

International Journal of Nursing Science Practice and Research

ISSN: 2455-6351 Volume 8, Issue 2, 2022 DOI (Journal): 10.37628/IJNSPR

http://nursing.journalspub.info/index.php?journal=IJNSPR&page=index

Research IJNSPR

A Quasi-experimental Study to Evaluate the Effectiveness of Spirulina with Lemon Juice on Anemia among Under-five Children in Selected Schools at Madurai, Tamil Nadu

R. Jothilakshmi^{1,*}, Nalini Jeyavanth Santha²

Abstract

Anemia among under-five children is an important concern for the health authorities in India. Untreated anemia in children can have a serious effect on their growth. Spirulina is blue-green algae. It contains protein, iron, vitamins, and minerals. It is aimed at a quasi-experimental study to evaluate the effectiveness of spirulina with lemon juice on anemia among under-five children in selected schools at Madurai, Tamilnadu. Quasi-experimental pre-test post-test control group design was used. 150 children were participated. All children were aged between 2-4 years. Pre-test was done. The researcher administered spirulina (1 gm) with lemon juice (50 ml) to the experimental group. Posttest I and II were conducted for both groups. Following apparatus was used for data collection such as Shali's haemoglobinometer. Observation checklist used to identify signs, and symptoms of anemia. Descriptive and inferential statistics were used to analyze the findings. In the experimental group, the signs and symptoms of anemia mean post-test I score was lesser (2.4) than mean pre-test score (4.56). The obtained (F value-18.8, p-value 0.000***) and the mean post-test I haemoglobin value was higher (9.77) than mean pre-test score (8.99). The obtained (F value-715, p-value 0.000***) were very highly significant. Based on haemoglobin (student's t-test = 7.194, p-0.000***) and observation checklist on signs, and symptoms of anemia (student's t-test = -4.690, p-0.019*), it concluded that spirulina with lemon juice was effective in reducing signs and symptoms of anemia, and improved haemoglobin level among under-five children.

Keywords: Anemia, hemoglobin, lemon, spirulina, under-five children

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Received Date: August 08, 2021 Accepted Date: August 16, 2021 Published Date: July 31, 2022

Citation: R. Jothilakshmi, Nalini Jeyavanth Santha. A Quasi-experimental Study to Evaluate the Effectiveness of Spirulina with Lemon Juice on Anemia among Under-five Children in Selected Schools at Madurai, Tamil Nadu. International Journal of Nursing Science Practice and Research. 2022; 8(2): 50–61p.

INTRODUCTION

"Children are the world's most valuable resource and it is best hope for the future".

—John F. Kennedy

Anemia is a condition in which the number of red blood cells or the haemoglobin concentration within them is lower than normal. Haemoglobin is needed to carry oxygen and if you have too few or abnormal red blood cells, or not enough haemoglobin, there will be a decreased capacity of the blood to carry oxygen to the body's tissues. Anemia is a serious global public health problem that particularly affects young children. WHO

estimates that 42% of children less than 5 years of age worldwide are anaemic (WHO) [1]. Ashraf M. Sharoba. (Dec 2014) reported that Spirulina which is one of the blue-green algae rich in protein 62.84% and contains a high proportion of essential amino acids (38.46% of the protein) and a source of naturally rich in vitamins especially vitamin B complex such as vitamin B12 (175 μ g / 10 g) and folic acid (9.92 mg / 100 g), which helps the growth and nutrition of the child brain, also rich in calcium and iron it containing (922.28 and 273.2 mg / 100 g, respectively) [2] Jennifer Adler. (2013, Feb 15) Spirulina grows mainly in subtropical and tropical countries, where there is year-round heat and sunlight. It is high in protein (up to 70%), rich in antioxidants and loaded with vitamins and minerals, particularly iron and vitamin B-12. And it has no cellulose-the cell wall of green plants-so its nutrients are easy for the body to digest and absorb [3]. Lemons are a good source of vitamin C. One lemon provides about 31 mg of vitamin C, which is 51% of the reference daily intake (RDI) [4].

STATEMENT OF THE PROBLEM

A quasi-experimental study to evaluate the effectiveness of spirulina with lemon juice on anemia among under-five children in selected schools at Madurai, Tamil Nadu.

OBJECTIVES

- 1. To assess the pre-test level of anemia among under-five children.
- 2. To evaluate the effectiveness of spirulina with lemon juice on anemia among under-five children.
- 3. To associate the pretest level of anemia among under-five children with selected demographic variables (Age, sex, number of siblings, family type, parent's monthly income, religion, dietary pattern, educational status of parent, occupation of parent).

HYPOTHESES

- 1. The mean post-test level of anemia score among under-five children who had spirulina with lemon juice will be significantly lower than their mean pre-test level of anemia score.
- 2. The mean post-test level of anemia score among an experimental group of under-five children will be significantly lower than the mean post-test level of anemia score in the control group.
- 3. There will be the significant association between anemia among under-five children with selected demographic variables (Age, sex, family type, parent's monthly income, and educational status of parent, the occupation of a parent, religion and dietary pattern).

OPERATIONAL DEFINITION

Effectiveness

It refers to an intended result formed by an action. In this study, it refers to the outcome of administering spirulina with lemon juice on anemia among under-five children which was measured through estimation of haemoglobin by Sahli's hemoglobinometer method and monitoring the signs and symptoms of anemia by using checklist. It is the dissimilarity in the mean post-test level of anemia score between the experimental group and the control group.

Spirulina

Spirulina or Arthrospira is blue-green algae. Spirulina is a minute and filamentous cyanobacterium that derives its family name from the twisting or helical nature of its filaments. In this study, it assign to the industry name spirulot. Two capsules 500 mg of spirulot (It contains 1 gm of spirulina with vitamins, raw materials and anti-oxidants) were administered daily to the experimental group. (1.8 mg of iron present in one gram of spirulina).

Lemon Juice

It means lemon extract with sugar and water. Lemon is the traditional resource of Vitamin C and is a citrus fruit. In this examination, lemon juice means 2 ml lemon extract plus 1 teaspoon of sugar (16 kcal) and added with 48 ml of water. 2.6 mg vitamin c in the 2 ml lemon extract.

Anemia

The WHO defines anemia as follows. It results from a fewer numeral of Red Blood Cells (RBCs) or a haemoglobin concentration lower established cut-off points in the blood. These values differs hinge on the age of child. Haemoglobin <11 gm/dl in children age is between 6 months to 5 years. In this investigation, haemoglobin measured by Sahli's haemoglobinometer and also to find out the clinical manifestations of anemia such as fatigue, lack of concentration, anorexia, shortness of breath, habit of pica eating, palpitation, getting irritated often, pale conjunctiva, pallor of tongue, pallor of nails and pallor of palms.

Under-five Children

Under-five children is the age group between one month to 4 years or children younger than 5 years old. In this study under-five children is the age group between 2–4 years.

METHODOLOGY

Research Approach

• Quantitative research approach.

The present study is designed to evaluate the effectiveness of spirulina with lemon juice among under-five children affected by anemia:

- o *Phase 1:* Survey approach was used for this study.
- o *Phase 2:* In this study quasi-experimental approach was used to find out the effectiveness of spirulina with lemon juice intake among under-five children who are affected by anemia.

Research Design

- Quasi-experimental pre-test-post-test, control group design was used.
- The intervention was carried to the experimental group only, after treatment both the groups' undergone post-test.

Table 1. Experimental and control group's pre-test, post-test and intervention symbol's.

| Groups | Measurement of | Measurement of | Measurement of | Measurement of |
|--------------------|---------------------------------|-----------------------------|---------------------------------------|--|
| | dependent | independent variable | dependent variable | dependent variable |
| | variable (Pre-test) Ist week | (manipulation) (8 weeks) | post-test-1 (6 th week) | post-test-2 (10 th week) |
| | 1st week | (o weeks) | (o week) | (10 week) |
| Experimental group | A_1 | XY | A_2 | A_3 |
| Control group | A_1 | = | A_2 | A_3 |

In Table 1 keys indicates that:

- XY Intervention strategy was spirulina with lemon juice, it was given for 8 weeks.
- A Anemia
- A₁ Pre-test assessment on anemia was assessed by measuring haemoglobin level of the selected group and observing signs and symptoms of anemia at Ist week in an experimental group and control group.
- A₂ 1st post-test assessment on anemia was assessed by measuring haemoglobin level of the selected group and observing signs and symptoms of anemia at 6th week in experimental group and control group.
- A₃ 2nd post-test assessment on anemia was assessed by measuring haemoglobin level of the selected group and observing clinical manifestations of anemia at 10th week in experimental group and control group.

Variables

- *Independent variable:* In the current study, the independent variables refer to intervention strategy which includes administration of spirulina with lemon juice to the selected group.
- Dependent variables: Anemia

Setting of the Study

The study was conducted among children less than five years with anemia selected from nursery and primary schools at Madurai:

- *Population:* The study population for this research was children under-five years.
- *Target population:* In this current study, the target population was children under-five years having anemia.
- Accessible population: In this study, the available population was children under-five years having anemia, in selected matric schools at Madurai.

Sample

In this study samples are children under-five years with anemia studying in selected primary schools that satisfied the inclusion and exclusion criteria.

Sample Size

Sample size calculation based on prevalence. The sample includes 150 children in the age group of 2–4 years. Within these 75 children were in the experimental group and remaining 75 subjects were in the control group.

Sampling Techniques

Convenience sampling techniques was used.

Criteria for Sample Selection

Inclusion Criteria

- 1. Both sexes were included
- 2. Hb 5–11 gms/dl
- 3. Children under-five years who were willing to participate.

Exclusion Criteria

- 1. 0–24 months of children.
- 2. Children under-five years with other disease condition.
- 3. Children with allergies to seafood
- 4. Children with severe anemia Hb < 5 gms / dl

Description of Tool

In this study, the tool consists of 2 parts.

Part-1

This part consists of demographic variables of children such as age, sex, the living status of parents, number of siblings, birth order, and type of family, monthly profits of the family, educational condition of parents, and the employment of parents, religion and nutritional pattern.

Part-2

Section A: self-reported practice interview schedule: It includes washing hands with soap and water after each defecation, washing hands before taking foods, iron and folic acid intake. Having the habit of using slipper, cutting nails weekly, taking medication for worm infestation once in six months and habit of taking tea or coffee with the meals, consuming green leafy vegetables once in a week.

Scoring procedure: The tool consists of 8 statements related to self-reported practice interview schedule. There were 3 options (always, sometimes and never), that is "Always" carries score "2", "Sometimes" carries score "1", "Never" carries score "0".

Section B: biochemical assessment: haemoglobin: haemoglobin was checked by using sahli's haemoglobinometer.

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Interpretation of haemoglobin value: (WHO)

>11 gm/dl : No anemia. 11–10 gm/ dl : Mild anemia 7–9.9 gm/ dl : Moderate anemia <7 gm/dl : Severe anemia

Section C: Clinical Evaluation

Observation checklist on signs and symptoms of anemia: It consists of 11 items such as fatigue, lack of concentration, anorexia, shortness of breath while doing physical activities, the habit of pica eating, palpitation while doing activities, getting irritated often, pale conjunctiva, pallor of the tongue, pallor of nails and pallor of palm.

Scoring process: The scores were set as per the occurrence of the manifestations. Score "1" was given for presence of symptoms and score "2" was given for presence of signs such as pale conjunctiva, pallor of the tongue, pallor of nails and pallor of palm, Score "0" was given in case of absence of particular signs and symptoms. Overall score given to the item was 15.

Interpretation:

1–5 : Mild anemia
 6–10 : Moderate anemia
 11–15 : Severe anemia

Content Validity of the Tool

The observation checklist was developed by the investigator based on the review of the literature. The tool was given to 11 experts in the field of pediatric medicine, nursing, and nutrition for their opinion and suggestion. Based on their valuable suggestion, reframing of tools was done and validity was established.

Reliability

The reliability of the tool was established after collecting data from 15 children under-five years. Reliability of the tool was established by using an inter-rater method which is used to assess the internal consistency of the tool. Cronbach's alpha used to assess the internal consistency. Observation checklist on signs and symptoms of anemia r = 0.74. Consistency of the Sahli's hemoglobinometer r = 0.98. Sahli's haemoglobinometer was calibrated and certified by quality system standards.

Pilot Study

The pilot study result was the significant dissimilarity between the mean post-test score of an experimental and the control group. Subjects of the pilot study did not comprise in the major study. Result further suggested that spirulina with lemon juice administration is feasible, practical and cost-effective. No issues or problems raised in implementing the intervention.

Data Collection Procedure

The researcher has obtained formal consent from the school organization where the research was conducted and the headmaster. Followed by that, the intervention was explained to the samples and their parents. Written consent was got from their parents and school establishment. Researcher built good relationship with every subject. Also, privacy of their responses was guaranteed to the subjects.

Step I

Inward number 28, 8 schools were homogeneous in settings, fees structure, socioeconomic background of parents and quality of education. Out of these four schools were selected by convenient sampling technique. Out of these 2 schools were randomly assigned to an experimental group (setting

I and II) and 2 schools were assigned to control group (setting III and IV). In the survey, 220 children under-five years participated from setting I and II and 228 under-five children were from setting III and IV at Madurai. The survey period was 24 days. Children's nutritional status was assessed by haemoglobin value. Haemoglobin was checked by Sahli's haemoglobinometer. Each day 18–20 students participated.

Among them, there were 171 (84 from experimental and 87 from the control group) children with anemia, their haemoglobin level below 11 gm/dl.

Step II

The pre-test was conducted by the researcher to the control group and experimental group through collecting the demographic data self-reported practice, measuring haemoglobin value and through observing the clinical manifestations of anemia among children under-five years. The selected samples were 171 from experimental group and control group de-wormed with the suspension Ivermectin-A 5 ml. which contains Albendazole 200 mg and ivermectin 1.5 mg.

Step III

The intervention was applied to the experimental group. The researcher prepared lemon juice. 50 gm lemon gives 18–20 ml of lemon extract, so researcher squeezed 175–200 gm of lemon and got 72 ml of lemon extract, added 1728 ml of water and 150 gm of sugar for 36 samples. Same procedure followed for remaining 39 samples. Cap. S pirulot 500 mg 2 (It contains spirulina 1 g with vitamins, minerals, and antioxidants) with 50 ml of lemon juice (2 ml lemon extract plus 48 ml of water and add one teaspoon of sugar (4.2 g)) was given to the experimental group every day at 11.30 am- 12.30 pm except Sunday and government holidays. The control group was maintained by regular dietary practices.

Step IV

The experimental group was maintained by regular dietary practices and continuous administration of spirulina (1 gm) with lemon juice (50 ml) for 8 weeks. Post-test I (6th week) and post-test II (10th week) were done. The researcher monitored the signs and symptoms of anemia by observational checklist. Haemoglobin was checked by Sahli's haemoglobinometer.

Step V

The control group was followed by usual nutritional practices. There was no interference for the control group. Post-test I (6th week) and post-test II (10th week) were done. The researcher monitored the signs and symptoms of anemia by observation checklist. Haemoglobin was checked by Sahli's haemoglobinometer. No problem was encountered during the data collection period.

RESULTS AND INTERPRETATIONS

It deals with the analysis and interpretation of collected data related to evaluating the effectiveness of spirulina with lemon juice on anemia among under-five children at Madurai, Tamilnadu.

The data were tabulated, analyzed and interpreted using descriptive and inferential statistics on the basis of objectives and hypothesis formulated for the purpose of this study.

Table 2 shows the distribution of samples based on observation checklist on signs and symptoms of anemia before intervention by group wise. In experimental group, the pre-test reveals that out of 75 under-five children, 36 (48%) had fatigue, 29 (38.66%) had anorexia, 29 (38.66%) had lack of concentration, 24 (32%) had shortness of breath, 30 (40%) had habit of pica eating, 24 (32%) had palpitation, 32 (42.66%) were getting irritated often, 16 (21.3%)had pallor of conjunctiva, 15 (20%) had pallor of tongue, 19 (25.33%) had pallor of nails, 18 (24%) had pallor of palms and soles.

ISSN: 2455-6351

Table 2. Frequency and percentage distribution of samples based on observation checklist on signs

and symptoms of anemia before intervention among experimental group and control group.

| S.N. | Variables | Experimental Group (N = 75) | | Control Group (N = 75) | | Chi-square test value | D.F. | P-Value |
|------|------------------------------------|-----------------------------|-------|------------------------------|-------|--------------------------|------|---------|
| | | F | % | F | % | | | |
| 1. | Fatigue | 36 | 48 | 24 | 32 | 4.000 | 1 | 0.046* |
| 2. | Anorexia | 29 | 38.66 | 22 | 29.33 | 1.456 | 1 | 0.228 |
| 3. | Lack of concentration | 29 | 38.66 | 22 | 29.33 | 1.456 | 1 | 0.228 |
| 4. | Shortness of breath | 24 | 32 | 19 | 25.33 | 0.815 | 1 | 0.367 |
| 5. | The habit of pica eating | 30 | 40 | 19 | 25.33 | 3.463 | 1 | 0.063 |
| 6. | Palpitation while doing activities | 24 | 32 | 19 | 25.33 | 0.815 | 1 | 0.367 |
| 7. | Getting irritated often | 32 | 42.66 | 19 | 25.33 | 5.021 | 1 | 0.025* |
| 8. | Pale conjunctiva | 16 | 21.33 | 14 | 18.66 | 0.135 | 1 | 0.713 |
| 9. | Pallor of tongue | 15 | 20 | 14 | 18.66 | 0.043 | 1 | 0.836 |
| 10. | Pallor of nails | 19 | 25.33 | 14 | 18.66 | 1.369 | 1 | 0.242 |
| 11. | Pallor of palms and soles | 18 | 24 | 14 | 18.66 | 0.636 | 1 | 0.425 |

^{*-}Significant

In the control group the frequency of anemia signs and symptoms, during the pre-test reveals that out of 75 under-five children, 24 (32%) had fatigue, 22 (29.33%) had anorexia, 22 (29.33%) had lack of concentration, 19 (25.33%) had shortness of breath, 19 (25.33%) had habit of pica eating, 19 (25.33%) had palpitation, 19 (25.33%) were irritated often. 14 (18.66%) had pale conjunctiva, 14 (18.66%) had pallor of the tongue, 14 (18.66%) had pallor of nails, 14 (18.66%) had pallor of palms and soles. The non-significant p-value of the Chi-square test infers that children's signs and symptoms of anemia before intervention had been equally distributed in both the groups except fatigue ($\chi^2 = 4.000$, p-value 0.046*) and getting irritated often ($\chi^2 = 5.021$, p-value 0.025*).

Level of Anemia

Figure 1 shows that distribution of samples based on the categorization of anemia by haemoglobin concentration of pre-test, post-test I, and post-test II of experimental group. In pre-test 26 (34.66%) had mild anemia, 30 (40%) had moderate anemia, 19 (25.33%) had severe anemia. In post-test I 16 (21.33%) had normal haemoglobin value (no anemia), 21 (28%) had mild anemia, 27 (36%) had

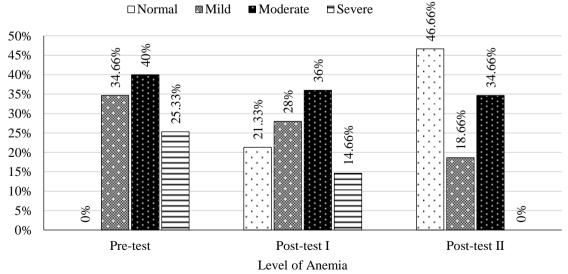


Figure 1. Comparison of pre, post-test I and II level of anemia by haemoglobin concentration among experimental group.

moderate anemia and 11 (14.66%) had severe anemia. In post-test II 35 (46.66%) had normal haemoglobin value, 14 (18.66%) had mild anemia, 26 (34.66%) had moderate anemia and no one had severe anemia.

Levels of Anemia by Hemoglobin Concentration among Experimental Group

Figure 2 shows that distribution of samples based on the categorization of anemia by haemoglobin concentration of pre-test, post-test I, and post-test II of control group In control group pre-test 23 (30.66%) had mild anemia, 38 (50.66%) had moderate anemia, 14 (18.66%) had severe anemia. In post-test I, no one had normal haemoglobin value, 25 (33.33%) had mild anemia, 37 (49.33%) had moderate anemia and 13 (17.33%) had severe anemia. In post-test II, 18 (24%) had mild anemia, 44 (58.66%) had moderate anemia and 13 (17.33%) had severe anemia.

Figure 1 and 2 findings proved that there was increased haemoglobin value in the experimental group than the control group. This effect was due to an intake of spirulina with lemon juice. Spirulina contains iron and lemon contains Vit - C. Vitamin C helps to absorb iron hence the increased haemoglobin level for the experimental group.

Table 3 shows the comparison of mean and standard deviation of pre-test anemia outcome-related variables by group wise. Based on haemoglobin value of anemia score (student's t-test = 0.079, p-0.937#) and based on observation checklist on signs and symptoms of anemia score (student's t-test = 1.178, p-0.241#). The non-significant p-value infers that before the intervention, haemoglobin value and the level of signs and symptoms of anemia had been similar in the two groups.

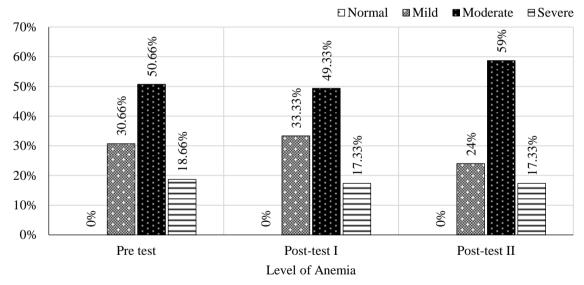


Figure 2. Comparison of pre, post-test I and II level of anemia by haemoglobin concentration among the control group

Table 3. Comparison between experimental group and control group outcome variables of anemia at pre-test.

| S. N. | Variables | Experimental group N = 75 | | Control group N = 75 | | Student's t-test value | D.F. | P-value | | | |
|----------|---|---------------------------|-------|-------------------------|-------|---------------------------|------|---------|--|--|--|
| | | Mean | SD | Mean | SD | | | | | | |
| 1. | Haemoglobin in gm | 8.997 | 1.244 | 8.183 | 1.228 | 0.079 | 148 | 0.937# | | | |
| 2. | Observation checklist on signs and symptoms of anemia | 4.5600 | 6.052 | 4.4133 | 4.865 | 1.178 | 148 | 0.241# | | | |

^{# -} Non-significant

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Table 4. Comparison between experimental group and control group outcome-related variables of anemia at post-test I.

| S.N. | S.N. Variables | | Experimental group N = 75 | | ol group = 75 | Student's t-test | D.F. | P-value | |
|------|---|-------|---------------------------|-------|------------------|---------------------|------|----------|--|
| | | Mean | SD | Mea | SD | value | | | |
| | | | | n | | | | | |
| 1. | Haemoglobin in gm | 9.779 | 1.2146 | 8.441 | 1.280 | 3.768 | 148 | 0.000*** | |
| 2. | Observation checklist on signs and symptoms of anemia | 2.403 | 4.393 | 4.382 | 6.231 | -2.378 | 148 | 0.019* | |

^{*** -} very highly significant, * - significant

Table 5. Comparison between experimental group and control group outcome variables of anemia at post-test II (N = 150).

| S.N. | Variables | Experimental group N = 75 | | | ol group = 75 | Student' s t-test | D.F. | P-value |
|------|--|------------------------------|--------|-------|------------------|----------------------|------|----------|
| | | Mean | SD | Mean | SD | value | | |
| 1. | Haemoglobin in gm | 10.536 | 1.2126 | 8.791 | 1.2478 | 7.194 | 148 | 0.000*** |
| 2. | Observation checklist on signs and symptoms of anemia. | 1.201 | 2.329 | 4.35 | 6.120 | -4.690 | 148 | 0.000*** |

^{***}very highly significant

Table 4 shows the comparison of mean and standard deviation of post-test I anemia outcome-related variables by group wise. The purpose of this table is to verify after the intervention, whether the outcome variables of the study are similar in both groups or any variation exists between them. Based on haemoglobin value, the significant p-value infers (student's t-test = 3.768, p-0.000***) that after the intervention, the haemoglobin value had been different among experimental group and control group. This indicates that this difference may be due to the effects of spirulina with lemon juice intake.

Based on observation checklist on manifestations of anemia, the significant p-value infers (student's t-test = -2.378, p-0.019*) that after the intervention, the appearance of anemia had been different among the two groups. This indicates that this difference is due to the effects of spirulina with lemon extract.

Table 5 shows the comparison of mean and standard deviation of post-test II anemia outcomerelated variables by group wise Based on haemoglobin value (student's t-test = 7.194, p-0.000***), and an observation checklist on signs and symptoms of anemia the significant p-value infers (student's t-test = -4.690, p-0.000***) that after the intervention, the haemoglobin value and on signs and symptoms of anemia has been statistically different among the two groups. This indicates that this difference may be due to the effects of spirulina with lemon juice intake.

Table 6 shows the comparison of haemoglobin value in the experimental group of children between pre-test, post-test I and II. The significant p-value of the comparisons between pre-test versus post test1 infers (F-value 715.731, p-value 0.000^{***}) that there was a significant difference in the haemoglobin value at post-test I compared to pre-test and the comparison between post-test I and post-test II infers (F-value 841.351, p-value 0.000^{***}) that there was a significant difference in the haemoglobin value at post-test II compared to post-test 1. This difference may be due to the effectiveness of spirulina with lemon juice intake.

Table 7 shows that the comparison of observation checklist on signs and symptoms of anemia in the experimental group between pre-test, post-test I and post-test II. The comparison of observation checklist on signs and symptoms of anemia in the experimental group between pre-test vs. post-test 1

Table 6. Comparison of hemoglobin value in the experimental group of children between pre-test, post-test I and II.

| Assessment | Mean | SD | ANOVA repeated test value | | Repeated contrast test result | | | | | | | |
|----------------|--------|--------|---------------------------|----------|-------------------------------|---------|----------|--|--|--|--|--|
| | | | F-value P-value | | Comparison | F-value | P-value | | | | | |
| 1. Pre-test | 8.997 | 1.244 | 1007.967 | 0.000*** | Pre-test vs post-test 1 | 715.731 | 0.000*** | | | | | |
| 2. Post-test 1 | 9.779 | 1.2146 | | | Post-test 1 vs post-test II | 841.351 | 0.000*** | | | | | |
| 3. Post-test 2 | 10.532 | 1.2126 | | | | | | | | | | |

^{*** -} Very highly significant

Table 7. Comparison of observation checklist on signs and symptoms of anemia in the experimental group between pre-test, post-test I and post-test II.

| Assessment | Mean | SD | ANOVA repeated test value | | Repeated contrast test result | | | |
|-------------------------------|--------------|----------------|---------------------------|----------|---|------------------|----------------------|--|
| | | | F-value | P-value | Comparison | F-value | P-value | |
| 1. Pre-test 2. Post-test 1 | 4.56 2.40 | 6.052 4.393 | 27.145 | 0.000*** | Pre-test vs post-test 1 Post-test 1 vs post-test 2 | 18.817 19.977 | 0.000*** 0.000*** | |
| 3. Post-test 2 | 1.20 | 2.329 | | | | | | |

^{*** -} Very highly significant

Table 8. Mean difference between pre-test and post-test II of haemoglobin value in the experimental group and the control group (N = 150).

| Group | Mean | SD | ANCOVA test result | | | | | |
|-----------------|---------|---------|--|---------|----------|--|--|--|
| | | | Source | F-value | P-value | | | |
| 1. Experimental | -1.5387 | 0.38621 | Father's education | 0.008 | 0.930 | | | |
| 2. Control | -0.613 | 0.17051 | Mother's education | 0.980 | 0.324 | | | |
| | | | Father's occupation | 0.116 | 0.734 | | | |
| | | | Washing hands with soap and water after each defecation | 0.593 | 0.443 | | | |
| | | | Washing hands before taking foods | 0.857 | 0.356 | | | |
| | | | Taking iron and folic acid supplementation | 0.505 | 0.479 | | | |
| | | | Wearing slipper | 2.950 | 0.088 | | | |
| | | | Cutting nails weekly | 0.015 | 0.903 | | | |
| | | | Taking treatment for worm infestation once in six months | 0.188 | 0.665 | | | |
| | | | Not consuming tea or coffee with the meal | 1.194 | 0.276 | | | |
| | | | Consuming green leafy | 0.307 | 0.580 | | | |
| | | | Group | 384.794 | 0.000*** | | | |

^{*** -} Very highly significant

infer (F-value 18.817, P-value 0.000***) and post-test 1 vs. post-test II infers (F- value 19.977, p-value 0.000***) that there was a significant difference on signs and symptoms of anemia score at post-test I compared to pre-test and post-test II compared to post-test 1. This difference may be due to the effectiveness of spirulina with lemon juice intake.

Table 8 shows that the Mean difference between pre-test and post-test II of haemoglobin value in the experimental group and the control group. All the co-variants were non-significant but "group" shows (F-value 384.794, p-value 0.000***) that there was a significant difference after controlling all the covariates. This is due to the administration of spirulina with lemon juice for the experimental group.

The Mean difference between pre-test and post-test II of an observation checklist on signs and symptoms of anemia in the experimental group and the control group ANCOVA test result shows that all the co-variants were non-significant, but "group" shows (F-value 19.258, P-0.000***) that there

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was a significant difference after controlling all the covariates. This is due to the administration of spirulina with lemon juice for the experimental group.

Kruskal-wallis test has been applied to find out the association between the anemia and demographic variables. Demographic variables such as age of the child (k = 1.938, p-value 0.379), sex (k = 1.407, p-value 0.235), siblings (k = 5.476, p-value 0.140), family type (k = 1.370, p-value 0.543), income (k = 2.031, p-value 0.362), religion (k = 4.515, p-value 0.105), diet (k = 1.185, p-value 0.276), father's education (k = 6.470, p-value 0.167), mother's education (k = 5.026, p-value 0.285), father's occupation (k = 9.133, p-value 0.068), mother's occupation (k = 5.428, p-value 0.246). The non-significant p-value infers that there was no association between the anemia and demographic variables.

DISCUSSION

The present study intervention was administration of spirulina with lemon juice, which had greater impact on increasing haemoglobin value and reducing the level of signs and symptoms of anemia among under-five children. The observational group mean post-test I signs and symptoms of anemia was decreased (2.40) than the control group (4.38). It was extremely significant (student's t-test= -2.378, p-0.019**). The experimental group mean post-test II was lesser (1.20) than control group (4.35). It was very highly significant (student's t-test = -4.690, p-0.000***). In the experimental group, the mean post-test I Haemoglobin score (9.779) and post-test II haemoglobin score (10.532) were significantly increased than the pretest mean (8.99). Contrast test results also showed that there was a significant difference between pre-test versus post-test I (F-715.731, P-0.000) and post-test I versus post-test II (F-841.351, P-0.000). Haemoglobin score was highly significant (p- 0.000***). It may be due to the effectiveness of spirulina with lemon juice intake. Fehmida Iyer Visnagarwala. (2017) were reported that 100 children who were given 1-2 gms of spirulina for 3 months that increase of 1.5-3 gms of Hb [5]. Abede (2016) analyzed that 3 grams of Spirulina supplementation significantly improved in hemoglobinvalue from 10.28 to 11.42 gm/dl (p<0.001) in the experimental group [6]. Ammatul Fathima and Dr. Sangita Srivastava (2016) were stated that the experimental group received iron rich biscuits fortified with spirulina for 45 days and control group did not receive such supplementation. Intervention program brought out the significant (P<0.05) increment in haemoglobin level as 1.66 gm/dl in experimental group and not significant increment noted in the control group [7]. Prashanth Thankachan et al. (2013) evaluated the effectiveness of ascorbic acid on iron absorption among Indian young women. Vitamin C added to the rice meal in the molar ratio of 2:1 or 4:1 In the experimental group, iron absorption was high (291% or 350%) than the control group (270% or 343%) [8]. Dorothy et al (2021) done a study about Impact of Spirulina corn soy blend (SCSB) on Iron deficient children aged 6-23 months. Children consuming SCSB were significantly higher than those consuming corn soy blend (CSB) (log-rank- $X^2 = 0.978$; CI: 0.954–1.033, P = 0.001) and the placebo (log-rankX2 = 0.971; CI: 0.943–0.984, P = 0.0001). Children consuming SCSB had a mean recovery time of 8 days (CI: 7-12 days) compared to those consuming CSB (19 days; CI: 20-23 days) and placebo (33 days; CI: 3 1–35 days). The recovery rate was 15.4 per 100 persons per day for children who consumed SCSB as opposed to 4.6 and 1.8 per 100 persons per day for those who consumed CSB and the placebo, respectively [9].

CONCLUSION

The following conclusions are drawn from this study:

- Anemia prevalence in young children continues to remain over 70% in most parts of India and Asia despite a policy being in place and a program that has been initiated for a long time [10]. So there is a vital to initiate and incorporate the nutritional based intervention and will be a better preventive strategy to stop the severe effect of anemia.
- It was concluded that intake of spirulina with lemon juice had beneficial effect on the haemoglobin level and reducing signs and symptoms of anemia and could be effectively used to treat anemia among under-five children.

- Spirulina is a natural dietary supplementation for children with anemia.
- This study has only 8 weeks intervention and no randomization; further studies are needed with long term follow up and proper randomization to provide more beneficial effect of spirulina with lemon juice intake on anemia among under-five children. It might be used in a wide range of settings.
- Awareness program can be conducted on manifestations and methods to overcome the anemia among under-five children.

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