

Article

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Cyclophosphamide induced anemia in Wistar rats: can it be prevented by Sajna (*Moringaoleifera*)?

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Abstract

Background: Chemotherapy-induced anemia is a multifactorial challenge to the treatment of malignancy. Herbal medicine may help to treat such anemia due to its natural compatibility. *Moringaoleifera* (Sajna), is well known for preventing and curing anemia. **Objective:** To evaluate the preventive effect of aqueous extract of *Moringaoleifera* leaf (AEMOL) on cyclophosphamide (CP) induced anemia in Wistar rats. **Method:** The experimental study was carried out in the Department of Physiology, Dhaka Medical College, Dhaka from July 2015 to June 2016. Thirty Wistar rats of both sexes weighing 175±25 gm were grouped as A [normal saline (1ml/kg); oral], and B [CP (3mg/kg); i. p.], C [AEMOL (300mg/kg); oral], D [AEMOL (300mg/kg); oral and CP (3 mg / kg) i. p. simultaneously for 14 consecutive days] and E [AEMOL (300mg/kg); oral for 14 consecutive days and CP (30mg/kg); i. p. on 12th, 13th, 14th day] with 6 rats in each group. All the rats had free access to food and distilled water during the period of acclimatization (seven days) and experiment (fourteen days). Total count (TC) of RBC, hemoglobin (Hb) level and packed cell volume (PCV) were estimated to assess anemia in all rats before and after experiment. The statistical analyses were done by one way ANOVA followed by Bonferroni post hoc test where p<0.05 was accepted as level of significance. **Result:** The mean TC of RBC, Hb% and PCV were significantly (p<0.001) lower in CP treated rats (group B), in comparison to all other rats of different groups (A, C, D and E). In addition, all these three variables were significantly higher in AEMOL treated group (C) compared to saline treated rats (A) but almost similar in rats with two groups of different dose schedule of AEMOL and CP (D, E). **Conclusion:** From the study, it can be concluded that CP induced anemia can be prevented by AEMOL used either simultaneously or in an anticipating manner.

Keyword: *Moringaoleifera*, Cyclophosphamide, anemia, Hb, PCV.

Introduction

Cyclophosphamide, is a synthetic anti-neoplastic drug, currently used for treatment of tumors, lymphomas, leukemias and as an immuno-suppressive agent before bone marrow and organ transplantation.¹ In addition to its common adverse effects, nausea, vomiting, alopecia, mucosal ulceration, nephrotoxicity and hepatic toxicity,² lowering of blood cell counts, as well as hemoglobin percentage has also been reported due to bone marrow suppression³. So, anemia is one of the common and recurrent consequence of patients receiving drugs like CP.⁴

To overcome this unwanted side effect (anemia), synthetic hematinics are used therapeutically, but they are costly, not user friendly and some of them have potential side effects of their own. On the contrary, medicinal herbs are cheap, easily available and usually free from any detrimental effects as well as better compatibility.⁵

Different medicinal plants like *Astragaliradix*, *Angelicae Radix*,⁶ *Vernonia amygdalina*⁷, *Jatropha curcas*⁸, *Zanthoxylum zanthoxyloide*⁹, *Jatropha tanjorensis*, *Betavulgaris*, *Solanum melongena*¹⁰, *Acalypha wilkesiana*¹¹, *Amaranthus cruentus*⁴, are studied widely against CP induced anemia in various animal models.

But recently, 'Sajna', [*Moringaoleifera* (Family: Moringaceae)], 'the miracle tree', is a medicinal plant, also has shown to possess hypocholesterolemic, hepatoprotective, anticancer, analgesic, anticonvulsant, antibiotic, anti-allergic and anti-inflammatory, antidiabetic, antioxidant effects.¹²⁻²⁰

In addition to these studies, 14 days oral supplementation of *Moringaoleifera* leaf (MOL) extract, was found to increase RBC count in healthy adults.²¹ Moreover, it also improved hemoglobin level of iron deficient women after 30 days of MOL supplementation.²² Furthermore, this medicinal plant extract was shown to increase RBC count and hemoglobin concentration after different dose schedules in sodium arsenite²³ as well as aluminium induced anemia²⁴ in two

different rat models. Moreover, RBC count, Hb concentration and PCV were found significantly higher in rats with 14 days MOL treatment in comparison to those of without treatment rats.²⁵

However, as far as it has been searched, no reported data regarding cyclophosphamide induced anemia prevention by MOL was available. On the basis of this background, this study was designed to evaluate the preventive role of aqueous extract of *Moringaoleifera* leaf (MOL) on cyclophosphamide induced anemia in Wistar rats.

Method

The prospective experimental study was carried out in the Department of Physiology, Dhaka Medical College and Hospital (DMCH), Dhaka from July 2015 to June 2016. Animal care and experiments were performed according to guidelines set in the 'The ethical guidelines for experimentation in laboratory animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh²⁶ (icddr, b 2002) and the ethical clearance was taken from Ethical Review Committee of Dhaka Medical College.

Procurement and maintenance of animals:

A total number of 30 rats of both sexes, weighing 175±25 gm were collected from the animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. The animals were housed in metallic cages in the animal house of Institute of Nutrition and Food Science, University of Dhaka, under a 12/12 hour light/dark cycle²⁷. The ambient room temperature was maintained at around 27 to 28°C, corresponding to the thermo neutral zone for rodents²⁸. They had free access to food and water ad libitum. The rats were kept for seven consecutive days for environmental acclimatization prior to the experiment.

Grouping and dose schedule:

After acclimatization for 7 days, the rats were divided into five groups (A, B, C, D and E) of six rats in each group. In addition to basal diet, they were treated as follows:

Table I: Grouping and dose schedule for Wistar rats (N=30)

| Groups rats/group) | Treatment | Route | Dose(per kg body weight) | Duration |
|--------------------|---------------|---------------------------|--------------------------|---|
| A(n=6) | normal saline | oral | 1ml | 14 consecutive days |
| B(n=6) | CP | i.p. | 3mg | 14 consecutive days |
| C(n=6) | AEMOL | oral | 300mg | 14 consecutive days |
| D(n=6) | AEMOL and CP | AEMOL(oral); CP (i.p.) | AEMOL300mg; CP3 mg. | 14 consecutive days simultaneously |
| E(n=6) | AEMOL and CP | AEMOL(oral); CP (i.p.) | AEMOL300mg; CP30 mg. | AEMOL for 14 consecutive days; CP on 12 th , 13 th , 14 th day |

CP= cyclophosphamide; AEMOL= aqueous extract of *Moringaoleifera* leaf; i.p. = intraperitoneal, N= total number of rats; n=number of rats in each group.

Table II: Study variables in different groups of rats and their statistical analysis (N=30)

| Parameters | Groups | | | | |
|-----------------------------------|---------------------|---------------------|---------------------|--------------|--------------|
| | A (n=6) | B (n=6) | C (n=6) | D (n=6) | E (n=6) |
| RBC count (x 10 ⁶ /μl) | 7.89 ± 0.47 | 3.86 ± 0.64 | 8.12 ± 0.58 | 7.41 ± 0.45 | 7.08 ± 0.51 |
| Hemoglobin(gm/dl) | 12.10 ± 0.83 | 8.10 ± 0.67 | 14.19 ± 1.02 | 13.86 ± 0.84 | 12.94 ± 0.91 |
| PCV (%) | 43.98 ± 2.25 | 19.06 ± 1.24 | 41.14 ± 1.43 | 40.53 ± 1.18 | 38.78 ± 1.05 |
| Groups | P value | | | | |
| | RBC | Hemoglobin | PCV | | |
| A vs B vs C vs D vs E | <0.001*** | <0.001*** | <0.001*** | | |
| A vs B | <0.001*** | <0.001*** | <0.001*** | | |
| A vs C | 0.049* | 0.003** | 0.030* | | |
| A vs D | 1.000 ^{ns} | 0.016* | 0.005** | | |
| A vs E | 0.091 ^{ns} | 1.000 ^{ns} | <0.001*** | | |
| B vs C | <0.001*** | <0.001*** | <0.001*** | | |
| B vs D | <0.001*** | <0.001*** | <0.001*** | | |
| B vs E | <0.001*** | <0.001*** | <0.001*** | | |
| C vs D | 0.001** | 1.000 ^{ns} | 1.000 ^{ns} | | |
| C vs E | <0.001*** | 0.188 ^{ns} | 0.110 ^{ns} | | |
| D vs E | 1.000 ^{ns} | 0.774 ^{ns} | 0.520 ^{ns} | | |

Results are expressed as mean ± SEM; The statistical analysis was done by one way ANOVA followed by Bonferroni post hoc test. N = Total number of rats, n = number of rats in each group; ns = not significant; */**/** = significant; A: [normal saline (1ml/kg); oral]; B [CP (3mg/kg); i. p.]; C [aqueous extract of *Moringaoleifera* leaf (300mg/kg); oral]; D [aqueous extract of *Moringaoleifera* leaf (300mg/kg); oral and CP(3 mg / kg) i.p. simultaneously for 14 consecutive days]; E [AEMOL(300mg/kg); oral for 14 consecutive days and CP(30mg/kg); i. p. on 12th, 13th & 14th day.]

Blood collection:

On 15th day of experiment, rats were deeply anaesthetized by 30% chloroform. Two (2) ml blood samples were drawn through cardiac puncture and collected in EDTA tube to be analyzed by Automated Hematology Analyzer XT-SYSMEX-2000i; in the Department of Hematology, DMCH.

Euthanasia:

Painless deaths of the deeply anesthetized rats were ensured by decapitation.²⁹

Statistical analysis:

Results were expressed as mean \pm SEM. Statistical analysis was done by one way ANOVA followed by Bonferroni post hoc test. In the interpretation of results, p value <0.05 was accepted as level of significance.

Results

As shown in Table II, the total count of RBC, hemoglobin percentage and PCV were significantly ($p < 0.001$) lower in group B (CP treated), in comparison to group A (normal saline treated). Again, these parameters were significantly ($p < 0.001$) higher in group D (AEMOL and CP simultaneously treated) and E (AEMOL and CP simultaneously treated) in comparison to CP treated (B) group. Besides, all these three parameters were almost similar in group D (AEMOL and CP simultaneously treated) and E (AEMOL pretreated and CP post treated). However, these parameters were significantly higher in group C, in comparison to those of group A ($p < 0.05$), B ($p < 0.01$).

Discussion

In this study, mean total count of RBC, hemoglobin level, PCV were estimated to assess hematological changes. Here, all these three parameters were significantly lower in CP treated rats in comparison to those of normal. This finding is similar to different studies.^{5,3,30} Cyclophosphamide induced production of free

radicals might be causing lipid peroxidation and subsequent cell damage¹ resulting anemia in our rats.

In addition, AEMOL prevented the CP induced anemia in our experimental rats. It has been suggested that MOL possesses antioxidants (polyphenols, flavonoids, vitamin A, C, E)³¹ exhibiting strong scavenging effect on different free radicals (superoxide, nitric oxide radical) inhibiting lipid peroxidation and preventing oxidative damage to major biomolecules.³²

Moreover, MOL also possess different hemopoietic factors, such as vitamins (A, B₁, B₂, B₁₂, C, E, K, folic acid), minerals (Mg, P, Na, K, Ca, Fe, Mn, Zn, Cu, Se), essential amino acids³³ (Okot-Asi et al. 2015) which might cause growth, differentiation, proliferation of hemopoietic stem cell as well as maturation of DNA and RBC³⁴. This hematinic potential of MOL might cause the prevention of CP induced anemia in our rats.

Moreover, in the present study, CP induced anemia was prevented by AEMOL, used either simultaneously with CP (group D) or in an anticipating manner (group E). Though induction of anemia by CP was with different dose schedule, but correction by AEMOL was almost similar in both groups D and E. However, the exact mechanism cannot be elucidated as free radical concentration produced by CP and phytochemical contents of *Moringaoleifera* leaf were not assessed in the present study.

Conclusion

The present study reveals that AEMOL has preventive role against cyclophosphamide induced anemia. So, it can be concluded that *Moringaoleifera* leaf has the potential to prevent anemia either in sudden or simultaneous exposure to bone marrow suppressing agent like CP.

Conflict of interest - None.

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