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Effect of Tea Polyphenols on Masugi Nephritis of Rabbit

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Abstract: Therapeutic effects of tea polyphenols (TP) on rabbit Masugi nephritis induced by way of subcutaneous immunization were determined by Dexamethasone as a control reagent. TP solutions at three doses (large dose 306mg/Kg; middle dose 102mg/Kg and small dose 34mg/Kg) were administered at 24h intervals for 2 weeks. Urinary protein within 24h, serum creatinine and urea nitrogen of different treatments were quantitatively determined before and after treatment, and kidney specimens were also observed from the euthanized animals. TP reduced the urinary protein excretion, serum creatinine and serum urea nitrogen significantly ($P < 0.01$) in TP-treated rabbits. There were also significant differences in cell number, diameter and also morphological characters of glomerulus, between the treated and non-treated individuals. The effects of TP at large dose were higher than that of Dexamethasone on Masugi nephritis. The above results suggest that TP treatment can reduce symptoms of Masugi nephritis of rabbits.

Key words: Tea polyphenols, therapy, masugi nephritis, serum creatinine, serum urea

Introduction

Tea is one of the most popular beverages all over the world because of its attractive flavour and physiological functions. Polyphenols are the most significant group of tea components, including the catechin groups such as (-)-epigallo- catechin-3-gallate (EGCG), (-)-epigallocatechin(EGC), (-)-epicatechin-3-gallate(EGCg), (-)-epicatechin (EC), and (-)-catechin (Fig. 1). Among these polyphenols, EGCG is the dominant component. Many functions of tea polyphenols (TP) have been studied (Lin *et al.*, 1996), including anti- inflammatory, antioxidative (Ho *et al.*, 1992; Katiyar *et al.*, 1993; Shiraki *et al.*, 1994), anti-mutagenic (Hung *et al.*, 1992) and anti-carcinogenic effects (Lu, 1998).

Immunity is a state of resistance or protection from pathogenic microorganisms and other foreigners with the response of a number of organs, tissues, cell types, and molecules that are scattered throughout the body. Usually, optimal immunity is benefit to health, but successive and excess immunization may cause tissue inflammation and organ dysfunction. Masugi nephritis, induced by continuous immunization with large dose antigen, is a typical inflammatory disease caused by hyperimmunifaction. Inflammation is the inducer of nephritis, but the subsequent generation of reactive oxygen species is just the mediator of the disease. As effective antioxidant and antiinflammationant, tea polyphenols may help to therapeutic effect on Masugi nephritis. The objective of the present study was to determine whether administration of TP would attenuate the renal injury.

Materials and Methods

The experiment was carried out in Wannan Medical College as well as in Department of Tea Science, Zhejiang University between September, 1997 and December, 1998.

Reagents: Tea polyphenols (> 95%): obtained from Tea Institute of Zhejiang University, China, Lot No. 97011104; Freund's adjuvant complete agent (10ml/unit), purchased from Sigma, Lot No. 026H8900; Cr reagent (Lot No. 961101), BUN reagent (Lot No: 960801) and total protein reagent (Lot No. 960801) were bought from Shanghai Changzheng Medi-science Limited Corp.; Sodium phosphate Dexamethasone agent (5mg/unit), Yangzhou Pharmaceutic Factory, Lot No. 960625. Other reagents were of analytical grade.

Animals: Forty eight, 8-10-weeks old New Zealand white rabbits weighing 2.3 ± 0.2 kg, male and female in half, were obtained from Shanghai experimental animal Farm (Qualified Card No.113), 36 rabbits for experimental Masugi nephritis and 10 controls. The rabbits were selected as the model in this study because it is a more practical mammalian animal than other animals that have been studied (e.g., sheep).

Experimental protocol and treatment

Screening of qualified animal: The rabbits were housed in animal facility for 24h, limited access to food but freely to water) for 12h. Collected 24-h urine and 1ml blood sample in the edge of ear for determination of a baseline blood urea nitrogen (BUN [mg/dL]), creatinine and 24-h Urine protein, only the rabbits with standard creatinine, BUN and Pr level (less than 100 μ mol/L, 10mmol/L and 50mg/24h respectively) were used as experimental animals.

Preparation of rabbit Masugi nephritis model: Kidney was removed from a sacrifice a rabbit weighting 2.5kg and rinsed repeatedly

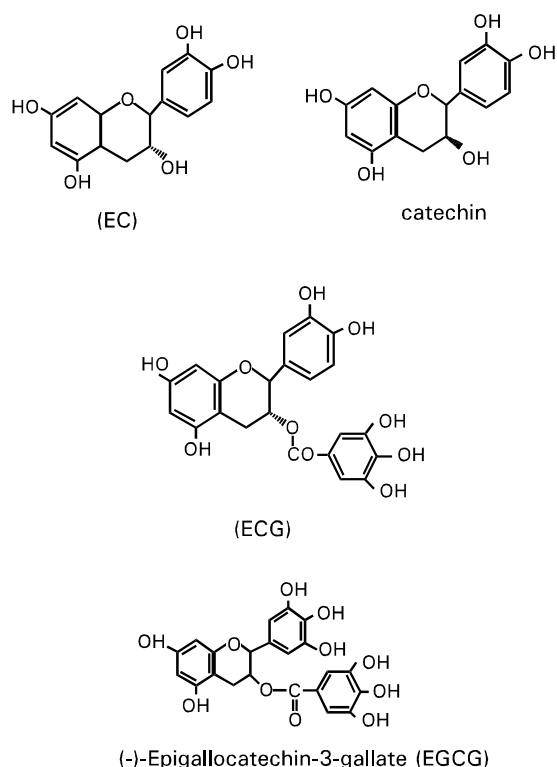


Fig. 1: Chemical structures of the catechins in tea polyphenols

with saline through a fine pipe inserted into the small entering artery. Five gram renal cortex was sampled and ground into serosity, then mixed with Freund's adjuvant complete agent and further ground into water in oil suspension with 20ml saline, thus the rabbit renal antigen was formed.

Goats were injected intra- peritoneally with 1ml rabbit renal antigen respectively in five sites at two-weeks intervals for four times. Fourteen days after the last immunization, sampled the blood from sheep's carotid under narcotism, serum was isolated and mixed with equal rabbit erythrocyte rinsed with saline for three times and kept to absorption in 4° C refrigerator for one night, centrifuged for 10min (2500rpm) and removed the blood cell absorbed with miscellaneous antibody, the suspension heated at 56° C for half an hour was the goat-anti-rabbit antigen.

A total of 40 rabbits (male and female halfly) were immunized in the edge of ear with 0.75ml goat-anti-rabbit antigen at 0.5h intervals for 3-5 times, the Masugi nephritis model was prepared when proteinuria immersed. Two days later, typical symptom of nephritis appeared such as increasing of urine protein, serum creatinine and urine nitrogen, swelling and dilatation of glomerulus, and proliferation of endodermis and mesangial cell. TP and other treatment was performed after perfect confidence of nephritis development (with urinary protein concentration more than 100 mg/dl).

Grouping, dose-setting and administration: Forty rabbits with Masugi nephritis were divided randomly into 5 groups according to the severity of nephritis (judged with 24h urine protein, serum creatinine and serum nitrogen). LDTP, MDTP, SDTP: treatment groups (n= 38); DS: Positive control (n = 8); MC: Model control (n = 8). Moreover, other 8 unimmunized rabbits were used as normal control (NC). All the groups were treated as follows:

- 1 Normal Control (NC): equal saline (NS)
- 2 Model Control (MC): equal saline (NS)
- 3 Large-dose tea polyphenols (LDTP): 306mg/kg tea polyphenols
- 4 Middle-dose tea polyphenols (MDTP): 102mg/kg tea polyphenols
- 5 Small-dose tea polyphenols (SDTP): 34mg/kg tea polyphenols
- 6 Positive control (DS): 0.1mg/kg Dexamethasone (0.01%)

The small dose of experimental tea polyphenols was converted from adult oral dose (600mg/day) according to weight ratio, and 3 times increasing gave the middle and further large dose. Tea polyphenol was prepared as 6, 2 and 0.6% solution with saline just before using. All the rabbits were treated through the stomach with 5ml/kg solution at 24h intervals for 14 days.

Assessment of kidney function:

Biochemical evaluation: Biochemical evaluation including Urine protein, creatinine and BUN was performed before and after preparation of identified nephritis model respectively. Twenty-four hour urine protein excretion was measured using precipitation by trichloroacetic acid and plasma creatinine using the alkaline picrate method (Bousnes and Taussky, 1945). Blood urea nitrogen were assessed by the oxime method (Evans, 1968) and serum total protein using the procedure of Lowry *et al.* (1951).

Pathological examination: Once the experiment ended, measured body weight, weight of both kidneys in all animals and calculated the kidney/body weight ratio. Both kidneys were removed and fixed with 10% formalin, then cut longitudinally to include both poles and the renal pelvis, followed by staining of the sections with hematoxylin and eosin. Stained kidney sections were examined using light microscopy and the severity of glomerular lesions were blindly evaluated parameters such as hyper cellularity, glomerular infiltration of PMN, fibrinoid necrosis, focal and segmental proliferation, and interstitial infiltration. Five largest glomerular sections were selected in each sample for measurement of diameter and cell sum.

Statistical analysis: Data were expressed as means $\bar{x} \pm$ S.D.

Statistical analysis was performed using Student's t test, and P values < 0.05 were considered significant.

Results

Effect of TP on urine proteins in rabbit Masugi nephritis: The changes of the mean levels of urinary protein excretion between LDTP, MDTP, SDTP and DS, MC, NC are shown in Table 1. All experimental animals had similar baseline urine protein before treatment. However, there was significant increase after immunization and marked drop after TP-treatment in urea protein. Two weeks after the last administration, the urine protein was less in TP-treatment animals than in non-TP-treatment control (P< 0.05 or P< 0.01), even less than in Dexamethasone treatments. TP had an obvious dose-dependent reduction of urea protein, which indicated the potent protection of TP in renal function.

Effect of TP on concentration of creatinine and BUN: In Table 2 and Table 3, the concentration of creatinine and BUN have been compared between different groups. Prominent enhancement of creatinine was observed in Masugi nephritis rabbits comparing with pro-reformation of nephritis (Table 2). However, TP reduced

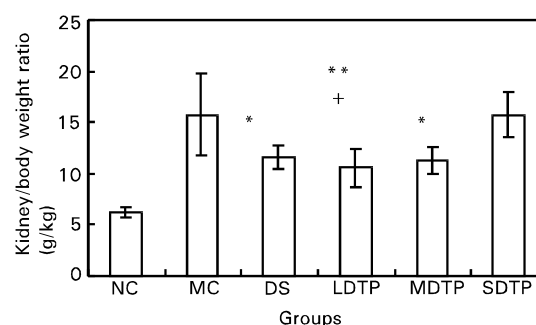


Fig. 2: Kidney/body weight ratio in Masugi nephritis rabbits two wks post medicine treatment (*P < 0.05, **P < 0.01 vs NC, + P < 0.05 vs SDTP)

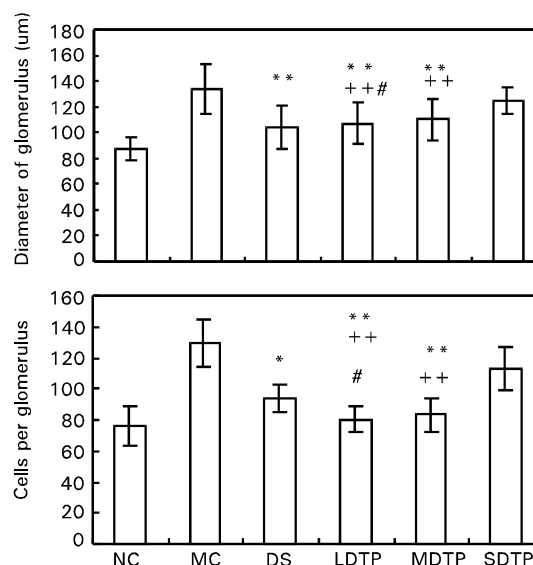


Fig. 3: Comparison of glomerulus and cell number in Masugi nephritis rabbits two wks post medicine treatment (* P < 0.05, ** P < 0.01 vs NC, ++ P < 0.01 vs SDTP, #P < 0.05 vs DS)

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Table 1: Effect of TP on urine protein in Masugi nephritis rabbits, 2 weeks after administration (mg/24h, $\bar{x} \pm SD$)

Groups	No. of rabbits	Pro-formation of nephritis	Postformation of nephritis		
			Before taking medicine	After taking medicine	Changes (%)
DS	7	27.6 \pm 1.2	805.5 \pm 34.9	428.1 \pm 23.9	46.9 ^{aa}
LDTP	6	29.5 \pm 1.6	782.6 \pm 34.7	149.9 \pm 14.7	80.8 ^{aabbc}
MDTP	7	34.2 \pm 1.2	748.3 \pm 26.0	363.4 \pm 15.1	51.4 ^a
SDTP	6	22.4 \pm 2.0	811.0 \pm 24.4	594.7 \pm 29.1	26.7 ^b
MC	7	30.2 \pm 1.6	796.2 \pm 22.3	683.9 \pm 23.1	14.1
NC	8	28.7 \pm 1.6	38.5 \pm 12.0	34.6 \pm 8.3	10.1

^a $p < 0.05$, ^{aa} $p < 0.01$ vs NC; ^{bb} $p < 0.01$ vs SDTP; ^c $p < 0.05$ vs DS.

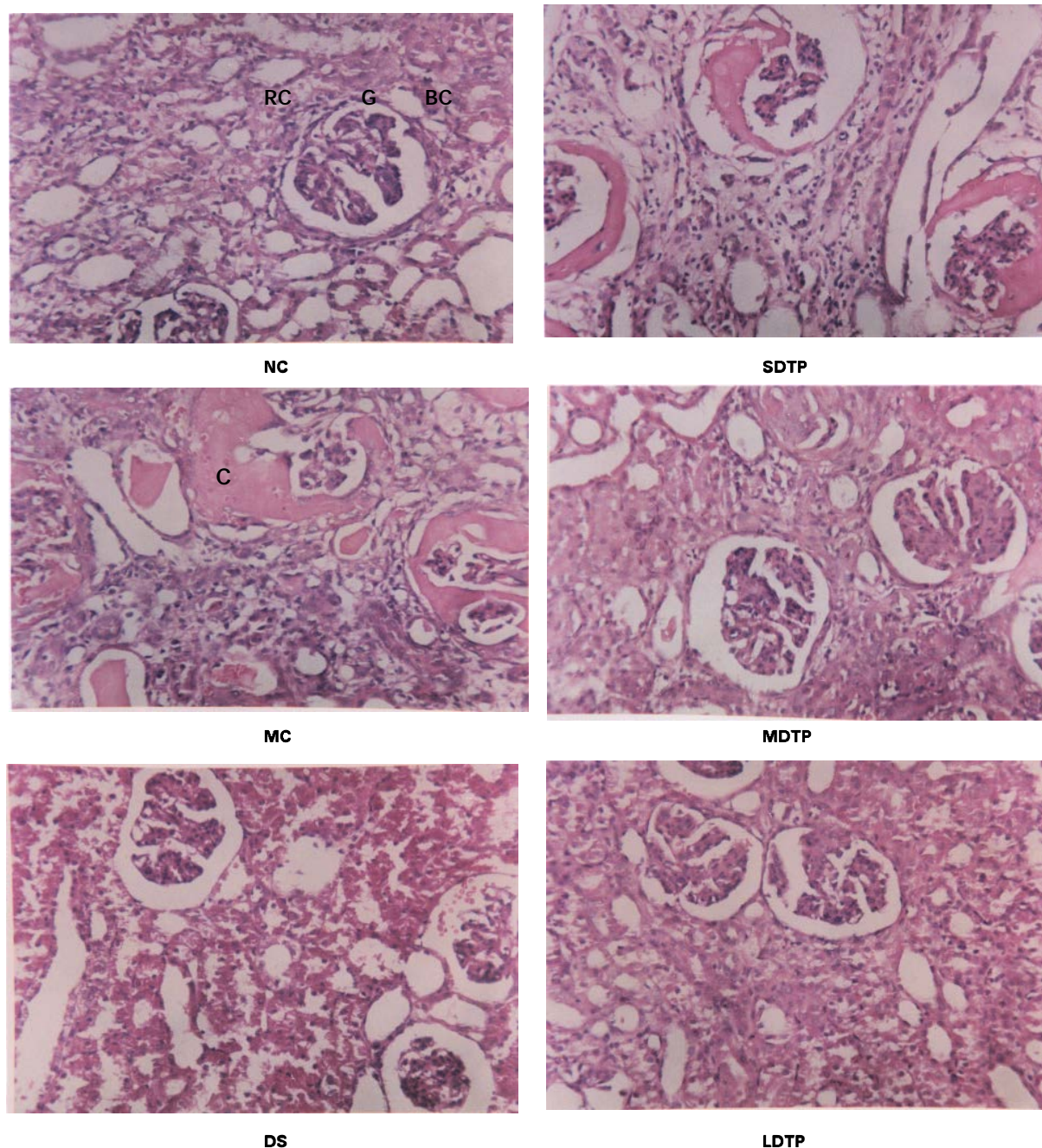


Fig. 4: Results observed with light microscope of renal sections for rabbits. (RC: renal corpuscle; G: glomerulus; BC: bowman's capsule; C: crescent)

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Table 2: Changes of BUN in Masugi nephritis rabbits two weeks post administration of medicine ($\mu\text{mol/L}$, $\bar{x} \pm \text{SD}$)

Groups	No. of rabbits	Pro-formation of nephritis	Postformation of nephritis		
			Before taking medicine	After taking medicine	Changes (%)
DS	7	84.3 \pm 2.44	281.9 \pm 7.94	202.7 \pm 9.76	28.1 ^{aa}
LDTP	6	89.5 \pm 5.54	342.6 \pm 8.77	162.0 \pm 3.63	52.7 ^{aabccc}
MDTP	7	72.3 \pm 3.15	294.2 \pm 5.61	177.0 \pm 8.78	39.8 ^{aa}
SDTP	6	79.8 \pm 7.35	307.7 \pm 7.92	236.6 \pm 6.77	23.1 ^a
MC	7	77.7 \pm 5.29	292.3 \pm 6.52	262.8 \pm 7.21	10.1
NC	8	88.4 \pm 5.56	83.6 \pm 4.25	89.8 \pm 12.13	-7.4

^a $p < 0.05$, ^{aa} $p < 0.01$ vs NC; ^{bb} $p < 0.01$ vs SDTP; ^{cc} $p < 0.01$ vs DS.

Table 3: Changes of BUN in Masugi nephritis rabbits two weeks post Administration of medicine (mmol/L , $\bar{x} \pm \text{SD}$)

Groups	No. of rabbits	Pro-formation of nephritis	Postformation of nephritis		
			Before taking medicine	After taking medicine	Changes(%)
DS	7	8.03 \pm 2.72	36.62 \pm 8.71	23.13 \pm 2.28	37.2 ^a
LDTP	6	8.44 \pm 2.17	39.22 \pm 7.06	15.47 \pm 5.13	60.6 ^{aabc}
MDTP	7	7.51 \pm 2.58	37.56 \pm 2.17	20.15 \pm 1.24	46.3 ^{aa}
SDTP	6	6.95 \pm 1.26	43.65 \pm 2.13	34.03 \pm 3.01	22.0
MC	7	7.74 \pm 1.93	41.50 \pm 3.43	38.40 \pm 1.39	7.5
NC	8	7.62 \pm 1.57	8.21 \pm 1.04	7.82 \pm 1.58	4.7

^a $p < 0.05$, ^{aa} $p < 0.01$ vs NC; ^b $p < 0.01$ vs SDTP; ^c $p < 0.01$ vs DS.

the creatinine in dose-dependent manner ($p < 0.01$) and the anti-creatinine effect of TP is better than that of Dexamethasone; here, LDTP vs. DS was also significant ($p < 0.01$). The changes of BUN in rabbits was similar to that of creatinine (Table 3). BUN decreased significantly after administration of TP, and 360mg/kg TP was more effective than Dexamethasone in anti-BUN ($p < 0.05$).

All the effects of TP on urine protein, creatinine and BUN revealed the potent biochemical protection of kidney in Masugi nephritis, and the following histological findings provided the similar results.

Histological changes

Effect of TP on kidney/body weight ratio: The much higher ratio of kidney/body weight in MC rabbits vs NC rabbits showed the proliferation of kidney (Fig. 2). Two weeks after administration of TP, the ratio debased in dose-dependent manner ($P < 0.05$, $P < 0.01$) and the effect of Large-dose-TP is better than that of Dexamethasone, indicating the beneficial detumescence of TP.

Effect of TP on glomerulus: The proliferation of nephritis rabbits and the detumescence of TP has been proved from Fig. 3. TP distinctly reduced the diameter and cell sum of glomerulus ($P < 0.01$). Large-dose TP inhibited the swelling and proliferation of glomerulus more effectively than Dexamethasone.

Histological changes: Light microscopic examination of renal tissue revealed the severity of hyper cellularity, glomerular infiltration of PMN, fibrinoid necrosis, focal and segmental proliferation, and interstitial infiltration in MC glomerular sectors. There was epithelial cellular proliferation in glomerular vesicle with red-stained deposits, severe fibrinoid necrosis of epithelial cells and dilatation with hyperaemia in capillary vessel and hyperplasia of extracellular fibrotissue with abundant infiltration of inflammatory cells including lymphocytes, plasma cells, polymorphonuclear cells, neutrophils and eosinophils. In addition, Hyperplasia, necrosis and multi-tubuloreticular was also investigated in renal tubules. Animals treated with TP showed significant alleviation of glomerular lesion than non-treated controls, the higher the dose, the more the effect (Fig. 4). Large dose TP markedly improve the glomerular pathology, and all the compartment of glomerulus was almost normal only with a little red-stained deposits, inflammatory inflation and tubuloreticular inclusions. Small dose TP and Dexamethasone also improved the renal pathology of rabbits, but is less effective than large dose TP.

Discussion

In recent years, polyphenols, especially tea polyphenols, have gained much attention, owing to their biochemical and pathological function. In the study presented here, we have demonstrated that TP can ameliorate biochemical and pathological elision in rabbit Masugi nephritis, indicating therapeutic effect of TP on renal dysfunction, which is in accord with previous documents (Yokozawa *et al.*, 1996; Takako *et al.*, 1997; Sano *et al.*, 1995). The mechanism of TP lies in dimensional ways.

Masugi nephritis was induced initially by activation of complement, followed by inflammatory response. In this context, immunomodulation and antiinflammation is a beneficial therapy for Masugi nephritis. Tea polyphenols has been ascribed the properties of being immunomodulation and antiinflammatory (Nakazato *et al.*, 1998). In our experiment, TP attenuated the formation and accumulation of inflammatory cells, which can be referred from the reduction of inflammatory cells in the masugi nephritis glomerulus. Therefore, antiinflammation may be the mechanism of therapeutic effect on nephritis.

In the past decade, inflammation-related endogenous production of reactive oxygen radicals has been suggested as a risk factor for cancer, nephritis and so on. During inflammation, a variety of reactive oxygen radicals (ROS) including NO, $\text{O}_2^{\cdot-}$, H_2O_2 , etc. released from monocytes-macro phages, endothelial cells, smooth muscle cells, and neutrophils, so the scavenging of these ROS helps to relieve inflammation and its damage. As to the ample document, the anti-inflammatory activity of TP has been generally attributed to its antioxidation based on its multi-pathway antioxidation. First, TP is an effective scavenger of free radicals including NO, H_2O_2 , $\text{O}_2^{\cdot-}$, $\cdot\text{OH}$ and so on. Next, TP inhibit the formation of reactive oxygen intermediates through an enhanced auto-oxidation of Fe^{2+} chelation with iron ion(Yona *et al.*, 2001) and down regulation of oxidase (xanthine oxidase). Both the inhibition of the formation and scavenging effect markedly relieve the direct or indirect harm of ROS to tissue.

Moreover, TP inhibited ROS through signal pathway. According to Lin and Lin (1997), TP decreased NO production through both reduction of iNOS (inducible NO synthase) gene expression and inhibition of enzyme activity, which will provide a distinctive advantage over inhibitors that may work at only one level. TP was also investigated to block activation of NF-Kb by blocking the signal-induced phosphorylation of Ikb, thereby inhibiting the induction of iNOS transcription. Thus, TP prevented the activation of transcription factor NF-kB, which in turn led to decreased

transcription of the iNOS gene. Dexamethasone was also observed to decrease the activity of iNOS promoter through inhibition of cytokine-induced NF- κ B complexes (Kleinert *et al.*, 1996). From these results, we can conclude that inhibition of NF- κ B activation and subsequent iNOS expression and NO production may be one of the important protective mechanism for both TP and dexamethasone in Masugi nephritis. Administration of TP at different doses could induce therapeutic effects in experimental Masugi nephritis. This novel antiinflammatory compound resulted in reduction of leukocytes infiltration in glomeruli due to inhibition of ROS and antioxidant in many processes, which may provide the reason that large dose TP was more effective than dexamethasone.

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References

- Bousnes and Tausky, 1945. The calorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.*, 158: 581-591.
- Evans, R.T., 1968. Manual and automated methods for measuring urea based on a modification of its reaction with diacetyl monoxime and thiosemicarbazide. *J. Clin. Pathol.*, 21: 527-532.
- Ho, C.T., Q. Chen, H. Shi, K.Q. Zhang and R.T. Rosen, 1992. Antioxidative effect of polyphenol extract prepared from various Chinese teas. *Prev. Med.*, 21: 520-525.
- Hung, M.T., C.T. Ho and Z.Y. Wang, 1992. Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. *Carcinogenesis*, 13: 947-954.
- Katiyar, S.K., R. Agarwal, M.T. Zaim and H. Mukhtar, 1993. Protection against N-nitroso- diethylamine and benzo[a]pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. *Carcinogenesis*, 14: 849-855.
- Kleinert, H., C. Euchenhofer, I. Ihrig-Biedert and U. Forstermann, 1996. Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor- κ B. *Mol. Pharmacol.*, 49: 15-21.
- Lin, Y.L. and J.K. Lin, 1997. (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor- κ B. *Mol. Pharmacol.*, 52: 465-472.
- Lin, Y.L., I.M. Juan, Y.L. Chen, Y.C. Liang and J.K. Lin, 1996. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J. Agric. Food Chem.*, 44: 1387-1394.
- Lowry, O.H., N.J. Rosebrough and A.L. Farr, 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
- Lu, Q., 1998. The antioxidative mechanisms of tea in the prevention of cancer. *Toxicol. Lett.*, 95: 52.
- Nakazato, K. and T. Takeo, 1998. Anti-inflammatory effect of oolong tea polyphenols. *Nippon Nogeikagaku Kaishi*, 72: 51-54.
- Sano, M., Y. Takahashi, K. Yoshino, K. Shimoi and Y. Nakamura *et al.*, 1995. Effect of tea (*Camellia sinensis* L.) on lipid peroxidation in rat liver and kidney: A comparison of green and black tea feeding. *Biol. Pharm. Bull.*, 18: 1006-1008.
- Shiraki, M., Y. Hara, T. Osawa, H. Kumon, T. Nakayama and S. Kawakishi, 1994. Antioxidative and antimutagenic effects of the aflavins from black tea. *Mutate Res.*, 323: 29-34.
- Takako, Y., D. Erbo and O. Hikokichi, 1997. Proof that Green Tea Tannin Suppresses the Increase in the Blood Methylguanidine Level Associated with Renal Failure. *Exp. Toxicol. Pathol.*, 49: 117-122.
- Yokozawa, T., H.Y. Chung, L.Q. Hae, H. Oura, H.Y. Chung and L.Q. He, 1996. Effectiveness of Green Tea Tannins on Rats with Chronic Renal Failure. *Biosci. Biotechnol. Biochem.*, 60: 1000-1005.
- Yona, L., B.H.Y. Moussa, M. Gila and M. Silvia, 2001. Attenuation of 6-hydroxy- dopamine (6-OHDA)-induced nuclear factor- κ B (NF- κ B) activation and cell death by tea extracts in neuronal cultures. *Biochem. Pharmacol.*, 63: 21-29.