

# Comparative Clinical Study of Nabayas Louha: An Ayurvedic Haematinic Preparation and a Conventional Iron Preparation in Female Anemic Patients

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## ABSTRACT

Anemia is common nutritional disorder and it affects one third of population around the globe. Assessing nutritional status of human is an inevitable process to lead a healthy life. Females are affected significantly by anemia compare to male. According to WHO report, developing herbs-based formulation to treat anemic patients is safe and less toxic. In this study a double-blind, cross group comparative clinical trial of Nabayas Louha (NBL) a Ayurvedic haematinic preparation with G-Iron Folic Acid (IFA) was undertaken on 66 female anemic volunteers with age between 20-30 years. It was seen that NBL after being administered at a daily dose of 500 mg for 30 days significantly increased the hemoglobin content of the treated volunteers. It produced an increase in serum iron content and decreases total iron binding capacity. The ESR level was also decreased. These effects of NBL were found to be comparable with IFA. There was a marked decrease in WBC count noticed, however statistically significant increase in the lymphocytes count was seen. Furthermore, the level of toxicity related enzymes SGOT and SGPT was not altered significantly in the NBL treated group which vividly confirm that supplementation of NBL is not toxic. In conclusion, these findings recommend use of NBL as supplement in the treatment of iron deficiency anemia and WBC disorders.

**Keywords:** Anemia; Ayurvedic; Haematinic; Nabayas louha

**Abbreviations:** World health organization (WHO); Nabayas louha (NBL); Iron folic acid (IFA); Erythrocyte sedimentation rate (ESR); White blood cell (WBC); Glutamic-pyruvic transaminase (GPT); serum glutamic-oxaloacetic transaminase (SGOT); Serum glutamic pyruvic transaminase (SGPT); Total iron binding capacity (TIBC); Iron deficiency anemia (IDA)

## INTRODUCTION

Iron deficiency is a quite common disorder around the world mainly due to malnutrition. Anemia is highly prevalent in developing and under developing countries. Bangladesh is developing nation where the number anemic cases increased significantly [1]. Anemia has been associated with significant negative clinical impacts such as decreased physical performance [2], increased number of falls [3], increased frailty, decreased cognition [4], increased [5]. dementia, hospitalization and mortality [6]. Treating anemia by synthetic drugs causes many secondary complications, the WHO

recommends the use of herbal based products for treatment of anemia as safe. In this study we used novel Nabayas Louha (NBL) is included (page 231-232) in the Bangladesh National Formulary of Ayurvedic Medicine 1992 and has been prescribed by practitioner of Ayurvedic medicine for scores of years for the treatment of anemia and jaundice [7].

It is a multicomponent drug and the main part of it is louha bhasma, is louha bhasma, a roasting iron, has been used as haematinic by ancient Indian. The other components are triphala, trimod and trikatu. In combination with iron, triphala (Embilica

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Officinals Gaertn, Syn. Or Phyllanthus emblica Linn., Terminalia bellerica Roxb. and Terminalia chebula Retz.) is used in anemia, jaundice and dyspepsia treatment [8]. Trikatu is a common herbal combination having Piper nigrum, P. longum, and Zingiber officinalis. Trimud includes Cyperus rotundus Linn., Embelia ribes Burm and Plumbago zeylanica. A clinical study conducted with alcoholic extract of berries of Embelia ribes showed improvement in haemoglobin percentage of the blood and a marked reduction of ova in stool. Plumbago zeylanica were found to decrease GPT levels in serum and increased alkaline phosphates, total protein and prothrombin time [9]. The pretense for using nine different plant components in association with Louha bhasma (iron) in treating this malnutritive condition attracted the interest of the scientist working in the traditional medicine preparation. There are few reports available on use of various individual ingredients; however, no scientific study has reported the combined effect of these ingredients. To the best of our knowledge this is the first report on clinical efficacy of NBL on anemic patients.

## MATERIALS AND METHODS

### Subjects

Subjects selected for this trial were all females most of them were nurses and health-workers of Gonoshasthya Medical Center, Nayarhat and the rest were the villagers of the villages next to the Jahangirnagar University campus, Savar. The study started with a total of 66 subjects. Two subjects did not complete the trial. Subjects were selected on the basis of the following criteria: the hemoglobin concentration were to be less than 12 gm/100 ml, they were free from other diseases, they were not taking any other medication and they were willing to participate in the trial (Ethical approval no. BBECJU/M2017 (3) 2).

It was a double blind; cross group comparative trial having a treatment period of one month. Subjects were randomly assigned to three different groups, each consisting of 22 people. Group A received placebo, twice daily and served as the control group. While Group B received, 500 mg Nabayas Louha (NBL) and group C received twice daily, G-Iron Folic Acid (Ferrous sulfate,

200 mg and folic acid, 200µm) manufactured by GPL Savar, Nayarhat. All the three ingredients, i.e. Placebo, NBL as well as G-Iron Folic acid(R) were administered in identical capsule shell.

### Method

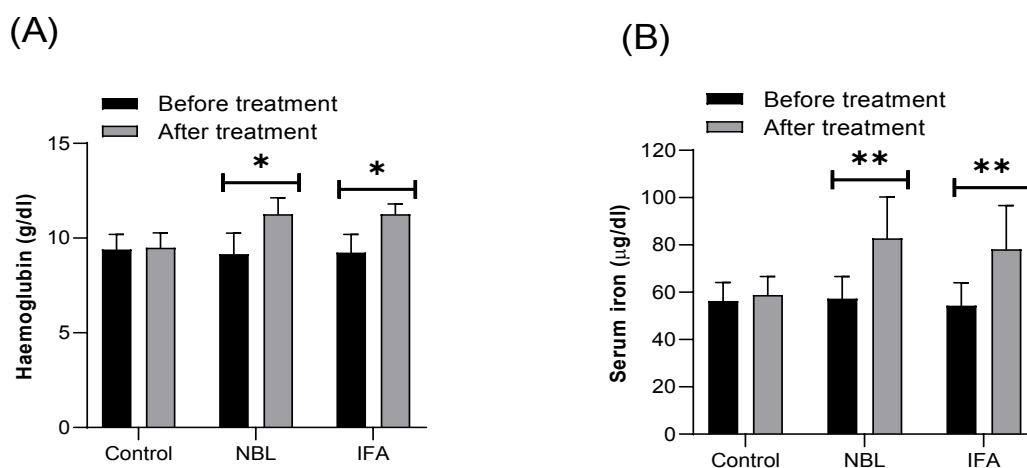
Cyanmethaemoglobin method was employed for estimation of hemoglobin and the procedure followed was that described by Thakur and Guttikonda [10]. WBC count was done according to standard method [11] and ESR was done by Westergren method [12]. Total iron binding capacity (TIBC) was estimated by using the Bathophenanthroline deproteinization method (calorimetric) and serum iron was estimated according to the technique developed by [10,13]. SGOT and SGPT were estimated by using a standard colorimetric method [13]. Blood analysis was carried out within 24 hours after collection of the blood specimens.

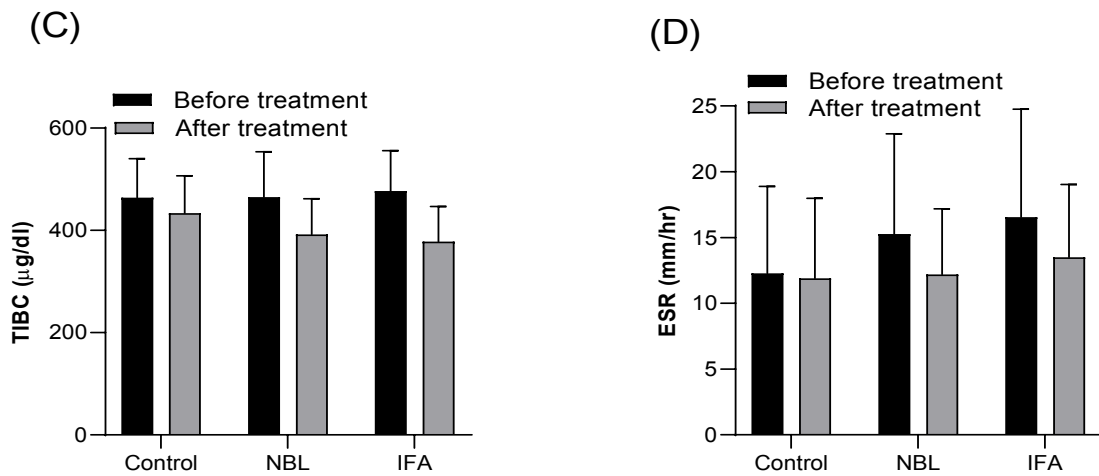
### Statistical Analysis

Statistical analysis tests were performed by using GraphPad Prism 8. The results are represented as mean  $\pm$  SEM. One-way ANOVA (Bonferroni) was done as the test of significance with the significant level of  $p < 0.05$ .

## RESULTS

The levels of haemoglobin, serum iron, TIBC and ESR are depicted in Figure 1 (A-D). Administration of NBL and IFA increased the hemoglobin levels from 9.16 g/dl to 11.25 g/dl and 9.23 g/dl to 11.27 g/dl ( $p < 0.05$ ) (Figure 1A) respectively. In contrast there was no change in hemoglobin levels in control group, receiving placebo. It is worth notice that efficacy of NBL is comparable to standard drug (G-Iron Folic Acid). All subject recruited in this study have lower serum iron level. Our data shows that significant ( $p < 0.01$ ) increase in serum iron content after the treatment, in both the group receiving NBL and the group receiving IFA (Figure 1B). Noticeably the subjects received NBL showed marked increase in serum iron level compared to standard drug. TIBC and ESR were reduced in both NBL and standard drug treatment (Figures 1C and 1D) however this is not statistically significant.

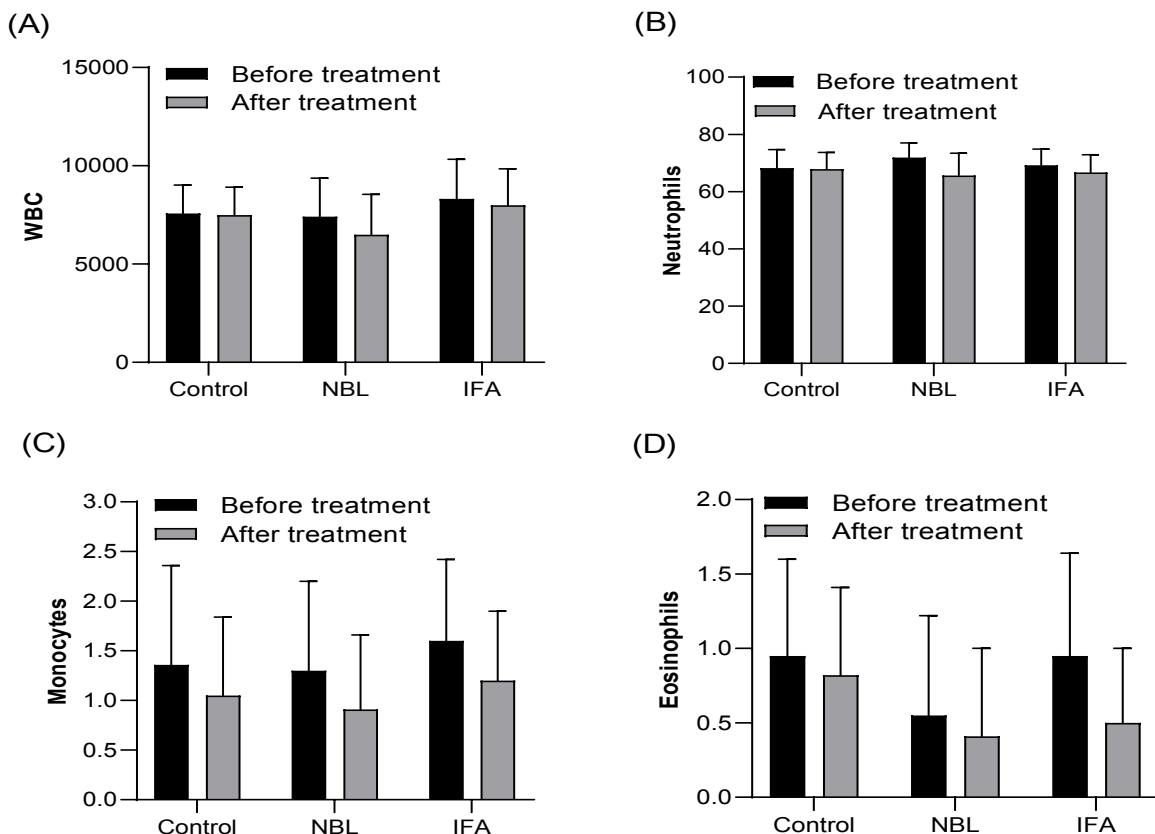


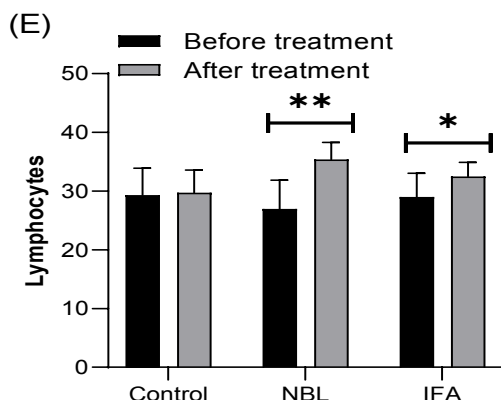


**Figure 1:** Effect of NBL & IFA on haemoglobin (A) Serum iron (B) Total iron binding capacity (TIBC) C) ESR D) After 30 days of treatment. Results are expressed as mean $\pm$ SEM. One-way ANOVA (Bonferroni) was done as the test of significance with the significant level of  $p < 0.05$ .

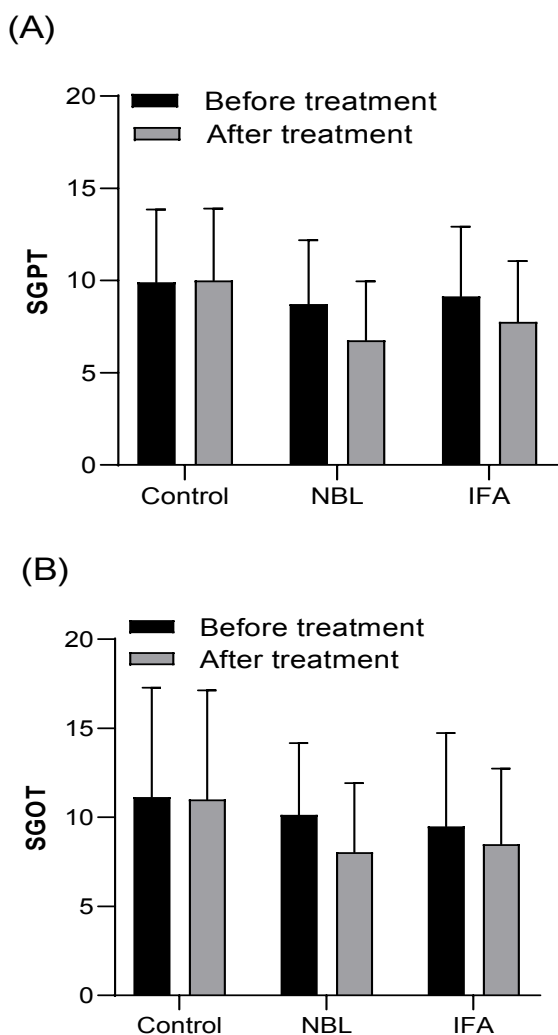
Data of blood cell count shows that NBL and standard drug treatment lowered the number of WBC, neutrophils, monocytes, and eosinophil but it is not statically significant (Figure 2A-2D). However, both NBL and standard drug administration increased lymphocytes count considerably ( $p < 0.05-0.001$ ) (Figure 2E). This

clearly says that both NBL and standard have immune boosting property. The level of toxicity related liver enzymes SGOT and SGPT were decreased in both NBL and standard drug treated group (Figure 3A and 3B).





**Figure 2:** Effect of NBL & IFA on WBC (A) Neutrophils (B) Monocytes (C) Eosinophils (D) Lymphocytes (E) After 30 days of treatment. Results are expressed as mean±SEM. One-way ANOVA (Bonferroni) was done as the test of significance with the significant level of  $p < 0.05$ .



**Figure 3:** Effect of NBL & IFA on SGPT (A) SGOT (B) After 30 days of treatment. Results are expressed as mean ± SEM. One-way ANOVA (Bonferroni) was done as the test of significance with the significant level of  $p < 0.05$ .

## DISCUSSION

Deficiency of iron is probably the most common nutritional disorder in the world and prevalence is higher in the developing countries [14]. The females are more susceptible to iron deficiency anemia (IDA) and this can be due to dietary lack, impaired absorption, social negligence, unawareness and menstrual causes [15]. Dietary lack is a rare cause of iron deficiency in industrialized countries where about two thirds of the dietary iron is in the readily assimilated in heme form [15]. The situation is quite different in developing countries where diets are predominantly vegetarian, having poorly absorbable inorganic iron [16]. Growing infants and children, adolescents, and pre-menopausal women have a greater requirement for iron than do non-menstruating adults. To address this issue, in this study, we recruited the female anemic patients and assessed the anti-anemic potential of NBL and standard drug. Post treatment, we assessed hemoglobin level, blood cells counts, ESR, and liver enzymes (SGOT and SGPT).

The NBL treated anemic patients showed a remarkable increase in hemoglobin level from 9.16 g/dl to 11.25 g/dl ( $p < 0.001$ ). These results clearly show that NBL supplementation increased hemoglobin content however the mechanism of action is not known. Our results are in agreement with the other ayurvedic formulation reported in the literature for anti-anemic property [17]. It is well known that anemic patients have very low level of serum iron [18]. Our result shows a marked increase in serum iron content after the treatment, in both the group receiving NBL and the group receiving IFA. Iron is the central metal ion of hemoglobin and the amount of serum iron determines level of hemoglobin level in the blood. Furthermore, it is known to clinical scientist that the iron binding capacity and total serum iron content are inversely related. This leads to the fact an increase in the iron binding capacity of a person decreases the total iron content as well as the hemoglobin [10]. In this study iron binding capacity decreased in NBL treated group and comparable with standard drug. Overall, our findings state that NBL increased iron content, hemoglobin level and decreased total iron binding

capacity. Similarly, other ayurvedic formulations reported for restoring anemic patient's capacity to maintain the normal hemoglobin level. ESR is a valid indicator of the composition of plasma and the relation of red cells to plasma. NBL treatment decreases ESR however it is not statistically significant. It implies that NBL treatment did not modulate blood physiology and it may not elicit significant toxicity to the system. However, detail mechanistic study must delineate the role of NBL in human system.

Blood cells (WBC, neutrophils, monocytes, eosinophils) playing pivotal role in body defense. Any drug formulation which adversely affects the counts of these blood cells may not be ideal drug candidate for treatment. In this study we used NBL ayurvedic formulation to treat anemic patients and it is imperative to assess its effect on blood cell count. Our data clearly shows that NBL treatment did not alter count of WBC, neutrophils, monocytes, eosinophil significantly but it enhances the count of lymphocytes. The exact molecular mechanism of this action is unknown. However, it is suggested that iron supplementation could nourish the bone marrow where hematopoiesis occurs. Our results agree with others reports where iron supplementation increases the count of blood cells [19].

The ayurvedic literature suggests that the drug NBL is used for the treatment of jaundice and this fact encouraged us to investigate the effects of drug on SGOT and SGPT. It is well established that level of these enzymes increased enormously during liver toxicity [20]. An array of literatures states that a drug formulation which does not elicit hepatotoxicity can be an ideal drug candidate [20]. In this study, our results show that NBL treatment did not alter SGOT and SGPT level at the end of experimental period. It is clearly shown that NBL is safe and it did not elicit sever hepatotoxicity. However, detailed molecular mechanistic studies must delineate the role of NBL on liver toxicity.

## CONCLUSION

NBL treated anemic patients recovered after the treatment period by restoring hemoglobin level, serum iron level, iron binding ability and ESR. Further NBL did not alter the blood cells count and level of liver enzymes (SGOT and SGPT) significantly in anemic patients. However before reaching to definite conclusive remarks on the anti-anemic effect of NBL, a large-scale study with inclusion of huge anemic patient sample size is needed.

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## Author contribution

MSKC and JMA were responsible for the conception and design of research and contributed equally to the supervision of the study; PA, HAB & HLF were responsible for reading and revising manuscript; P.A., SA & MS performed the experiments, analysed

the data, interpreted the results, and drafted the manuscript with JMAH; PA & HAB edited the revised manuscript; All authors approved the final version of the manuscript.

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