INFLUENCE OF SPIRULINA ON THE PHENYTOIN INDUCED HAEMATOLOGICAL CHANGES

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ABSTRACT:
Phenytoin is indicated for the control of tonic clonic seizures and status epilepticus. Antiepileptics are known to deplete vital nutrients such as calcium, folic acid, vitamin D, vitamin K, biotin, carnitine, copper, selenium and zinc. Depletion of nutrients is known to cause adverse effects such as ataxia, nystagmus, lethargy, slurred speech and hematological disturbances. Spirulina is a rich source of vital nutrients including iron. It is proposed to study the effect of spirulina on the hematological disturbances induced by phenytoin. Seven groups of male albino rats weighing 130-150g were used. Each group consisted of six animals. Phenytoin at a dose of 20mg/kg/day dissolved in water, spirulina 50, 100, 200mg/kg/day suspended in 1% tween 80 alone or in combination with phenytoin was administered for 30 days. Hemoglobin content, total leucocyte and erythrocyte count were determined on 30th day. Phenytoin significantly decreased the hemoglobin content, total erythrocyte and leukocyte count. Spirulina did not show any effect at the lower dose of 50 and 100mg/kg and higher dose of 200mg/kg significantly elevated hemoglobin content. Spirulina at a dose of 200mg/kg/day in combination with phenytoin reversed the phenytoin induced decrease in hemoglobin content, total erythrocyte and leukocyte count. The results of this study indicates that supplementation of phenytoin with spirulina may reverse the hematological disturbances induced by phenytoin.

Key words: hematological changes hemoglobin, erythrocytes, leucocytes, phenytoin, anemia

INTRODUCTION
Phenytoin is indicated for the control of tonic clonic seizures and status epilepticus. Antiepileptics are known to cause aplastic\(^1\), megaloblastic\(^2\) and haemolytic\(^3\) anemia. Anemia is a common symptom of many diseases. It is a symptom or disease recognized by reduced oxygen carrying capacity. The symptoms of anemia are paleness, weakness, breathlessness, restlessness and loss of appetite. Indirectly it is recognized by reduction in hemoglobin percentage or total erythrocyte count or both. Phenytoin is known to deplete vital nutrients such as calcium, folic acid, vitamin D, vitamin K, biotin, carnitine, copper, selenium and zinc\(^2-6\). Depletion of nutrients is known to produce adverse effects such as cognitive impairment, nystagmus, lethargy, slurred speech\(^7\) and hematological disturbances\(^3,8\).
induced hematological disturbance may be due to nutrient depletion and spirulina is a rich source of proteins, vitamins, minerals and antioxidants. It is proposed to study the effect of spirulina on the hematological disturbances induced by phenytoin.

MATERIALS AND METHODS

Animals

Pathogen free adult male albino rats weighing 130-150g were used. In order to eliminate estrous cycle induced hematological differences, the study was carried out in male animals. The animals were housed in groups in cages at room temperature (25±3°C) with 12/12 hours light and dark cycle and were fed with a balanced diet and tap water ad libitum.

Study Protocol: The study protocol was approved by institutional animal ethical committee. The rats were divided into seven groups. Each group consisted of six animals.

Control Group: Received 1% tween 80 orally daily for 30 days between 9 am and 11 am.

Phenytoin Group: Received 20mg/kg phenytoin dissolved in water orally daily for 30 days between 9 am and 11 am.

Spirulina-I group: Received spirulina 50mg/kg, p.o suspended in 1% tween 80 between 9 am and 11 am for 30 days.

Spirulina-II group: Received spirulina 100mg/kg, p.o suspended in 1% tween 80 between 9 am and 11 am for 30 days.

Spirulina group-III: Received spirulina 200mg/kg, p.o suspended in 1% tween 80 between 9 am and 11 am for 30 days.

Phenytoin and Spirulina group-I:

Received 100mg/kg spirulina suspended in 1% tween 80 orally one hr before the administration of 20mg/kg phenytoin dissolved in water orally daily for 30 days between 9 am and 11 am.

Phenytoin and Spirulina group-II:

Received 200mg/kg spirulina suspended in 1% tween 80 orally one hr before the administration of 20mg/kg phenytoin dissolved in water daily for 30 days between 9 am and 11 am. Hematological parameters were determined on the last day of study. Blood samples were collected from the retro-orbital plexuses under light ether anesthesia.

Hematological Profile included:

1. Hemoglobin estimation
2. Total erythrocyte count
3. Total leukocyte count
Hemoglobin estimation

Hemoglobin content of each rat was estimated by Sahli’s method. The graduated diluting tube was filled with N/10 HCl up to the mark of 2gm. Blood was sucked up to 20 cumm mark of Hb diluting pipette and deposited at the bottom of the graduated tube. The tube was rinsed two to three times with HCl. Blood was mixed with the help of a stirrer and then solution was allowed to stand for 3 minutes so that all hemoglobin was converted to acid haematin. The mixture was diluted by adding distilled water in drops until it matched exactly with the standard glass tubes. Stirrer was taken out of the diluting tube and matched with standard glass color tubes and the scale was read on the side of the tube. 

Total erythrocyte count

The counting chamber was adjusted and observed under high power of microscope keeping the Thomas cover slip resting on the platform of the slide. Blood was sucked up to mark 1.0 of RBC diluting pipette and RBC dilution fluid was sucked up to mark 101, then the pipette was brought to horizontal position and the finger was placed over the tip of the pipette. A simple knot was given to rubber tube and pipette was rolled between the palms to mix blood with dilution fluid. Few drops were discarded and then pipette was held at an angle of 45° to the counting chamber and the tip was applied to the narrow slit between the counting chamber and the cover slip. A drop was placed which ran into the capillary space because of capillary action, and care was taken to ensure that the drop should not be large enough to float the cover slip. The fluid was allowed to settle for two minutes. RBC chamber was located and RBCs in 80 central smallest squares were counted and total RBC count was calculated.

Total leukocyte count

The counting chamber was adjusted and observed under low power of microscope keeping the Thomas cover slip resting on the platform of the slide. Blood was sucked up to mark 0.5 of WBC diluting pipette and WBC dilution fluid was sucked up to mark 11, then the pipette was brought to horizontal position and the finger was placed over the tip of pipette. A simple knot was given to rubber tube and the pipette was rolled between the palms to mix blood with dilution fluid. Few drops were discarded and then pipette was held at an angle of 45° to the counting chamber and the tip was applied to the narrow slit between the counting chamber and the cover slip. A drop was placed which ran into the capillary space because of capillary action and care was taken to ensure that the drop should not be large enough to float the cover slip. The fluid was allowed to settle for two minutes, WBC chamber was located, WBCs in 16 smallest squares were counted and count was continued in four corners.
similarly. Thus total WBC count was calculated 10.

**Statistical Analysis**

The data is expressed as mean ± SEM. The data was analyzed statistically using one way ANOVA, followed by Dunnett’s T test. P values less than 0.05 were considered significant.

**RESULTS**

Phenytoin significantly decreased the hemoglobin content, total erythrocyte count and leukocyte count (P<0.05). Spirulina by itself at the dose of 50 and 100mg/kg did not show any significant effect but dose of 200mg/kg significantly elevated the hemoglobin content, erythrocyte and leucocyte count when compared to control group (P<0.05). Spirulina (100mg/kg) did not reverse the phenytoin induced hematological changes, a higher dose of spirulina 200mg/kg (co-administration with phenytoin) reversed the phenytoin induced hematological disturbances (P<0.05). Results are summarized in table-1.

**DISCUSSION**

It has been reported that antiepileptic drugs (AEDs) including phenytoin decreased the folate levels 2,11,12, primarily by impaired folate absorption, which results in macrocytic anemia. The mechanism of interaction is complex, but there is data suggesting that phenytoin induces pH changes in the gut, which affect the enterohepatic circulation of folate 2. Secondarily, it was suggested that folate deficiency resulted from accelerated metabolism of folate consequent upon induction of liver enzymes by anticonvulsants 11,12. In addition phenytoin is known to deplete vital nutrients such as calcium, folic acid, vitamin D, vitamin K, biotin, carnitine, copper, selenium and zinc 6,2,4 which are essential for erythropoiesis.

The second cause for anemia with all major AEDs except gabapentin (specifically felbamate, carbamazepine, phenytoin, valproate, ethosuximide, phenobarbital, lamotrigine and primidone) is aplastic anaemia 3,14. Total destruction of the hematopoietic elements leads to the clinical entity known as aplastic anemia. Aplastic anemia can arise by several mechanisms immunologic, infectious, constitutional, and idiopathic and physico-chemical 14. Chemicals/drugs can cause idiosyncratic bone marrow suppression or dose-related suppression. In the present study, a prominent decrease in Hb content and total RBC count indicating anemia in rats was observed. Iron, folic acid, B-complex calcium, copper and proteins are essential for the erythropoiesis. Spirulina is blue green algae, considered as an ideal nutritional supplement because over half of it consists of amino acids 15. It is also rich source of other nutrients including B-
complex vitamins, beta-carotene, vitamin E, carotenoids, manganese, zinc, copper, iron, selenium and an essential fatty acid, α-linolenic acid (16, 9, 17). The iron level in spirulina is equivalent to that contained in beef 18. In the previous studies phycocyanin, a pigment of spirulina and polysaccharides of spirulina were reported to have erythropoietin 19, phycocyanin, a pigment of spirulina also showed protection of human erythrocytes against lysis by peroxyl radicals 13. In the present study also spirulina reversed the phenytoin induced decrease in Hb content and total RBC count.

There is evidence of depressed immunological function in phenytoin treated patients 20, 21, 22, 23. The hematological abnormalities induced by phenytoin also include leucocytosis with atypical lymphocytes, eosinophilia 24, leucopenia 25 and agranulocytosis 26, 27. An immune mechanism with phenytoin dependent antigranulocyte antibody may cause leucopenia, which resolves on discontinuing therapy. Phenytoin may cause a direct toxic effect with pancytopenia and agranulocytosis 28. In the present study also phenytoin caused significant decrease in total leucocytes, may be due to agranulocytosis and leucopenia. Hayashi et al. 1991 and Cheng-Wu et al., 1994 29, 19, reported immuno-potentiating effect of spirulina in mice. In the present study also spirulina reversed the leucopenia induced by phenytoin in rats.

In summary, the results of our study lead to the conclusion that the spirulina reversed the leucopenia and anemia induced by phenytoin.

| Table 1: Effect of Spirulina on Phenytoin induced haematological changes |
|-------------------------------|-----------------|-----------------|-----------------|
| Treatment                     | RBC million /mm³ | Hb gm/dl        | WBC/mm³         |
| Control                       | 8.698 ± 0.196   | 15.6 ± 1.691    | 8074 ± 271      |
| Phenytoin                     | 7.16 ± 0.356    | 13.6 ± 1.208*   | 7001 ± 308.3*   |
| Spirulina (50mg/kg)           | 8.670 ± 0.193   | 15.8 ± 1.772*   | 7999 ± 242.6*   |
| Spirulina (100mg/kg)          | 8.726 ± 0.356   | 15.6 ± 1.905    | 7948 ± 308.3†   |
| Spirulina (200mg/kg)          | 8.986 ± 0.356*  | 16.1 ± 1.208    | 8306 ± 308.3†   |
| Phenytoin and Spirulina (100mg/kg) | 7.357 ± 0.482* | 13.8 ± 1.691*   | 7144 ± 308.3*   |
| Phenytoin and Spirulina (200mg/kg) | 8.859 ± 0.196†  | 16.8 ± 1.828***| 8138 ± 106.8***|

Values are Mean ± SEM of 6 animals
*P< 0.05 Vs control group
+P< 0.05, ++P< 0.001 Vs phenytoin group
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