



Evaluation of Memory and Cognition Enhancing Activity of *Glycyrrhiza glabra* Linn. Formulation Along with Yoga in Teenage (13-16 years) Students

Mrudul Chitrakar¹, Supriya Bhalerao², Vinay Pawar¹ and Vedvati Bhapkar¹

¹School of Ayurveda, DY Patil Deemed to be University, Navi Mumbai - 400706, Maharashtra, India; mrudulmlonkar@gmail.com, vinay.pawar@dypatil.edu, drvedvati@gmail.com

²IRSHA, Bharati Vidyapeeth Deemed to be University, Pune – 411030, Maharashtra, India; supriya.bhalerao@gmail.com

Abstract

Yashtimadhu (*Glycyrrhiza glabra* Linn.) is one of the important neuro-nutrient herbs (*Medhya Rasayana*) mentioned in Ayurveda classics. Also, its usefulness in memory and cognition has been shown in many studies. Yoga, a part of the rich Indian heritage has also shown promising results in the memory enhancement of children and adults. Thus, a study was planned to assess the efficacy of their combined schedule in teenage students. *Glycyrrhiza glabra* Linn. was converted into an herbal formulation '*Yashtimadhu Kalpa*' (YK) as per principles of Ayurveda pharmaceutics. The objective of this study was to evaluate the effect of YK and Yoga schedules on the memory and cognitive performance of teenage (13-16 years old) students. In this open-labeled, randomized, placebo-controlled study, 100 assenting healthy students between 13-16 years of age were enrolled and randomized into trial and control groups, after obtaining EC approval and parent consent. Those in the trial group received YK and Yoga, while control group participants received a placebo (Ragi malt) and Yoga. The YK and placebo were to be taken with cow milk in the morning. A 30-minute Yoga schedule was also taught to them. The duration of treatment was of 90 days. Effect on memory and cognition was assessed by using cognitive ability and mental ability tests devised by Jnana-Prabodhini's Institute of Psychology, Pune. These assessments were done on randomization visits (Day 0) and end-of-study visits (Day 90). Appropriate statistical tests were applied to the data to obtain results. Both groups showed significant improvement in cognition and memory after 90 days of treatment duration. However, YK group showed more significant improvement in cognition of figural classes and cognition of figural system in cognitive ability and memory of figural transformations, the memory of symbolic transformations, total memory, and mental ability among memory test parameters when compared to the control group. The inclusion of a Yoga schedule in both groups was found to be effective in the improvement of memory and cognition. YK (formulation of *Glycyrrhiza glabra* Linn.) along with Yoga can be used in children to enhance their memory and cognition.

Keywords: Intelligence, *Medhya Rasayana*, Pranayama, Shavasana, Yashtimadhu

1. Introduction

Memory is the mental capacity of retaining and reviving facts, events, impressions, etc., or of recalling or recognizing previous experiences¹. Cognition pertains to how a person understands the world and acts in it. It is a set of mental abilities or processes that are part of nearly every human action while we are awake².

In school health programs, mostly physical health is considered, though mental and social health are equally important. Many parents are unaware of the importance of memory-boosting medicines or good conduct of life such as yogic procedures to be followed by children for improving cognitive performance³.

According to Ayurveda classics, *Rasayana* is defined as the means of achieving the finest quality of body tissues

and the drugs act as a neuro-nutrient by promoting intelligence and functions of the brain as *Medhya Rasayana* (neuro-nutrients)^{4,5}. Neuro nutrients are useful for the prevention and treatment of mental disorders of all age groups. They produce neuro-nutrient effects by improving cerebral metabolism⁶.

Ayurveda has mentioned four *Medhya Rasayana* and their usage in different dosage forms is also described. Among these, *Yashtimadhu* (powder of *Glycyrrhiza glabra* Linn) with cow milk has been prescribed⁷. Previous experimental studies have shown satisfactory effects of *Glycyrrhiza glabra* Linn on memory and cognition. In an experimental study conducted by Chakravarthi *et al.*, it was concluded that the aqueous root extract of *Glycyrrhiza glabra* was beneficial in improving spatial learning and memory in rat models. The Diazepam-induced amnesia was even found to be reversed⁸. It was also proven to be beneficial in improving inflammation-induced cognitive dysfunction by down-regulating inflammatory proteins in a study conducted by Cho *et al*⁹.

The dynamic nature of Ayurveda, allows the alteration of medicinal forms keeping the basic principles intact. For the present study, a formulation was developed named *Yashtimadhu Kalpa* (YK)¹⁰. A conventional way of powdered *Yashtimadhu* (*Glycyrrhiza glabra* Linn.) neuro-nutrients herb mentioned in Ayurveda classics given with milk has been replaced by novel YK for the present study. It is milk-dissolvable, stable, and palatable¹¹.

The role of yoga in memory boosting as a lifestyle modification is well-known to the scientific fraternity¹². It aims to attain the unity of mind, body, and spirit through *Asana* (exercise), *Pranayama* (breathing), and meditation¹². Research studies for exploring the effects of practicing yoga in educational settings on young adolescents have shown potential benefits in various domains¹³. Brain activity is associated with different cognitive processes and plays a critical role in different yogic practices¹⁴. Thus, this study was to evaluate the effect of YK and Yoga schedule on the memory and cognitive performance of teenage (13-16 years old) students.

2. Materials and Methods

2.1 Study Sites

This two-arm, open-labelled, randomized, prospective-controlled clinical trial was conducted at D. Y. Patil Deemed to be University School of Ayurveda, Navi Mumbai in collaboration with R. M. V. School, Navi Mumbai.

2.2 Ethical Considerations

Ethical approval was obtained from the Institutional Ethics Committee of the trial site (Letter no. DYPUSA/18/426(A)). The study was registered in Clinical Trials Registry-India (CTRI/2019/07/020034) on 4th July 2019. Administrative permission from the head, R. M. V. School, Navi Mumbai, was obtained from where the study participants were enrolled. Written informed consent was obtained from the parents of the students participating in the study along with assent from the students before the trial.

2.3 Inclusion Criteria

The students of either sex between the age group of 13-16 years who were willing to participate and follow the schedule were included in the study.

2.4 Exclusion Criteria

Teenage students with illnesses like juvenile type 1 diabetes mellitus, protein-energy malnutrition, tuberculosis, human immunodeficiency virus, cancer, or any other major physical illnesses and disorders and students with genetic disorders like Down's syndrome, congenital malformations, with neurological disorders like epilepsy, autism, meningitis, cerebral palsy and psychiatric disorders, Attention Deficit Hyperactivity Disorder (ADHD), mood disorders and anxiety were excluded from the study. Also, students who were not willing to participate in the study or who were not fit for yoga, or not willing for a yoga schedule were excluded from the study.

2.5 Sample Size

As it was an exploratory study, an empirical sample size of a minimum of 50 students in each group was considered appropriate for the study. Considering 10% dropout rate, 56 students were randomized in each group. Teenage students coming under the inclusion criteria were selected for the study using the purposive sampling method. A dropout rate of 20% was considered for fixing the sample size. Enrollment, allocation, follow-up, and analysis scenarios of the study are presented in the CONSORT flow diagram (Figure 1).

2.6 Plan of Study

Enrolled study participants were randomly divided into two groups, namely trial and control groups, by using computer-generated randomization list. Clinical history, general physical examination, and systemic examinations were carried out to rule out any illness.

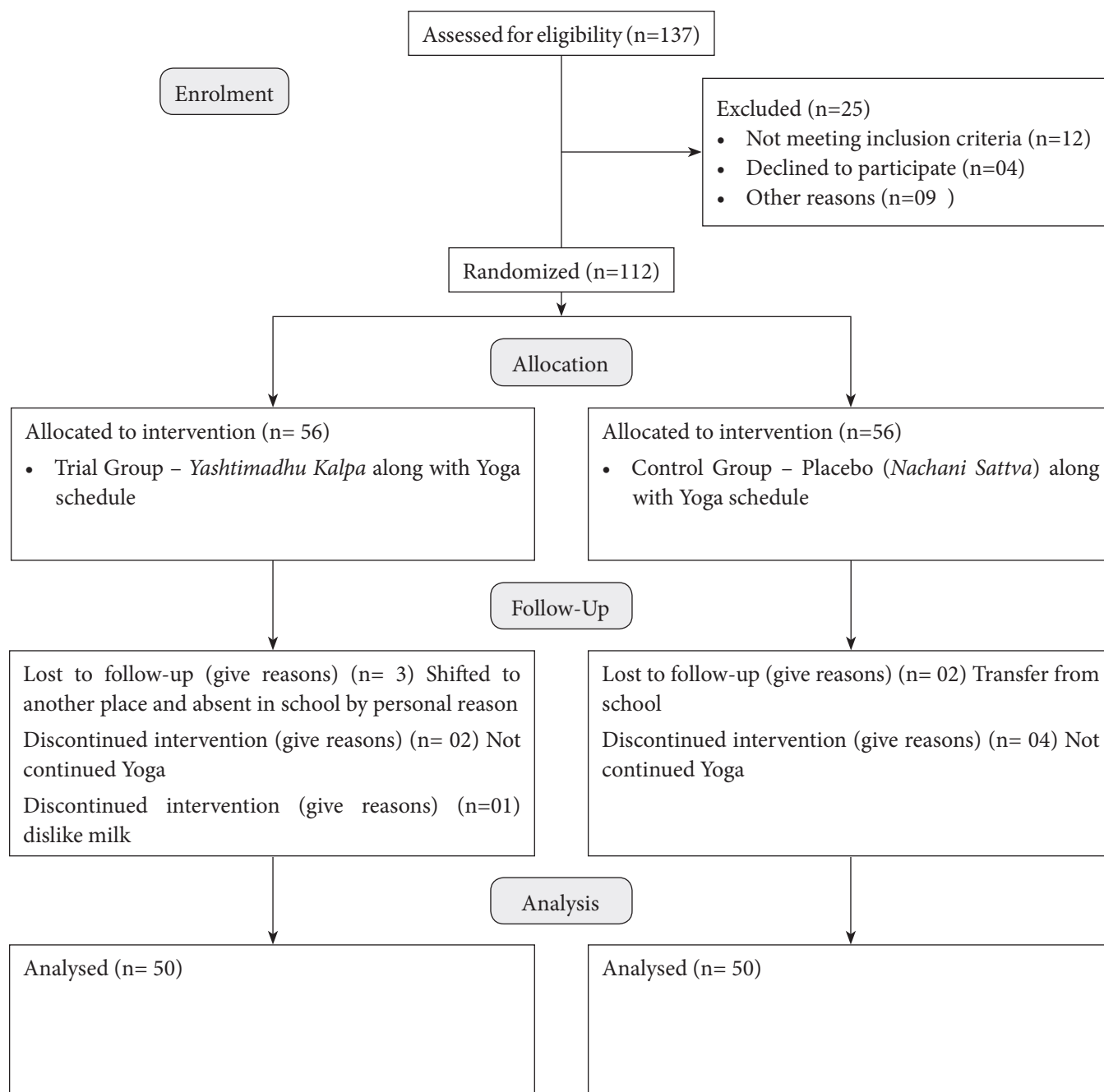


Figure 1. CONSORT flow diagram.

2.7 Interventions

Study participants in the trial group were given YK, 6 mg with cow milk in the morning along with a yoga schedule for continuous 90 days. YK was prepared in a GMP-certified pharmacy¹⁵. Study participants in the control group were given a placebo (Ragi malt), 6 mg with cow milk in the morning along with the same yoga schedule 5 days a week minimum for 90 days. The

preferred dose in students depends on the age of the child (Table 1).

2.8 Follow-up

The participants were tested after every 15 days for memory and for cognitive performance after three months of the study. The evaluation report of study participants before and after the study was assessed. Grading was given as per the guidelines of psychological assessment.

Table 1. Study plan

	Trial Group	Control Group
Sample Size (n)	50	50
Intervention	<i>Yashtimadhu Kalpa (YK)</i>	Placebo (Ragi malt)
Dosage	6 gm	6 gm
<i>Anupana</i>	Cow milk	Cow milk
Time of administration	Morning	Morning
Supplementary schedule (common to both the groups)	Yoga schedule Loosening and stretching exercise 1) Loosening of all joints, 5 rounds each daily 2) <i>Suryanamaskar</i> , 6 rounds 3) <i>Bhramari</i> , 5 rounds 4) <i>Omkar</i> , 5 rounds 5) <i>Shavasana</i> , 2 minutes Yoga schedule for 18-20 minutes approximately 5 days in a week minimum for 90 days	

2.9 Tools for Assessment of Efficacy Parameters

The following tests were administered to the study participants: A battery of Cognition Function Tests (CFTs), an Indian adaptation of Guilford's structure of intellect model, devised by the Jnana-Prabodhini's Institute of Psychology, Pune, India, was administered to each student¹⁶. This test was suitable for use in children of 9 to 16 years of age.

Mental ability assessment test kit: This battery contains nine tests based on J. P. Guilford's Structure of Intellect (SOI) model. It involves observing and identifying similarities and differences between figures and numbers, observing the developmental sequence between figures and numbers as well as observing and identifying transformations occurring in these two. It involves the Cognition of Figural Classes (CFC), Cognition of Figural Systems (CFS), Convergent Production of Figural Classes (NFC), Evaluation of Figural Classes (EFC), Convergent Production of Figural relation (NFR), Convergent Production of Figural Systems (NFS), Evaluation of Figural Relation (EFR), Evaluation of Figural Systems (EFS) and Evaluation of Figural Implications (EFI).

Memory assessment test kit: This test battery involves immediate recognition after correctly identifying and understanding the common characteristics of a group of figures, common letters, feelings or emotions expressed, and meaningful relations between two words. It contains four tests based on J. P. Guilford's Structure of Intellect (SOI) model. It involves Memory of Figural Transformations

(MFT), a Memory of Symbolic Transformations (MST), Memory of Symbolic Implications (MSI) and Memory of Behavioral Classes (MBC).

2.10 Plan for Statistical Analysis

All baseline and demographic data were summarized descriptively. All continuous variables were summarized using a mean \pm standard deviation. All categorical variables were summarized using frequency and percentages as well as median and range. GraphPad In Stat Version 3.6 (www.graphpad.com) software was used for the statistical analysis of data. The primary and secondary outcomes were analyzed by applying appropriate statistical tests like paired and unpaired 't' test when data passed the normality test. Wilcoxon matched pairs, signed ranks test and Mann-Whitney test were used when data failed the normality test. p value < 0.05 was considered significant.

3. Results

3.1 Demographic Details

All study participants were enrolled from a private English medium school located in Navi Mumbai, Maharashtra, India. It was observed that most of the study participants belonged to middle and upper middle class only.

3.2 Cognitive Ability

A statistically significant difference was observed in almost all cognitive ability tests in both the groups except EFS between both groups.

In the trial group, the average CFC score before treatment was 7.50 ± 1.49 which got changed to 9.04 ± 1.26 ($p < 0.0001$) whereas in the control group, the average CFC score before treatment was 7.14 ± 1.65 which got changed to 8.34 ± 1.55 ($p < 0.0001$).

In the trial group, the average CFS score before treatment was 5.94 ± 2.45 which got changed to 8.42 ± 2.11 ($p < 0.0001$) whereas in the control group, the average CFS score before treatment was 5.78 ± 2.62 which got changed to 7.42 ± 2.16 ($p < 0.0001$).

On comparison, the improvement in CFC and CFS scores in the trial group was better than that in the control group (Table 2).

Cognition of Figural Classes (CFC), Cognition of Figural Systems (CFS), Convergent Production of Figural Classes (NFC), Evaluation of Figural Classes (EFC), Convergent Production of Figural Relation (NFR), Convergent Production of Figural Systems

(NFS), Evaluation of Figural Relation (EFR), Evaluation of Figural Systems (EFS) and Evaluation of Figural Implications (EFI).

3.3 Mental Ability Quotient (MAQ)

In the trial group, the average Mental Ability Quotient (MAQ) score before treatment was 80.98 ± 11.10 with median 84.5 (range 60–98) which got changed to 96.02 ± 13.48 with median 96 (range 62–124) whereas in the control group, the average MAQ score before treatment was 81.02 ± 11.53 with median 84.5 (range 60–98) which got changed to 90.52 ± 14.35 with median 93 (range 60–125). The difference in the medians of MAQ was statistically significant ($p < 0.0001$) in both groups. On inter-group comparison, the difference in the medians of MAQ after treatment was found to be statistically insignificant ($p = 0.0824$).

Table 2. Comparative cognitive ability between two groups

Sr. No.	Test	Group	Score (Mean \pm SD)	
			BT	AT
1	Test 1 (CFC)	Trial Group	7.50 ± 1.49	$9.04 \pm 1.26^{***##}$
		Control Group	7.14 ± 1.65	$8.34 \pm 1.55^{***}$
2	Test 2 (CFS)	Trial Group	5.94 ± 2.45	$8.42 \pm 2.11^{***##}$
		Control Group	5.78 ± 2.62	$7.42 \pm 2.16^{***}$
3	Test 3 (NFC)	Trial Group	3.25 ± 1.62	$5.55 \pm 2.32^{***}$
		Control Group	3.27 ± 1.74	$4.98 \pm 2.34^{***}$
4	Test 4 (EFC)	Trial Group	6.12 ± 2.62	$7.06 \pm 1.99^{***}$
		Control Group	5.84 ± 2.80	$6.72 \pm 2.17^{***}$
5	Test 5 (NFR)	Trial Group	5.52 ± 2.70	$6.80 \pm 1.78^{***}$
		Control Group	5.06 ± 2.93	$6.10 \pm 2.12^{***}$
6	Test 6 (NFS)	Trial Group	6.24 ± 3.71	$7.38 \pm 2.63^{***}$
		Control Group	6.90 ± 3.38	$7.44 \pm 2.85^{***}$
7	Test 7 (EFR)	Trial Group	5.75 ± 1.93	$6.08 \pm 1.79^{***}$
		Control Group	5.42 ± 2.40	$5.77 \pm 2.12^{***}$
8	Test 8 (EFS)	Trial Group	4.90 ± 1.76	$5.14 \pm 1.95^{***}$
		Control Group	4.80 ± 2.08	$5.02 \pm 2.10^{***}$
9	Test 9 (EFI)	Trial Group	2.98 ± 2.11	$4.98 \pm 2.25^{***}$
		Control Group	2.92 ± 2.15	$4.14 \pm 2.18^{***}$

Intra-group comparison—compared to baseline, $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$

Inter-group comparison—compared to control group, $p < 0.05^{\#}$, $p < 0.01^{\#\#}$, $p < 0.001^{\#\#\#}$

3.4 Memory Tests

A statistically significant difference was observed in all memory tests in both groups. In the trial group, the average MFC score before treatment was 4.29 ± 2.57 which got changed to 7.90 ± 2.55 ($p < 0.0001$) whereas in the control group, the average MFC score before treatment was 4.42 ± 2.97 which got changed to 7.78 ± 2.35 ($p < 0.0001$).

In the trial group, the average MSC score before treatment was 4.94 ± 2.58 which got changed to 7.78 ± 2.35 ($p < 0.0001$) whereas in the control group, the average MSC score before treatment was 4.84 ± 2.42 which got changed to 6.94 ± 2.28 ($p < 0.0001$).

On comparison between the groups, improvement in MFC and MFS scores in the trial group was better than that in the control group (Table 3).

Cognition of Figural Classes (CFC), Cognition of Figural Systems (CFS), Convergent Production of Figural Classes (NFC), Evaluation of Figural Classes (EFC), Convergent

Production of Figural Relation (NFR), Convergent Production of Figural Systems (NFS), Evaluation of Figural Relation (EFR), Evaluation of Figural Systems (EFS) and Evaluation of Figural Implications (EFI).

4. Discussion

The present clinical study was conducted to evaluate the neuro-nutrient effect of YK on memory and cognitive performance of teenager (13-16 years) students. A significant improvement was observed in all cognitive and memory parameters after treatment over a period of 90 days in both groups. The group treated with YK showed more improvement compared to control group with statistically significant difference in Cognition of Figural Classes (CFC) and Cognition of Figural System (CFS) in Cognitive Ability and Memory of Figural Transformations (MFT), Memory of Symbolic Transformations (MST), total memory and mental ability among memory test parameters.

Table 3. Comparative results of mental ability between two groups

Sr. No.	Test	Group	Score (Mean \pm SD)	
			BT	AT
1	Test 1 (CFC)	Trial Group	7.50 \pm 1.49	9.04 \pm 1.26***##
		Control Group	7.14 \pm 1.65	8.34 \pm 1.55***
2	Test 2 (CFS)	Trial Group	5.94 \pm 2.45	8.42 \pm 2.11***##
		Control Group	5.78 \pm 2.62	7.42 \pm 2.16***
3	Test 3 (NFC)	Trial Group	3.25 \pm 1.62	5.55 \pm 2.32***
		Control Group	3.27 \pm 1.74	4.98 \pm 2.34***
4	Test 4 (EFC)	Trial Group	6.12 \pm 2.62	7.06 \pm 1.99***
		Control Group	5.84 \pm 2.80	6.72 \pm 2.17***
5	Test 5 (NFR)	Trial Group	5.52 \pm 2.70	6.80 \pm 1.78***
		Control Group	5.06 \pm 2.93	6.10 \pm 2.12***
6	Test 6 (NFS)	Trial Group	6.24 \pm 3.71	7.38 \pm 2.63***
		Control Group	6.90 \pm 3.38	7.44 \pm 2.85***
7	Test 7 (EFR)	Trial Group	5.75 \pm 1.93	6.08 \pm 1.79***
		Control Group	5.42 \pm 2.40	5.77 \pm 2.12***
8	Test 8 (EFS)	Trial Group	4.90 \pm 1.76	5.14 \pm 1.95***
		Control Group	4.80 \pm 2.08	5.02 \pm 2.10***
9	Test 9 (EFI)	Trial Group	2.98 \pm 2.11	4.98 \pm 2.25***
		Control Group	2.92 \pm 2.15	4.14 \pm 2.18***

Intra-group comparison—compared to baseline, $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$

Inter-group comparison—compared to control group, $p < 0.05^{\#}$, $p < 0.01^{\#\#}$, $p < 0.001^{\#\#\#}$

A significant improvement in cognitive and memory test parameters in the control group is shown due to regular practice of yoga. Yogic practices assisted in cognitive development in the participants of both the groups¹⁷. In the control group, as placebo Ragi malt was used. It has the highest amount of calcium (344 mg) and potassium (408 mg). Also, it has higher dietary fibre, minerals, and sulphur containing amino acids compared to white rice¹⁸. However, no studies or classical references have mentioned its use for cognition or memory enhancing. No adverse events were seen in any groups.

The concept of herbal neuro-nutrient is appealing as it is being analyzed through different angles relating to their target, mode of action, applications in various stages, etc¹⁹. *Glycyrrhiza glabra* Linn. has anxiolytic, immunomodulatory, anti-oxidant, anti-stress and adaptogenic properties²⁰. Anti-inflammatory and antioxidant properties of *Glycyrrhiza glabra* Linn. may be contributing favorably to the memory enhancement effect²¹. In various phyto-chemical studies, *Glycyrrhiza glabra* Linn. has shown the presence of alkaloids, carbohydrates, glycosides, phytosterols, saponins, phenolic compounds, tannins, proteins, amino acids and flavonoids²².

Various studies have also shown the efficacy of *Glycyrrhiza glabra* Linn. in powder and cow milk combination. However, its use can be inconvenient for children and they may be less compliant. Thus, for present study a special formulation was developed consisting of *Glycyrrhiza glabra* Linn. and candy sugar, and cow milk was used as its adjuvant. The preparation of YK was completed using and analyzed as per traditional method mentioned in Ayurvedic Pharmacopeia of India (API)^{23,24}. The role of yoga in memory boosting as a lifestyle modification is well known and children can practice yoga too. Thus, the study was conducted to assess efficacy of their combined schedule in teenage students. Small sample size, purposive sampling and single centre study are the major limitations of the present study, which can be overcome by a large sample size, coverage of a larger geographical area and probability sampling techniques with a longer duration in future studies to draw more reliable conclusions.

5. Conclusion

The novel preparation YK (formulation of *Glycyrrhiza glabra* Linn.) as an herbal neuro-nutrient along with yoga can be safely used with children to enhance their memory and cognition. Further large-scale studies with

larger sample size are warranted to validate and confirm the clinical efficacy as well as the mode of action of *Yashtimadhu Kalpa*.

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