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Anti-Anemic Activity of *Raphanus sativus* Leaves on Phenylhydrazine Induced Anemia in Wistar Albino Rats



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ABSTRACT

- "*Raphanus sativus* was chosen for this study based on literature reviews highlighting its various activities. This study aimed to evaluate the effect of an ethanolic extract of *Raphanus sativus* leaves on phenylhydrazine-induced anemia in Wistar albino rats by assessing hematological parameters and in vivo enzymatic antioxidant activity."

- "Male Wistar albino rats were divided into five groups (n=6). Group I (normal control) received normal feed and water. Anemia was induced in Groups II-V by intraperitoneal administration of PHZ (40 mg/kg). Group II (negative control) received phenylhydrazine for two days. Group III (positive control) received hematinic syrup (0.68 ml/kg). Groups IV and V received 200 mg/kg and 400 mg/kg of the ethanolic extract of *Raphanus sativus* leaves, respectively, for 12 days."

The *in-vivo* anti-anemic activity was evaluated by measuring the biochemical, hematological parameters and RBC morphological microscopic examination. The enzymatic antioxidant potential was evaluated by superoxidase dismutase and glutathione peroxidase enzyme. The results were analysed using one-way ANOVA and p value were calculated to find the significance of the results.

In-vivo antioxidant results showed that ethanolic extract of *Raphanus sativus* leaves had high anti-oxidant activity. The treatment with Ethanolic extract of *Raphanus sativus* leaves had been effective in increasing the count of RBC, Hemoglobin and hematocrit. There is significant decrease in MCV, MCH and MCHC. In addition, it was confirmed that treatment with ethanolic extract of *Raphanus sativus* leaves reversed the decreased serum level of glutathione peroxidase and superoxide dismutase which confirmed its antioxidant potential against oxidative stress in erythrocytes.



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1 INTRODUCTION

Anemia is a severe worldwide public health issue that primarily affects young children, teenage girls who are menstruation, and women who are pregnant or just gave birth. According to WHO estimates, anaemia affects 40% of children aged 6-59 months, 37% of pregnant women, and 30% of women aged 15-49 globally.⁽⁴⁾

Anemia is a condition in which the number of red blood cells or the hemoglobin concentration within them is lower than normal. Hemoglobin is needed to carry oxygen and if you have too few or abnormal red blood cells or not enough hemoglobin, there will be decreased capacity of the blood to carry oxygen to the body's tissues. Symptoms of anemia include fatigue, weakness, dizziness, shortness of breath.

Common types of anemia include iron deficiency anemia, vitamin deficiency anemia, aplastic anemia, hemolytic anemia, sickle cell anemia and anemia caused by other disease. People at greater risk for anemia include disease condition such as Rheumatoid arthritis or other autoimmune diseases, kidney disease, cancer, liver disease, thyroid disease and inflammatory bowel disease.

Hemolytic Anemia: Haemolytic anaemia is a condition in which red blood cells are destroyed and removed from the bloodstream before their normal lifespan is up. Haemolytic anaemia can affect people of all ages, races and sexes. Hemolytic anaemia can lead to various health problems such as fatigue, pain, arrhythmias, an enlarged heart and heart failure. Inherited haemolytic anaemias include Sickle cell anaemia, Thalassaemia, hereditary spherocytosis, hereditary elliptocytosis, Glucose-6-phosphate dehydrogenase (G6PD) deficiency, Pyruvate kinase deficiency. Acquired hemolytic anaemias include Immune haemolytic anaemia, Autoimmune hemolytic anaemia, Alloimmune hemolytic anaemia, Drug-induced hemolytic anaemia, Mechanical hemolytic anaemias, nocturnal hemoglobinuria, Certain infections and substances can also damage red blood cells and lead to hemolytic anaemia.

Symptoms: The most common symptom of anaemia is fatigue. A low red blood cell count can also cause shortness of breath, dizziness, headache, coldness in your hands or feet, pale skin, gums and nail beds, as well as chest pain. Symptoms of haemolytic anaemia include Jaundice, Pain in the upper abdomen, Leg ulcers and pain, A severe reaction to a blood

transfusion. Treatments for haemolytic anaemia include blood transfusions, medicines, plasmapheresis, surgery, blood and marrow stem cell transplants and lifestyle changes. ⁽⁷⁾

Raphanus sativus is used as a home remedy for the treatment of many diseases such as jaundice, gallstone, liver diseases, rectal disorder, indigestion, and other gastric pains. The leaves which generally have been discarded are containing 10 times more vitamin C than roots thus containing more anti-oxidant properties. This research focuses on the efficacy of Leaves of *Raphanus sativus* on hemolytic anemia induced by phenyl hydrazine. Hemolytic anemia is a disorder in which the red blood cells are destroyed faster than they are made. Include types such as inherited and acquired. It can be diagnosed by complete blood count, serum iron and ferritin level.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant material

The leaves of *Raphanus sativus* Linn. were purchased from the Local market, in Ambattur, Chennai-600053. Tiruvallur district, Tamil Nadu in the month of December, 2023. The plant was identified and authenticated by Prof. Dr.S.Sankaranarayanan M.sc, M.Phil., Ph.D., HOD, Department of Medicinal Botany and Pharmacognosy, Government Siddha Medical College, Arumbakkam, Chennai-600106.

"The leaves were dried under shade, powdered, and stored in airtight containers. The ethanolic extract was prepared using a Soxhlet extractor and concentrated with a rotary evaporator."

2.2 Preparation of ethanolic extract

The leaves powder was extracted with ethanol (99.9%) by hot percolation method using Soxhlet extractor. The extract was concentrated by using a rotary evaporator. The semisolid residue obtained were weighed and stored in desiccator. The Percentage yield of the ethanolic extract of *Raphanus sativus* leaves was calculated using the following formula. ⁽⁹⁾

$$\text{Yield value} = \frac{\text{Weight of the residue}}{\text{Weight of the dried plant}} \times 100$$

2.3 Experimental animals

The present study was conducted after obtaining approval from the Institutional Animal Ethics Committee and this protocol met the requirements of national guidelines of CPCSEA/IAEC approval no: 1917/GO/ReBi/S/16/CPCSEA/25.10.2016 and 1/AEL/IAEC/MMC, Date: 26.12.2023. For this investigation, 30 male Wistar albino rats were purchased from the Madras Medical College Animal House in Chennai, India. In a quarantine period, animals were kept apart from those already housed in the facility while their health as well as their microbiological condition are being assessed. The newly procured male Wistar albino rats were quarantined for a period of one week to minimize the chance of introduction of pathogens into established animals and allowed to develop psychological, physiological and nutritional stabilization before their use. The animals were housed in a well-ventilated condition which was maintained at a constant temperature and relative humidity of 55 to 60%. The animals were housed in spacious polypropylene cages and paddyhusk was utilized as bedding material. The bed material was changed twice a week. The animals were maintained on standard pellets and purified water. The animals were provided with food ad libitum except during fasting. All animal cages used in the study had proper identification i.e., labels. Each animal in the cage was marked on the tail with picric acid for their appropriate identification. ⁽²⁾

2.4 Acute toxicity studies (as per the OECD guidelines no. 423):

Acute toxicity study has been carried out already and the animals did not show any toxic effects up to dose of 2000 mg/kg-5000 mg/kg and hence 1/10th and 1/5th of the maximum dose administered (i.e., 200 mg/kg and 400 mg/kg) was selected for the present study. ⁽⁶⁾

2.5 Experimental design

All the animals were weighed and separated into 5 groups each consisting of n= 6. Anemia was induced by intraperitoneal administration of phenyl hydrazine (40 mg/kg) in all groups except normal control group rats on day 1 & day 2.

Group I: Normal control received normal feed and water.

Group II: Negative control received normal feed and water from day 3 to day 14.

Group III: Positive control received hematonic syrup (0.68 ml/kg) from day 3 to day 14.

Group IV: Test group received ethanolic extract of *Raphanus sativus* (EERS) 200 mg/kg from day 3 to day 14.

Group V: Test group received ethanolic extract of *Raphanus sativus* (EERS) 400 mg/kg from day 3 to day 14.

After 14 days of treatment, whole blood sample were collected from Lateral tail vein under anesthesia. Samples were added to tube containing ethylenediaminetetraacetic acid after 2 weeks of treatment. Blood samples were subjected to analyze hematological parameters ⁽²⁾, serum biochemical parameters ⁽⁴⁾ and Antioxidant potential such as glutathione peroxidase (GPx) and superoxide dismutase (SOD) was also analyzed using the standard procedure. ⁽²⁾

2.6 STATISTICAL ANALYSIS

The results were expressed as mean \pm SEM. The data was statistically analyzed by means of one way ANOVA followed by Dunnett's multiple comparison test using Graphical Pad Prism software version 8.0.2. One way ANOVA was used to correlate the statistical difference between the variables. P value ($P < 0.05$), ($P < 0.01$), ($P < 0.001$) was considered statistically significant.

3. RESULTS

3.1 Percentage yield of ethanolic extraction of *Raphanus sativus*

The Percentage yield of the ethanolic extract of *Raphanus sativus* leaves was found to be 7.14 % w/w.

3.2 Effect of EERS on Hematological parameters

Induction of anemia after the administration of phenyl hydrazine (I.P) at the dose of 40 mg/kg for 2 days caused significant ($P < 0.0001$) reduction of RBC Count, hemoglobin, hematocrit in disease control rats when compared with normal control rats. It was due to the presence of oxidative stress within RBC due to formation of ROS, subsequent hemolysis and premature splenic destruction. The RBC count, hemoglobin and hematocrit were increased significantly ($P < 0.0001$) after treatment with low dose (200 mg/kg) and high dose (400 mg/kg) as well as in standard drug (hematinic syrup, 0.68 ml/kg) treated rats and EERS treated rats as shown in the table 1.

There was significant increase ($P < 0.0001$) of mean cell volume, mean cell hemoglobin,

mean cell hemoglobin concentration in anemia induced rats when compared with normal control rats. while on treatment with low dose (200 mg/kg), high dose (400 mg/kg), as well as standard drug (hematinic syrup, 0.68 ml/kg) showed significant reduction of mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentration in standard treated rats and EERS treated rats as shown in the table 1.

Table 1. Haematological Parameters:

S.N O	PARAMETERS	GROUP I (Normal Control)	GROUP II (Disease control)	GROUP III (standard control)	GROUP IV (Low dose)	GROUP V (High dose)
1.	RBC Count ($\times 10^{12}/L$)	9.22±0.13	6.48±0.11	8.64±0.06	7.47±0.06	8.52±0.09
2.	Haemoglobin (g /DL)	16.15±0.13	11.05±0.12	15.18±0.11	14.97±0.12	15.05±0.12
3.	Haematocrit (%)	51±0.21	42.98±0.16	48.65±0.11	45.83±0.23	46.05±0.14
4.	Mean Corpuscular Volume (fL)	56.38±0.29	61.81±0.20	57.12±0.32	60.88±0.17	59.66±0.30
5.	Mean Corpuscular Haemoglobin (pg)	14.23±0.41	19.78±0.27	15.2±0.28	18.55±0.09	16.35±0.31
6.	Mean Corpuscular Haemoglobin Concentration (g/dl)	24.3±0.56	35.15±0.34	26.17±0.52	32.26±0.30	29.36±0.45

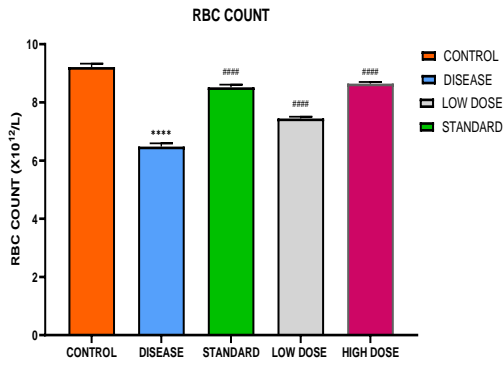


Figure 1a. RBC Count

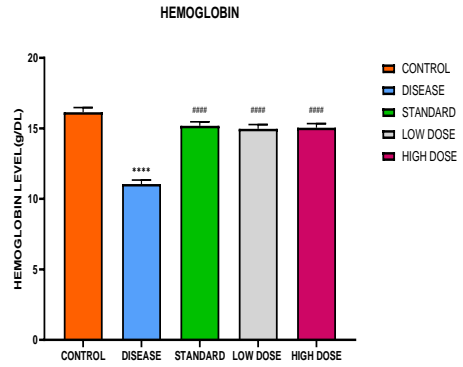


Figure 1b. Hemoglobin

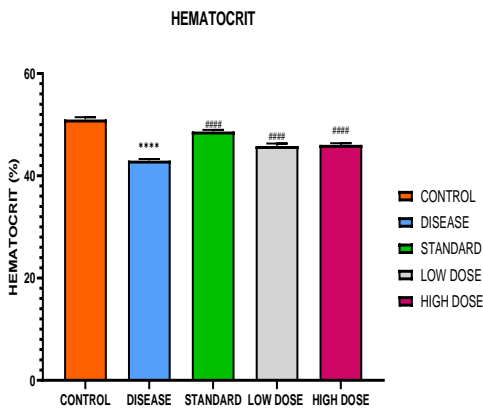


Figure 1c. Hematocrit

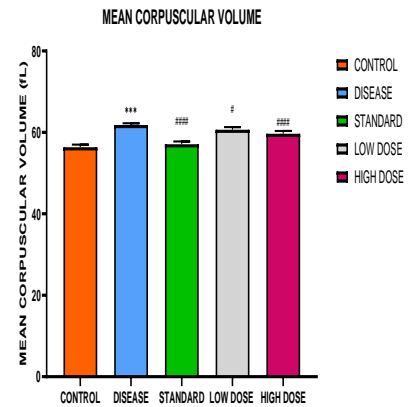


Figure 1d. Mean Corpuscular volume

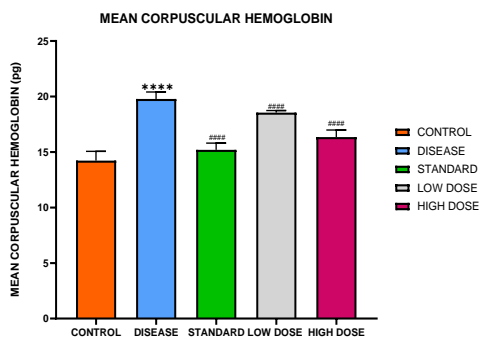


Figure 1e. Mean Corpuscular hemoglobin

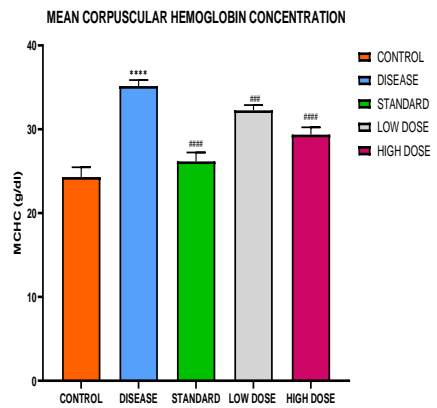


Figure 1f. Mean Corpuscular Hemoglobin Concentration

Figure 1: Effect of EERS on Hematological parameters.

- "All values are expressed as mean \pm SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Significance levels were set at $P<0.05$, $P<0.01$, and $P<0.001$."

3.3 Effect of EERS on serum biochemical parameters

Anemia was induced with phenylhydrazine (I.P) at the dose of 40 mg/kg for 2 days caused significant ($P<0.0001$) increase of serum iron level in disease treated rats when compared with normal control rats, due to the alteration in iron metabolism by phenylhydrazine which resulted in increased expression of DMT1 transporter in the spleen, duodenum and liver. DMT1 promotes the expression of genes related to iron metabolism such as ferric reductase DCytb, Ireg1 and DMT1 in humans and rats. Increased mRNA expression of DCytb, DMT1, Ireg1 and IFR1 in spleen and liver would increase the iron demand. On treatment with low dose (200 mg/kg), high dose (400 mg/kg) and standard drug (hematinic syrup, 0.68 ml/kg) in respective groups showed significant decrease ($P<0.0001$) of serum iron level in standard drug rats and EERS treated rats as shown in the table 2.

After the induction of anemia by intraperitoneal administration of phenylhydrazine (40 mg/kg) at day 1 and day 2, there is significant ($P<0.0001$) increase of serum ferritin level in disease treated rats when compared with normal control rats. While on treatment with standard drug (hematinic syrup 0.68 ml/kg) and EERS at both the doses had showed significant decrease ($P<0.0001$) of serum ferritin level as shown in the table 2.

There was significant ($P<0.0001$) increase of serum erythropoietin level in disease treated rats when compared with normal control rats after the intraperitoneal administration of phenylhydrazine (40 mg/kg). On treatment with standard drug (hematinic syrup, 0.68ml/kg), low dose (200 mg/kg) and high dose (400 mg/kg) of EERS showed significant decrease ($P<0.0001$) of serum erythropoietin level as shown in the table 2.

Table 2: Effect of EEDQ on serum biochemical parameters at the end of 14th day

Groups	Iron	Ferritin	Erythropoietin
Normal control	145.35±0.25	19.6±0.07	17.67±0.07
Disease control (Phenylhydrazine 40 mg/kg for 2 days)	276.10±1.07 ****	31±0.0 ****	30.42±0.14 ****
Standard control (hematinic syrup 0.68 ml/kg)	155.11±0.55 #####	20.4±0.15 #####	18.1±0.15 #####
Low dose (200 mg of <i>Raphanus sativus</i>)	169.22±0.86 #####	27.28±0.17 #####	25.75±0.19 #####
High dose (400 mg of <i>Raphanus sativus</i>)	159.85±0.28 #####	24.08±0.15 #####	19.9±0.14 #####

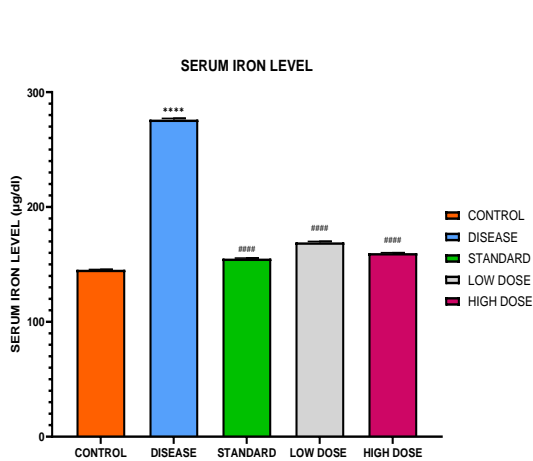


Figure 2a. Serum iron

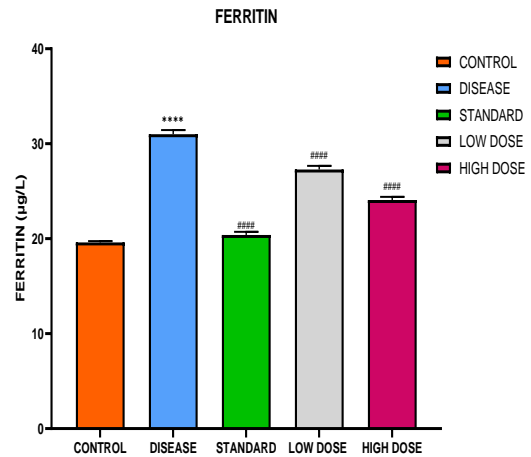


Figure 2b. Serum ferritin

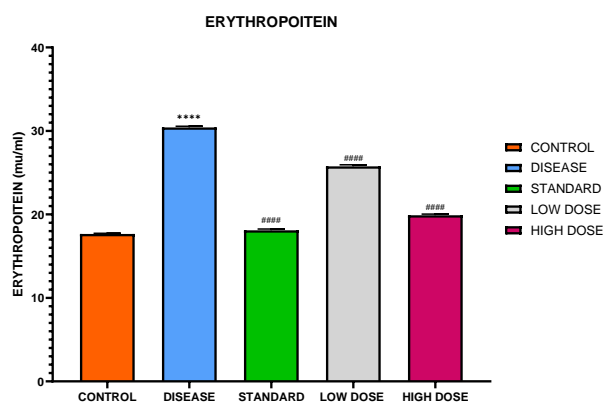


Figure 2c: Serum erythropoietin

Figure 2: Effect of EERS on serum biochemical parameters

All the values are plotted as mean \pm sem (n=6). Analysed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett t'test.

P<0.0001 compared with disease control group; **** P<0.0001 compared with normal control group.

3.4 Antioxidant enzyme parameters

After the administration of phenylhydrazine (40 mg/kg in I.P.) at day 1 and day 2, there was significant (P<0.0001) reduction of the Superoxide dismutase and Glutathione peroxidase concentration of disease control rats when compared with the normal control group. There was significant increase (P<0.0001) of the Superoxide dismutase and Glutathione peroxidase concentration in standard drug treated rats and EEDQ treated rats on treatment with hematinic syrup and EERS (low dose 200 mg/kg and high dose 400 mg/kg) at the end of 14th day as shown in table 3.

Table 3: Antioxidant enzyme level at the end of 14th day

Groups	Superoxide dismutase	Glutathione peroxidase
Normal control	3.58±0.06	0.044±0.002
Disease control (Phenylhydrazine 40 mg/kg for 2 days)	1.35±0.08 ****	0.019±0.0016 ****
Standard control (hematinic syrup 0.68 ml/kg)	3.02±0.12#####	0.034±0.0016#####
Low dose (200 mg of <i>Raphanus sativus</i>)	2.38±0.05 #####	0.024±0.0006 ##
High dose (400 mg of <i>Raphanus sativus</i>)	2.44±0.06 #####	0.026±0.0012#

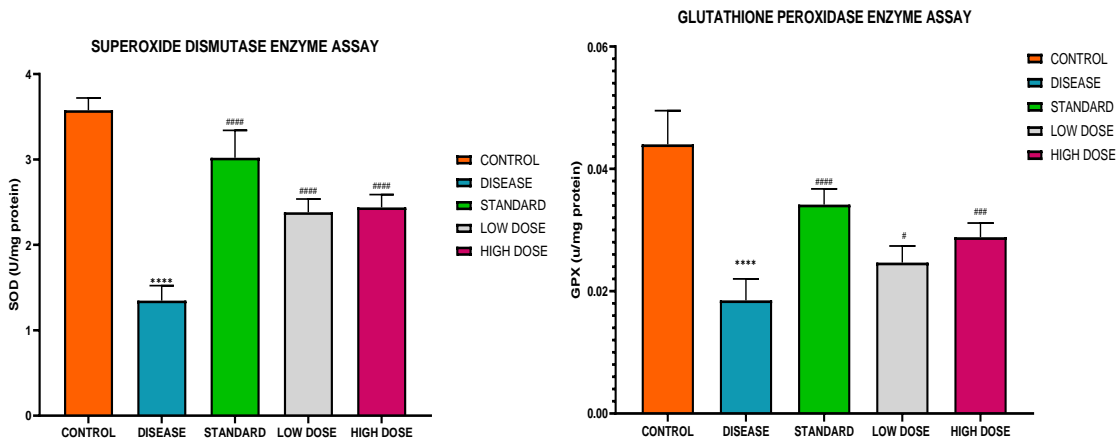


Figure 3a. Superoxide dismutase

Figure 3b. Glutathione peroxidase

Figure 3: Antioxidant enzyme level

All the values are plotted as mean ± sem (n=6). Analyzed by One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett t’ test.

P<0.0001 compared with disease control group; **** P<0.0001 compared with normal control group.

4. CONCLUSION

Hence it was suggested that the leaves extract of *Raphanus sativus* Linn., possessed significant anti-anemic effect. This may probably due to the anti-oxidant property of *Raphanus sativus* Linn, which was confirmed by *in-vivo* anti-oxidant enzyme activity by measuring the parameters of superoxide dismutase and glutathione peroxidase, which shows significant increase after the administration of leaves extract of *Raphanus sativus*, when compared with the disease control group of animals.

- "The ethanolic extract of *Raphanus sativus* leaves significantly improved hematological parameters and antioxidant enzyme levels in phenylhydrazine-induced anemic rats. These findings suggest the potential use of *Raphanus sativus* leaves as a natural remedy for anemia."

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Conflict of interest statement: The authors declared no conflict of interest.

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