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Antioxidant activity of extract from *Polygonum aviculare* L.

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ABSTRACT

Free radicals induce numerous diseases by lipid peroxidation, protein peroxidation, and DNA damage. It has been reported that numerous plant extracts have antioxidant activities to scavenge free radicals. Whether *Polygonum aviculare* L. (Polygonaceae) has antioxidant activity is unknown. In this study, dried *Polygonum aviculare* L. was extracted by ethanol, and the extract was lyophilized. The antioxidant activities of extract powder were examined by free radical scavenging assays, superoxide radical scavenging assays, lipid peroxidation assays and hydroxyl radical-induced DNA strand scission assays. The results show that the IC50 value of *Polygonum aviculare* L. extract is 50 μg/ml in free radical scavenging assays, 0.8 μg/ml in superoxide radical scavenging assays, and 15 μg/ml in lipid peroxidation assays, respectively. Furthermore, *Polygonum aviculare* L. extract has DNA protective effect in hydroxyl radical-induced DNA strand scission assays. The total phenolics and flavonoid content of extract is 677.4 ± 62.7 mg/g and 112.7 ± 13 mg/g. The results indicate that *Polygonum aviculare* L. extract clearly has antioxidant effects.

Key terms: antioxidant activity, free radical, phenolics, lipid peroxidation, DNA damage *Polygonum aviculare* L.

INTRODUCTION

Reactive oxygen species produced by ultraviolet light, ionizing radiation, chemical reactions, and metabolic processes have numerous pathological effects, such as causing lipid peroxidation, protein peroxidation, DNA damage, and cellular degeneration related to cardiovascular disease, ageing, cancer, inflammatory diseases, and a variety of other disorders (2, 6, 9, 16, 20, 28). They include superoxide radical anion ($^{\bullet}O_{2}^{-}$), hydroxyl radicals ($^{\bullet}OH$), singlet oxygen ($^{1}O_2$), and hydrogen peroxide (H_2O_2). In cellular oxidation reactions, superoxide radical normally is formed first, and its effects can be magnified because it produces other kinds of cell-damaging free radicals and oxidizing agents. The damaging action of the hydroxyl radical is the strongest among free radicals (18).

Phenolics have been reported to have a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants and have multiple biological effects, including antioxidant activity (13, 27). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential (22). Phenolics, such as flavonoids, phenolic acids, stilbenes, lignans, lignin, and tannins, are especially common in leaves, flowering tissues, and woody parts, such as stems and barks (17). They have been suggested to play a preventive role in the development of cancer, heart disease, and ageing-related diseases.

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from ageing-related diseases has intrigued scientist for a long time. I have screened the

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antioxidant activity of a variety of wild plants and other substances used in traditional Oriental medicine by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays. The results show that *Polygonum aviculare* L. (Polygonaceae) exhibits a DPPH free radical scavenging effect. *Polygonum aviculare* L. is used in traditional Oriental medicine and belongs to the "li-shui-shen-shih" category of drugs. It is used traditionally to treat ailments caused by high humidity, because of its diuretic property. In the present study, I wish to study the antioxidant effects of *Polygonum aviculare* L. on superoxide radical scavenging, lipid peroxidation, and DNA damage.

METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitroblue tetrazolium chloride (NBT), 2-thiobarbituric acid (TBA), ΦX174 RF1 supercoiled DNA, Folin-Ciocalteu reagent, L-ascorbic acid, and (+)-catechin were purchased from Sigma Chemical Co. The other chemicals and solvents used in this experiment were of the highest quality available. Dried *Polygonum avicular* was purchased from a local drugstore.

Preparation of plant extract

Dried *Polygonum aviculare* L. was made into powder form. 5 g of dried powder were extracted in 50 ml 50% ethanol solution at 25°C for 30 min with shaking. The extract was centrifuged at 15000 rpm for 3 min, and the supernatant was collected. The supernatant was concentrated in a rotary evaporator and then lyophilized. The resulting powder extract was used in this study (4).

DPPH assay

1 mg extract powder was dissolved in 1 ml of 50% ethanol solution to obtain 1000 μg/ml sample solution. 1000 μg/ml solutions were series diluted into 1 μg/ml, 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml, 500 μg/ml, and 1000 μg/ml with 50% ethanol. In each reaction, the solutions were mixed with 1 ml of 0.1 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 mM Tris-HCl buffer (pH 7.4), and 0.05 ml samples at room temperature for 30 min. 50% ethanol solution was used as control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH, a purple-colored, stable free radical is reduced to the yellow-colored diphenylpicrylhydrazine when antioxidants are added. L-ascorbic acid and (+)-catechin were used as positive controls. The inhibition ratio (percent) was calculated from the following equation: % inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100%. The antioxidant activity of each sample was expressed in terms of IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve (5, 8, 30).

NBT (superoxide scavenging) assay

The superoxide anion radical scavenging activity was performed by using the methods of Liu and Ng (18). Superoxide radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 μM β-nicotinamide adenine dinucleotide (reduced form, NADH), 50 μM nitroblue tetrazolium (NBT), 10 μM phenazin methosulfate (PMS), and test samples in 50% ethanol solution (final concentrations were 1, 5, 10, 20, 50, and 100 μg/ml, respectively). The color reaction of superoxide radicals and NBT was detected at OD 560 nm. (+)-catechin was used as a positive control. The inhibition ratio (%) was calculated from the following equation: % inhibition = [(absorbance of control – absorbance of test sample)/ absorbance of control] x 100%.

Lipid peroxidation assay

The brain of young adult male Balb/c mice were dissected and homogenized with a homogenizer in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate.

The homogenate was centrifuged at 12000g for 15 min at 4 ºC, and the supernatant was used for in vitro lipid peroxidation assay. A 1 ml aliquot of liposome was incubated with the test samples (final concentrations were 1, 5, 10, 20, 50, and 100 μg/ml, respectively) in the presence of 10 mM $FeSO_4$ and 0.1 mM ascorbic acid at 37ºC for 1 h. The reaction was terminated by the addition of 1.0 ml of trichloroacetic acid (TCA; 28%, w/v) and 1.5 ml of TBA (1%, w/v), followed by heating at 100ºC for 15 min. The absorbance of the malondialdehyde (MDA)-TBA complex was measured at 532 nm. (+)-catechin was used as a positive control. The inhibition ratio (%) was calculated from the following equation: % inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100% (4).

DNA strand scission assay

The assay was performed according to the method of Keum et al., with minor modifications (15). The reaction mixture (30 μl) contained 10 mM Tris-HCl, 1 mM EDTA buffer (pH 8.0), ΦX174 RF1 supercoiled DNA (0.6 μg), and H_2O_2 (0.04M). Various amounts of the test extract samples dissolved in 10 μl of ethanol (final concentrations of the plant extract in each assay were 1, 10, 100, 500, and 1000 μg/ml, respectively) were added prior to H_2O_2 addition. Hydroxyl radicals were generated by irradiation of the reaction mixtures at a distance of 5 cm with a 12 W UV lamp. After incubation at room temperature for 20 min, the reaction was terminated by the addition of a loading buffer (0.25% bromophenol blue tracking dye and 40% sucrose), and the mixtures were then analyzed by 0.8% submarine agarose gel electrophoresis (50eV, 1.5 h). The gel was stained with ethidium bromide, destained in water, and photographed on a transilluminator (4).

Determination of total flavonoid

1 mg samples were added in 1ml of 80% ethanol. A aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 min at room temperature. Total flavonoid concentration was calculated using quercetin as standard (19).

Determination of Total Phenolics

Total phenolics content was determined according to the Folin-Ciocalteu method (23), using gallic acid as a standards 1 mg extract powders were dissolved in 1 ml 50% methanol solution. 0.5 ml extract solution was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent. The mixture was let sit for 2-5 min before the addition of 1.0 ml of 20% Na_2CO_3. The mixture was centrifuged at 150 g for 8 min after 10 min of incubation at room temperature. The absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample (4).

RESULTS

The free radical scavenging activity of *Polygonum aviculare* L. extract was assessed by 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay. (+)-catechin and L-ascorbic acid were used as controls. Both are well-known antioxidant compounds. The result is shown in Figure 1. The IC_{50} values (the concentration required to inhibit radical formation by 50%) of *Polygonum aviculare* L. extract are 50 μg/ml. The IC_{50} values of (+)catechin and ascorbic acid are 35 μg/ml and 50 μg/ml, respectively; they exhibit a similar curve of antioxidant activity compared to (+)-catechin and L-ascorbic acid. The IC_{50} value of *Polygonum aviculare* L. extract is lower than that of (+)-catechin and is similar to that of L-ascorbic acid. This result demonstrates that *Polygonum aviculare* L. extract has an inhibitory effect on the DPPH radical.

The superoxide scavenging activity of *Polygonum aviculare* L. extract was evaluated by NBT (Superoxide Scavenging) assay. (+)-catechin served as a control. The result is shown in Figure 2. The IC_{50} value of *Polygonum aviculare* L. extract is 0.8 μg/

ml. The IC_{50} value of (+)-catechin is 40 μg/ml. Almost all superoxide radicals were inhibited by 10 μg/ml *Polygonum aviculare* L. extract. The superoxide scavenging activity of *Polygonum aviculare* L. extract is apparently higher than that of (+)-catechin. Therefore, *Polygonum aviculare* L. seems to be a potential source of superoxide radicals scavenging. This result shows that *Polygonum aviculare* L. extract has a significant superoxide scavenging activity.

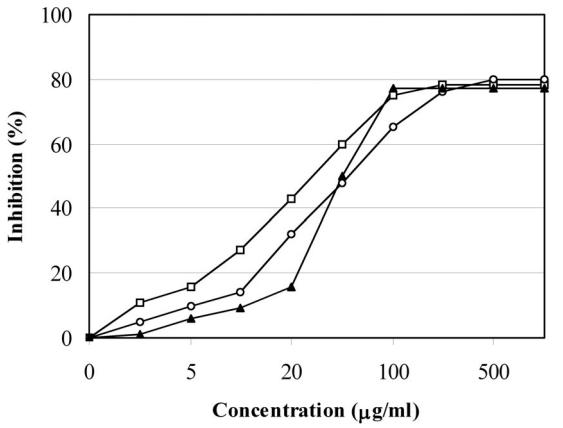

Figure 1: Free-radical scavenging activity of *Polygonum aviculare* L. extract are measured by using the DPPH assay: (◊) (+)catechin; (O) ascorbic acid; (▲) *Polygonum aviculare* L. extract. Results are mean ± SD (N=5).

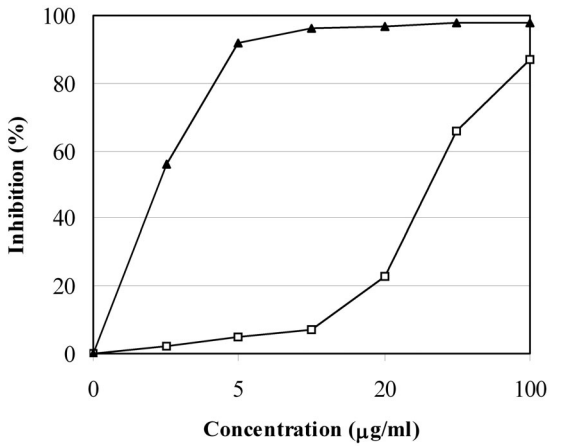

Figure 2: Superoxide scavenging activity of *Polygonum aviculare* L. extract are measured by using the NBT assay: (◊) (+)catechin; (▲) *Polygonum aviculare* L. extract. Results are mean ± SD (N =5).

The lipid peroxidation suppressing activity of *Polygonum aviculare* L. extract was estimated by TBA assay. (+)-catechin was employed as control. The result is shown in Figure 3. The IC_{50} values of *Polygonum aviculare* L. extract is 16 μg/ml. The IC_{50} value of (+)-catechin is 17 μg/ml. Compared to (+)-catechin, they exhibit the similar curve of antioxidant activity. The lipid peroxidation suppressing activity of *Polygonum aviculare* L. extract is identical to that of (+)-catechin. This result indicates that *Polygonum aviculare* L. extract has suppressing activity on lipid peroxidation.

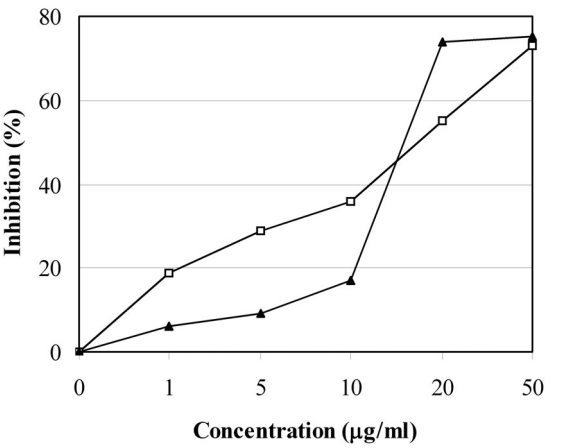

Figure 3: Effects of *Polygonum aviculare* L. extract on both ferric ion and ascorbic acid induced lipid peroxidation on mouse brain homogenates: (◊) (+)catechin; (▲) *Polygonum aviculare* L. extract. Results are mean ± SD (N =5).

ΦX174 RF1 DNA strand scission induced by UV photolysis of H_2O_2 elevated the protective effect of DNA of *Polygonum aviculare* L. extract. The result is shown in Figure 4. ΦX174 RF1 supercoiled DNA was utilized as control (lane 1). UV illumination alone did not cause DNA strand cleavage (lane 2). The treatment of supercoiled DNA with UV plus H_2O_2 led to the conversion of the DNA to open circular form (lane 3). The treatment of supercoiled DNA with UV, H_2O_2 plus the different concentration of

Polygonum aviculare L. extract led to the maintenance of the DNA in the supercoiled form (lanes 4-8). Almost complete protection was expressed at a dose of 1000 μg/ml. This protective effect of DNA exhibits dose-dependency. This result shows that *Polygonum aviculare* L. extract has DNA protective activity under oxidative stress.

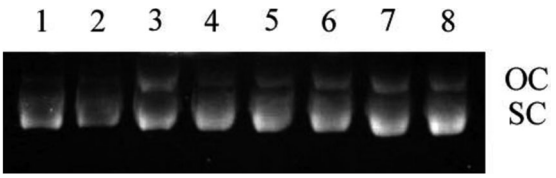

Figure 4: Protection effect of *Polygonum aviculare* L. extract on DNA strand scission induced by H_2O_2 and UV. ΦX174 RF1 supercoiled DNA as control (lane 1), ΦX174 RF1 supercoiled DNA was exposed to UV alone (lane 2), UV plus H_2O_2 (lane 3), or plus H_2O_2 in the presence of final concentration of 1000 μg/ml (lane 4), 500 μg/ml (lane 5), 100 μg/ml (lane 6), 10 μg/ml (lane 7), 1 μg/ml (lane 8) of *Polygonum aviculare* L. extract. Lane 1 represents native ΦX174 RF1 supercoiled DNA without any treatment. OC: Open circular; SC: Super coiled.

Plant phenolics are widely distributed in plants. They are highly effective free radical scavengers and exhibit strong antioxidant activity. The content of total phenolics in the *Polygonum aviculare* L. was determined spectrometrically according to the Folin-Ciocalteu procedure and calculated as gallic acid equivalent contents. The result is shown in Table 1. The total phenolic content of *Polygonum aviculare* L. extract is 677.4 ± 62.7 mg/g. The content of total flavonoid in the *Polygonum aviculare* L. also was determined spectrometrically and calculated as quercetin equivalents content. The result is shown in Table 1. The total flavonoid content of *Polygonum aviculare* L. extract is 112.7 ± 13 mg/g. These results imply that *Polygonum aviculare* L. extract contains a high quantity of phenolics and flavonoids.

TABLE 1

Total phenolic content and total flavonoid content of ethanolic extract from *Polygonum aviculare*

Extract	Total phenolic (mg of GAE/g)	Total flavonoid (mg/g of samples)
Polygonum aviculare	677.4 ± 62.7 (N=8)	112.7 ± 13 (N=10)

Total phenolics are expresed as gallic acid equivalent (GAE).
Total flavonoid are expresed as mg of total flavonoid content / g of samples based on quercetin as standard.
Values represent mean ± S.D.

DISCUSSION

Antioxidant activity of *Polygonum aviculare* L. extract has been found by means of free radical scavenging assays, superoxide radical scavenging assays, lipid peroxidation assays, and hydroxyl radical-induced DNA strand scission assays. In addition, *Polygonum aviculare* L. extract has high phenolics and flavonoid contents. This study indicates that *Polygonum aviculare* L. extract obviously has antioxidant effects.

DPPH is a stable radical that has been used widely to evaluate the antioxidant activity of various natural products (12). In this study, DPPH scavenging activity has been found in *Polygonum aviculare* L. extract. The maximum inhibition of *Polygonum aviculare*, (+)catechin and ascorbic acid is about 80% in this study. The maximum inhibition concentration of *Polygonum aviculare* L. and (+)-catechin is approximately 100 μg/ml. The maximum inhibition concentration of ascorbic acid is approximately 200 μg/ml. The inhibitory curve of DPPH scavenging activity of *Polygonum aviculare* L. is similar to that of *Acacia confusa* (4), Cat's claw (*Uncaria tomentosa*) (24), and *Anthriscus cerefolium* (5). However, the IC_{50} value of *Polygonum aviculare* L. (50 μg/ml) is less than that of *Acacia confusa* (5 μg/ml), Cat's claw *(Uncaria tomentosa)* (18 μg/ml), and *Anthriscus cerefolium* (45 μg/ml) (4, 24, 5). Nevertheless, *Polygonum aviculare* L.

extract is a potential source of natural antioxidants.

In cellular oxidation reactions, superoxide radicals normally are formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents (18). Additionally, xanthine oxidase is one of the main enzymatic sources of those reactive oxygen species in vivo. In this study, superoxide radicals scavenging property has been found in *Polygonum aviculare* L. extract. The IC_{50} value of *Polygonum aviculare* L. extract is 0.8 μg/ml, whereas the IC_{50} value of (+)-catechin is 40 μg/ml. The maximum inhibitory effect of *Polygonum aviculare* L. is about 100%. The maximum inhibition concentration of *Polygonum aviculare* L. is approximately 10 μg/ml. However, the maximum inhibition concentration of (+)-catechin is higher than 100 μg/ml. It is noteworthy that the superoxide scavenging activity of *Polygonum aviculare* L. extract is superior to that of (+)-catechin. Moreover, the IC_{50} value of *Polygonum aviculare* L. (0.8 μg/ml) is larger than that of *Paeonia suffruticosa* (50 μg/ml) (18). In other words, *Polygonum aviculare* L. has better superoxide radicals scavenging activity than *Paeonia suffruticosa*. These results show that *Polygonum aviculare* L. is an important source for superoxide radical scavenging.

In the current study, lipid peroxidation of mouse brain homogenates was induced by ferric ion plus ascorbic acid. Lipid peroxidation scavenging activity has been found in *Polygonum aviculare* L. extract. The IC_{50} value of *Polygonum aviculare* L. extract is about 16 μg/ml. The IC_{50} value of (+)-catechin is about 17 μg/ml. The maximum inhibitory effect of *Polygonum aviculare* L. is about 75%. The maximum inhibition concentration of *Polygonum aviculare* L. is approximately 20 μg/ml. The inhibitory effect of *Polygonum aviculare* L. is higher than that of (+)catechin when the concentration is higher than IC_{50} values. In other words, *Polygonum aviculare* L. extract has better scavenging effect than (+)-catechin when the concentration is higher than 17 μg/ml. This result indicates that *Polygonum aviculare* L. extract is a good source of lipid peroxidation scavenging.

The cellular damage resulting from hydroxyl radicals is strongest among free radicals. Hydroxyl radicals can be generated by biochemical reactions. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which subsequently can produce extremely reactive hydroxyl radicals in the presence of transition metal ions, such as iron and copper or by UV photolysis. Hydroxyl radicals can attack DNA to cause strand scission. That is, incubation of ΦX174 RF1 supercoiled DNA with H_2O_2 and then UV radiation resulted in complete conversion of supercoiled DNA to the open circular form. In this study, the administration of *Polygonum aviculare* L. extract to the reaction mixture substantially decreased the DNA strand scission induced by both H_2O_2 and UV radiation. It shows a dose-dependent protection of DNA under oxidative stress. The higher the concentration of *Polygonum aviculare* L. extracts, the better the DNA protection. There is almost complete protection at a dose of 1000 μg/ml. The effect of DNA protection of *Polygonum aviculare* L. is similar to that of *Acacia confusa* (4). These results reveal that *Polygonum aviculare* L. extract is an excellent DNA protector.

Phenolics are found in large quantities in the plant kingdom, and they have been shown to have multiple biological functions, including antioxidant activity (21, 25, 14). In this study, we examined the content of phenolics from the extract of *Polygonum aviculare*. The result showed that *Polygonum aviculare* L. extract contains 677.4 ± 62.7 mg/g phenolics. It indicated that the *Polygonum aviculare* extract contained a higher amount of phenolics than the bark and heartwood extracts of *Acacia confusa* based on Folin-Ciocalteu procedures (4). Therefore, *Polygonum aviculare* L. is a significant source of phenolics. The results in this study suggest that the effectiveness of the antioxidant activity of *Polygonum aviculare* extract is probably related to the high contents of phenolics, and the observed antioxidant activities of the extract may be due to the hydroxyl groups in phenolics (10).

A similar finding has been demonstrated in the plant extracts of *Eucommia ulmoides* (Du-zhong) and *Acacia confusa* in which enriched phenolics correlated well with their antioxidant activities (4, 29).

It also has been reported that *Polygonum aviculare* L. can be employed supportively in the therapy of gingivitis by oral rinse (7). It was suggested that this phenomenon was attributed to the flavonoid components that decrease capillary fragility and exert a cortisone-like effect on gingival tissues (7). In this study, we examined the content of flavonoids from the extract of *Polygonum aviculare*. The result showed that *Polygonum aviculare* L. extract contains high flavonoids, 112.7 ± 13 mg/g. It has been suggested that the therapeutic effect of *Polygonum aviculare* L. on gingivitis is derived from its high flavonoid. Additionally, the flavonoid content of *Polygonum aviculare* L. also is higher than that of propolis (29).

It is well known that free radicals are the principal cause of several diseases, including Parkinson's disease, coronary heart disease, cancer, and Alzheimer's disease (3, 1, 11, 26). This study demonstrated that *Polygonum aviculare* L. has high phenolics contents and excellent antioxidant activity. It would be interesting to investigate further the potential effectiveness of *Polygonum aviculare* L. for treating diseases caused by the overproduction of free radicals. Also, the antimicrobial effect, bioavailability and potential toxicity of *Polygonum aviculare* L need to be studied *in vivo*.

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