# Antioxidant Activity of a New Dry Plant Extract Nephrophyt

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Antioxidant activity of a new plant preparation nephrophyt was evaluated by its effect on the rate of superoxide anion radical generation *in vitro*, level of $Fe^{2+}$-induced chemiluminescence, and content of lipid peroxidation products (malonic dialdehyde and conjugated dienes) in the blood of control and experimental animals. This preparation produces a direct antioxidant effect and possesses no prooxidant activity.

**Key Words:** *nephrophyt; lipid peroxidation; antioxidant*

Nephroprotective activity of some new phytopreparations is associated with the ability of their components to inhibit free radical processes in intact and damaged cells and tissues [1,2,4,7,10,13]. Experiments with polyphenylalanine and oligo- and polyphenols added to cell-free translation systems based on kidney tissue microsomes showed that phenol compounds act as free radical-trapping and antioxidant agents. However, little is known about medicinal properties of these substances [1,3,5,6,8,9].

Our previous studies showed that the preparation nephrophyt containing dry extracts from bearberry leaves, *Orthosiphon stamineus*, and knotgrass includes a considerable amount of phenol compounds and *in vivo* produces a nephroprotective effect [2]. The study of the chemical composition [2] and published data [3,5,8,9] suggest that nephrophyt has antioxidant properties. Here we evaluated the effects of nephrophyt on the rate of superoxide anion radical ($O_2^{\bullet-}$) generation, content of lipid peroxidation (LPO) products malonic dialdehyde (MDA) and conjugated dienes, and level of $Fe^{2+}$-induced chemiluminescence (CL).

## MATERIALS AND METHODS

Experiments were performed on male outbred rats aging 3 months and weighing 160-180 g. The animals fed a standard diet and were deprived of food for 24 h before the experiment. The suspension of nephrophyt in physiological saline was given perorally (150 mg/kg, 0.03 $LD_{50}$) [1,10]. Control rats received an equivalent volume of physiological saline.

The concentration of conjugated dienes in rat plasma was measured spectrophotometrically [1,13]. Plasma MDA level was determined by the formation of colored complexes with thiobarbituric acid [1].

$Fe^{2+}$-induced CL in the suspension of multilayered liposomes was recorded by the modified method on a PXL-01 luminescence spectrophotometer [2]. Kinetic characteristics of CL were evaluated. Measuring cell contained 1 ml phosphate buffer (20 mM $KH_2PO_4$ and 15 mM KCl, pH 7.4), 0.5 ml buffer solution with 50 μl plasma, and 0.5 ml eosin (2.7 mM). Intrinsic antioxidant activity of the plant extract was estimated in the model system. The incubation medium contained 1 ml phosphate buffer, 0.5 ml chicken embryo liposomes, 0.5 ml eosin, and nephrophyt in an experimental therapeutic dose [7]. Incubation was performed in a dark chamber for 2 min under constant mixing. $FeSO_4 \times 7H_2O$ (0.2 ml, 38.5 mM) served as the inductor. Spontaneous CL was recorded for 30 sec. CL of control samples not containing plant extract was determined. We evaluated the effect of nephrophyt on kinetic characteristics of $Fe^{2+}$-induced CL. The data were processed on an Olivetti QRL07 digital analytic device (Olivetti) using Sigma Fluor Chem DX400 software [1].

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The effect of nephrophyt on the rate of $O_2^{\bullet-}$ generation was studied *in vitro* [1]. The measuring cell contained 1.9 ml sodium phosphate (10 mM, pH 7.8), 0.7 mM NADPH, and 0.1 ml plant extract in the corresponding concentration, 0.5 ml 0.01% nitroblue tetrazolium, and 0.15 ml 0.005% phenazine methosulfate were placed in a well and thoroughly mixed. Changes in optical density were recorded at 560 nm. The intensity of $O_2^{\bullet-}$ generation was determined by the rate of nitroblue tetrazolium reduction (tangent of the slope of the kinetic curve in a blank sample, 100%).

The differences between control and experimental measurements and correlation coefficients ($r$) were evaluated by nonparametric tests for $n \leq 6$ [7,10,12].

## RESULTS

Antioxidant activity of nephrophyt was determined by the relative rate of $O_2^{\bullet-}$ trapping in accordance with recommendations for pharmacological and toxicological assays of multicomponent bioorganic compounds [3,9]. The addition of nephrophyt to the model redox system with nitroblue tetrazolium promoted inhibition of its reduction (Table 1). The degree of $O_2^{\bullet-}$ trapping 5.6-fold surpassed the control level. These data indicate that nephrophyt exhibits high antioxidant activity. Our results are consistent with *in vitro* studies of the antioxidant effect of nephrophyt in another conventional test system ($Fe^{2+}$-luminescing suspension of liposomes, Table 2). A strong positive correlation was revealed between the concentration and antioxidant activity of nephrophyt ($r$=0.86). The high-amplitude response and significant differences between control and experimental samples indicate that nephrophyt *in vitro* acts as a potent inhibitor of free radical reactions (Tables 1, 2).

Screening for aromatic xenobiotics using test systems based on nitroblue tetrazolium and technique of liposome CL were successfully used in veterinary practice to search low-toxicity antioxidants [5,9,14]. Particular attention was given to oligo- and polyphenol compounds as most potent "catchers" of free radicals [9].

Taking into account high content of phenol compounds in nephrophyt and results of our experiments (Tables 1, 2), we evaluated the effects of this preparation on the content of LPO products (MDA and conjugated dienes) in the blood from experimental animals.

Single treatment with nephrophyt in therapeutic doses had no effect on MDA concentration, but sharply decreased the content of conjugated dienes in the plasma (Table 3). Similar results were obtained previously in studying antioxidant activity of synthetic oligophenylalanine. The inhibitory effect of oligophenylalanine on LPO was probably accompanied by exhaustion of MDA reserves due to rapid metabolic transformation by tetrahydrofolate-dependent alkylation [3,6,9,11].

Further studies are required to evaluate whether the influence of nephrophyt is mediated by the same mechanism.

We conclude that nephrophyt *in vitro* produces a direct antioxidant effect in test systems for toxicological and pharmacological assays and *in vivo* inhibits LPO. It should be emphasized that this preparation is characterized by low toxicity [2]. It is necessary to perform detailed clinical, pharmacological, and biochemical studies of nephrophyt.

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**TABLE 1.** Rate of $O_2^{\bullet-}$ Trapping in Model Redox System of Nitroblue Tetrazolium and Nephrophyt ($M \pm m$, $n$=8)

| Nephrophyt, mg/ml | Inhibition of nitroblue tetrazolium reduction, % |
|---|---|
| 0 | 16.4±2.3 |
| 0.25 | 78.0±7.7* |
| 0.50 | 86.0±8.6* |
| 1.00 | 91.2±9.8* |

**Note.** *$p$<0.01 compared to the control. Rank correlation coefficient 0.78.

**TABLE 2.** Effect of Nephrophyt on $Fe^{2+}$-Induced CL of Liposome Suspension ($M \pm m$, $n$=8)

| Nephrophyt, mg/ml | Antioxidant activity, arb. U |
|---|---|
| 0 (control) | 26.80±3.55 |
| 0.05 | 38.40±5.26*** |
| 0.10 | 62.90±6.15** |
| 0.25 | 88.8±6.1* |
| 0.50 | 109.60±7.72* |

**Note.** *$p$<0.01, **$p$<0.05, and ***$p$=0.05 compared to the control. Rank correlation coefficient 0.86.

**TABLE 3.** Effect of Peroral Treatment with Nephrophyt in a Single Therapeutic Dose of 150 mg/kg on the Amount of MDA and Total Content of Conjugated Dienes in Rat Plasma ($M \pm m$, $n$=8)

| Group | MDA, μM | Conjugated diene $A_{233}$ |
|---|---|---|
| Control | 1.95±0.06 | 4.35±0.38 |
| Experiment | 1.88±0.07** | 1.22±0.15* |

**Note.** *$p$<0.01 and **p<0.05 compared to the control.

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