutive days prior to ischemia and reperfusion demonstrated sufficient inhibition of apoptosis. Although precise quantitative analysis of the extent of DNA fragmentation was not conducted, semiquantitative detection showed a significant difference between the controls and the groups treated with Sanguisorbae Radix extract. The percentage of DNA fragmentation was lower in the treated rats than in the controls. This result indicated that Sanguisorbae Radix was able to inhibit apoptotic cell death.

Gobe et al.[17] obtained pathologic evidence for both necrosis and apoptosis of renal epithelial cells during the first 2 to 8 days after occlusion of the renal artery, whereas from 10 to 28 days when the renal mass was markedly reduced, cell death continued, but only apoptosis was observed. Other experiments have also yielded similar results. These results demonstrate that apoptosis plays an important role in ischemic cell injury and is responsible for renal dysfunction in ischemic acute renal failure. On the other hand, Hellberg and Kallskog[18] have reported that ischemia-reperfusion causes aggregation of polymorphonuclear cells in the glomeruli and stimulates them to release chemical mediators including free radicals, which then undergo ultrafiltration and subsequently injure the tubule cells from the luminal side. They explained that the initial change therefore occurs at the brush border, followed by deciduation of tubule cells into the lumen, forming casts in the distal part, resulting in tubule occlusion and hence decreased renal function. In this regard, we observed reversal of the decrease in renal function (in terms of increased levels of blood urea nitrogen and Cr) after administration of Sanguisorbae Radix extract.

The mechanism by which Sanguisorbae Radix inhibits apoptosis and protects against renal failure is not clear. However, reactive free radicals and oxidative stress have been extensively implicated in ischemia-reperfusion injury. As Sanguisorbae Radix contains a large amount of tannin as its major constituent, a species shown to have marked antioxidant and radical-scavenging activity,[19,20]

and furthermore in a preliminary study we have verified that Sanguisorbae Radix extract significantly scavenges superoxide and hydroxyl radical (data not shown), we speculate that these properties of Sanguisorbae Radix might contribute to its protective effect on renal function and inhibition of apoptosis.

NO plays an important role in the kidney, both under normal and pathological conditions. NO is produced from L-arginine by the action of NO synthase. In the kidney, three isoforms of NOS, located in different regions of the kidney and expressing different functions, have been found. The neuronal isoform (nNOS) is found in macula densa cells and the epithelium of Bowman's capsule; epithelial NOS (eNOS) is present in the endothelium of the glomerular capillaries, the afferent and efferent arteriole, the intrarenal arteries and the medullary vasa recta, and iNOS is found in the proximal tubule and the glomerulus[21]. NO generated by these NOS isoforms has been shown to exert different effects on renal physiology and pathology. Both nNOS and eNOS release a low and constant amount of NO, which plays a major role in the modulation of renal vascular tone and sodium excretion. Under normal conditions, iNOS also generates a physiological level of NO, which may participate in the modulation of vascular tone by an indirect mechanism in mesangical cell relaxation. However, as iNOS is induced by certain cytokines and hypoxia, it generates NO in a large quantities and for a prolonged period.[22] Excessive NO has been shown to be strongly cytotoxic, injuring cells and tissues. In freshly isolated rat proximal tubules, it has been demonstrated that NO and its metabolic product, peroxynitrite ($ONOO^-$) mediate tubularhypoxia-reperfusion injury[23]. Cattell et al.[24] also provided experimental evidence for the effects of NO in accelerated nephrotoxic nephritis using isolated rat glomeruli. In our study, LPS-treated rats showed a rapid decline in renal function, which was indicated by large increases in two renal function parameters, blood urea nitrogen and Cr. The serum nitrite/nitrate level, an indicator of NO formation, was also seento be markedly increased in LPS-treated rats compared with that seen in normal rats. As a more direct indicator, we monitored the activity of iNOS in renal homogenate using the method of Suh et al.[11] LPS-treatment resulted in an approximately 1.9-fold

increase in the activity of iNOS, suggesting the possible association of additional induction of iNOS with renal dysfunction in situ.

Various inhibitors of NO or NOS have been used in attempts to improve or attenuate the pathology involved in excessive generation of NO. However, conflicting results have been obtained. Using isolated renal proximal tubules, Yu et al.[15] reported that the NOS inhibitor, N-nitro-L-arginine methyl ester, protected the renal tubular epithelium against hypoxic injury. Weinberg et al[25] demonstrated that oral administration of N^G-monomethyl-L-arginine prevented the development of glomerulonephritis and reduced the intensity of inflammatory arthritis in MRL-lpr/lpr mice. In contrast to these observed beneficial effects, NOS inhibitors have been shown to aggravate renal dysfunction in several in vivo models of acute renal failure[26,27]. It is speculated that these contradictory results were attributable to a lack of selective NOS inhibitors.

As excessive generation of NO in renal disease is mainly associated with the induction of iNOS, the therapeutic strategy has concentrated on developing effective iNOS inhibitors. During systematic studies of the traditional drugs used in the treatment of renal diseases related to free radical injury, we found that in vitro Sanguisorbae Radix, a traditional crude drug which contains a large amount of tannin as its major constituent, has a strong scavenging effect on NO induced by sodium nitroprusside, an NO donor[5]. We also recently observed that Sanguisorbae Radix extract effectively inhibited the activity of iNOS in activated macrophages induced by LPS (data not shown). These findings prompted us to see if Sanguisorbae Radix extract could improve impaired renal function related to excessive generation of NO in vivo. We, therefore, conducted the present experiment and found that Sanguisorbae Radix extract significantly improved the impairment of renal function caused by LPS. The raised levels of serum urea nitrogen and Cr were markedly reduced in the two groups treated with different doses of Sanguisorbae Radix. Reduced serum nitrite/nitrate levels and renal iNOS activity demonstrated a protective action against the renal dysfunction caused by LPS, although these effects were weaker than those produced by aminoguanidine, a selective iNOS inhibitor.

In summary, reactive free radicals, particularly reactive oxygen species and

NO play an important effect in initiating and aggravating the impairment of renal function, and mediating cell death and DNA fragmentation. This toxic effect may be attenuated by administrating Sanguisorbae Radix extract. The results suggested a potential of Sanguisorbae Radix extract in protecting renal cells and renal function against free radicals injury.

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Table 2 Effect of Sanguisorbae Radix extract on blood urea nitrogen and creatinine.

Group	Dose (mg/kg B.W./day)	urea nitrogen (mg/dl)	Cr (mg/dl)
Normal	-	20.9 ± 1.0	0.36 ± 0.03
Ischemic and reperfused			
Control	-	138.4 ± 13.1[a]	2.81 ± 0.47[a]
Sanguisorbae Radix extract	100	82.1 ± 9.9[a,b]	1.65 ± 0.20[a,b]
Sanguisorbae Radix extract	200	54.9 ± 5.5[a,b]	1.05 ± 0.16[a,b]

Statistical significance: [a]p<0.001 *vs.* normal values, [b]p<0.001 *vs.* control values with ischemia-reperfusion.

Table 1 Effect of Sanguisorbae Radix extract on DNA fragmentation.

Group	Dose (mg/kg B.W./day)	Fragmentation (%)
Normal	-	-
Ischemic and reperfused		
Control	-	14.6 ± 2.7
Sanguisorbae Radix extract	100	7.1 ± 1.4[a]
Sanguisorbae Radix extract	200	6.5 ± 1.3[a]

Statistical significance: [a]p<0.001 *vs.* control values with ischemia-reperfusion.

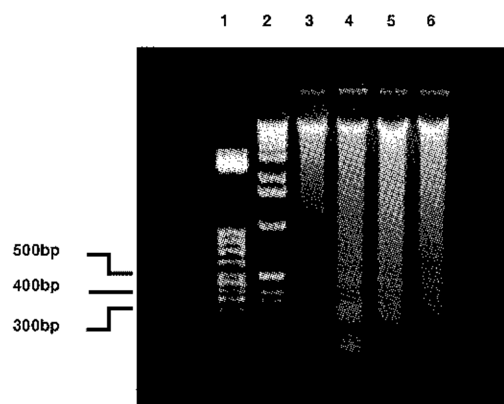

Fig. 1 Agarose fel electrophoresis of DNA. Lane 1: 50-bp marker DNA; lane 2: 1-kp marker DNA; lane 3: normal; lane 4: ischemic and reperfused control; lane 5: ischemic and reperfused Sanguisorbae Radix extract-treated (100 mg/kg B.W./day); lane 6: ischemic and reperfused Sanguisorbae Radix extract-treated (200 mg/kg B.W./day).

Table 3 Effect of Sanguisorbae Radix extract on urea nitrogen and Cr.

Group	Urea nitrogen (mg/kg B.W./day)	Cr (%)
Normal	21.3 ± 2.0	0.36 ± 0.01
LPS-treated		
Control	38.1 ± 2.9[a]	1.20 ± 0.08[a]
Sanguisorbae Radix extract		
(50 mg/kg B.W./day)	33.8 ± 3.1[a]	0.78 ± 0.10[a,d]
Sanguisorbae Radix extract		
(100 mg/kg B.W./day)	31.6 ± 2.3[a]	0.68 ± 0.08[a,d]
LPS-treated		
Control	37.8 ± 1.6[a]	1.18 ± 0.06[a]
Aminoguanidine		
(5 mg/kg plus 5 mg/kg/h)	32.4 ± 2.8[a,c]	0.66 ± 0.14[a,d]

Statistical significance: [a]$p<0.001$ *vs.* normal value, [b]$p<0.05$, [c]$p<0.01$, [d]$p<0.001$ *vs.* LPS-treated control values.

Table 4 Effect of Sanguisorbae Radix extract on Nitrite/Nitrate level and iNOS Activity.

Group	Nitrite/Nitrate in serum (μM)	iNOS in kidney (pmol/mg protein/min)
Normal	1.78 ± 1.02	1.94 ± 0.01
LPS-treated		
Control	6.50 ± 1.35[b]	3.67 ± 0.27[b]
Sanguisorbae Radix extract		
(50 mg/kg B.W./day)	4.39 ± 1.82[b,c]	2.69 ± 0.10[a,c]
Sanguisorbae Radix extract		
(100 mg/kg B.W./day)	3.72 ± 0.89[a,c]	2.58 ± 0.06[a,c]
LPS-treated		
Control	6.39 ± 1.24[b]	3.64 ± 0.29[b]
Aminoguanidine		
(5 mg/kg plus 5 mg/kg/h)	3.13 ± 1.28[c]	2.02 ± 0.18[c]

Statistical significance: [a]$p<0.01$, [b]$p<0.001$, *vs.* normal value, [c]$p<0.001$ *vs.* LPS-treated control values.