Combination of *Spirulina* with glycyrrhizin prevents cognitive dysfunction in aged obese rats

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**ABSTRACT**

**Objectives:** To evaluate the cognition enhancing effect of the combination of *Spirulina* and glycyrrhizin in monosodium glutamate (MSG)-induced obese aged rats.

**Materials and Methods:** Obesity was induced in rats by administration of MSG (intraperitoneally, 4 mg/g body weight) for 14 consecutive days from day 1 after birth. Subsequently, the animals were allowed to grow for 18 months with food and water *ad libitum*. Hypercholesterolemia, hyperglycemia, leptin resistance, were monitored in these animals. Cognitive status was assessed by Barne’s maze task and hippocampal acetylcholinesterase (AChE) levels. Further, the animals were treated with *Spirulina* (Sp) (oral route, 1 g/Kg body weight, for 30 days) alone or glycyrrhizin (Gly) alone (intraperitoneal route, 0.1 mg/Kg, on day 15 and day 21), or their combination (SpGly). Counting of the treatment days was done by considering first day of Sp administration as day 1. After the completion of 30 days of *Spirulina* treatment or 2 doses of Gly administration or the combination (SpGly) treatment, the animals were left for 3 weeks. They were then assessed for their biochemical and cognitive changes.

**Results:** The combination of Sp with Gly showed a significant reduction (*P* < 0.0001) in glucose, cholesterol, leptin levels in the serum with improvement in cognitive functions with concomitant reduction in AChE activity in the hippocampal tissue homogenates (*P* < 0.0001) of the obese rats.

**Conclusion:** SpGly combination has a potential role in reversing cognitive dysfunctions associated with aging and obesity.

**KEY WORDS:** Acetyl cholinesterase, cognition, glycyrrhizin, leptin, memory, obesity, *Spirulina*

**Introduction**

The global epidemic of obesity is rapidly evolving as one of the major economic and public health issues. A number of other associated diseases such as diabetes, hypertension, stroke, and myocardial infarction increase mortality and morbidity. Obesity can further accelerate the aging process with increased oxidative stress and leptin resistance.[1] Obesity is also a risk factor for cognitive decline with loss of memory in elderly humans.[2] Due to the undesirable side effects often encountered with most of the synthetic anti-obesity drugs,[3] efforts are being made in selecting natural products as safer alternatives. Encouraged with the findings documented in the scientific literature, we have chosen *Spirulina* (Sp) for its anti-oxidant function[4] and glycyrrhizin (Gly) for its anti-diabetic, anti-lipidemic properties.[5] Dietary supplementation with *Spirulina* has earlier been shown to ameliorate deterioration of memory, reduced oxidative stress damage, and augment the catalase activity in senescence accelerated 3-month-old mice.[6] In an independent study, Sp exhibited anti-hyperglycemic, anti-hyperlipidemic, and hepatoprotective activity and corrected the metabolic abnormalities seen in excessive fructose administered Wistar rats.[7] Anti-inflammatory and neuroprotective functions of Sp have recently been elucidated in *α*-synuclein models of Parkinson’s disease.[8] In another study, Gly ameliorated insulin resistance, hyperglycemia, dyslipidemia, and oxidative stress...
in fructose-induced metabolic syndrome rat model. More recently, the memory-enhancing activity of Gly has been shown in rodent models. Therefore, it is reasonable to expect that controlling obesity induced changes might reduce cognitive dysfunction. To test this hypothesis, first, we developed monosodium glutamate (MSG)-induced obese rats. We confirmed the cognitive dysfunction by measuring the acetylcholinesterase (AChE) levels in the hippocampal extracts and learning and memory assessment by Barne’s maze task. We used this experimental design for further studies. The study aims to evaluate both metabolic abnormalities and deterioration of memory in the same animal model. Accordingly, we analyzed the effects of Sp and Gly individually and in combination in reversing obesity and the consequent cognitive dysfunctions in the MSG-induced obese rats.

Materials and Methods

Materials

Monosodium L-glutamate (MSG), 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholine (ATC), glucose oxidase, peroxidase, Cholesterol standard, 4-aminophenazone, thiobarbituric acid (TBA), bovine serum albumin (BSA), glycyrrhizin were purchased from Sigma-Aldrich, Bangalore. Spirulina was purchased from Parry Nutraceuticals, Chennai. Enzyme-linked immunosorbent assay (ELISA) kit for estimation of rat serum leptin levels was purchased from SPI bio Bertin Pharma, France. All other reagents used were of analytical grade and obtained locally.

Animals and Experimental Protocols

Male Sprague Dawley rat pups were chosen for the study. They were maintained under controlled temperature and light. Neonatal rat pups were injected with MSG (4 mg/g body weight) once daily for 14 consecutive days after birth through intraperitoneal route. Rats of the same age and strain, receiving saline served as control. After 14 days of drug administration, the rats were allowed to grow for 18 months with ad libitum access to pellet food and water. Animals were divided into following seven groups (n = 6): Group I included 18-month-old, untreated control rats (CM). Group II contained control rats administered with Sp (oral route, 1 g/Kg body weight, for 30 days) (CM Sp). Group III control rats received glycyrrhizin (intraperitoneal route, 0.1 mg/Kg, injected on day 15 and day 21) (CM Gly). MSG-treated 18-month-old rats served as Group IV (MSG). Group V (MSG Sp) and Group VI (MSG Gly) received Sp and Gly, respectively, at the concentrations mentioned above. Group VII (MSG SpGly) received a combination of Sp and Gly as detailed above. Counting of the treatment days was done by considering first day of Sp administration as day 1. After the completion of 30 days of Sp treatment or 2 doses of Gly administration or the combination (SpGly) treatment, the animals were left for 3 weeks. They were then were assessed for their biochemical and cognitive changes.

After completing the behavioral assessment trials by Barnes maze task, the animals were sacrificed by cervical dislocation. Approximately 1 ml of blood was collected from each animal through cardiac puncture, serum separated and stored at −20°C until analysis. The brain was quickly removed, hippocampi dissected, weighed, and stored frozen at −20°C for further analysis. All procedures were approved by the Institutional Animal Ethics Committee (CPCSEA registration no. of the Institute: Reg. No. 12/GO/ac/99/CPCSEA; IAEC Approval no. AEC/43/258/NC dated Apr 21, 2011).

Behavioral Assessment

Before sacrifice, subjects underwent testing in the Barnes maze task to evaluate the spatial learning and memory. Each rat was assigned an escape hole number; assigned hole numbers were alternated across rats by 90 (i.e., hole positions 3, 6, 9, and 12) to eliminate odour cues for consecutively tested rats. The escape box location remained constant for any individual rat across test trials. Behavioral testing consisted of two shaping trials on the first day, and 15 evaluation trials (3 trials/day) over a period of 5 days. Each day the animals were transferred from their cage room to the testing room 30 min prior to the start of testing; a trial began by placing the rat under a black, opaque starting box positioned in the center of the platform. After 60 seconds, the box was lifted and the rat had a maximum of 2 min (120 seconds) to find and enter the escape box. Latency (time taken by the rat to find the escape box) and total errors (nose pokes into non-escape holes) were recorded. If the rat did not find the escape box within 2 min, it was gently guided there by the experimenter’s hand. After 30 seconds, the rat was removed from the escape box and returned to its home cage. The platform and escape box were cleaned after every trial with 20% ethanol solution. After the fifth day, testing abated for 5 days, after which retention was evaluated for three additional trials (1 day), conducted as described above.

Analysis of the Serum Parameters

Total cholesterol was estimated by the method of Zlalkis et al. Serum glucose was measured by the method of Trinder et al. Levels of leptin in the serum were measured by sandwich ELISA method as per manufacturer’s instructions. The sensitivity of the assay for leptin was 2 ng/ml and the detection range was 2–10 ng/ml. Thiobarbituric acid reactive substances (TBARS) were estimated by spectrophotometric method. The concentration of TBARS was expressed in terms of nmol of malondialdehyde/ml of serum.

Measurement of AChE Activity

Hippocampi were dissected and homogenized in ice-cold 50 mMTris–HCl buffer, pH 7.4 containing 150 mMNaCl, 2 mM EDTA, 1 mM PMSE and 0.5% Triton X-100, followed by sonication (10 seconds × 2 cycles). Homogenates were then centrifuged at 13 000 × g for 20 min. The clear extracts obtained were used for estimation of total protein content by using bovine serum albumin as standard.

The esterase activity was measured in the hippocampal extract by using an artificial substrate, acetyltiocholine (ATC). Thiococholine released because of the cleavage of ATC by AChE was allowed to react with the -SH reagent 5, 5-dithiobis-2-nitrobenzoic acid, which was reduced to thionitrobenzoic acid, a yellow colored anion with an absorption maxima at 412 nm. All samples were run in triplicate and the enzyme activity was expressed as μ mol ATC/h/mg protein.

Statistical Analysis

The data were expressed as mean ± standard error of mean (SEM), and differences between groups were analyzed by repeated measures analysis of variance (ANOVA), followed by Tukey’s multiple comparisons using GraphPad Version 5 (Prizm;
GraphPad Software Inc, San Diego, California, USA). Correlation analysis using Pearson’s correlation coefficient was performed. For analysis of memory acquisition in the Barnes maze, the area under the latency curve (AUC) was calculated for each animal and group comparisons were performed by two-way ANOVA. Probability values less than 0.05 were considered statistically significant.

**Results**

Effects on Body Weight, Serum Glucose, and Cholesterol Levels

The effects of neonatal administration of MSG in 18-month-old rats are summarized in Table 1 along with age-matched saline-treated control rats. The effects of Sp, Gly, and their combination on body weights, serum glucose, serum cholesterol, and leptin levels are shown in Table 2. The treatments, either alone or in combination, showed marginal reduction in body weight and body mass index (BMI), with MSG Sp exhibiting 3.2% reduction in body weight and the MSG Gly group reduced the body weight by 7.4%. However, the combination of Sp and Gly significantly reduced serum glucose (*P* < 0.0001), cholesterol (*P* < 0.0001), and leptin levels (40%) in comparison to the elevated levels of these three parameters seen in the serum of MSG group. There was significant elevation in TBARS content (*P* < 0.0001) in MSG group, as compared to CM group [Table 2]. The change in TBARS content was significantly (*P* < 0.0001) reversed by combination of SpGly treatment in MSG SpGly group.

**Effects on Hippocampal AChE Activity**

The AChE activity in the hippocampus was not attenuated significantly in MSG Gly group but increased significantly (*P* < 0.0001) in MSG group, compared to CM group. The combination of Sp with Gly significantly (*P* < 0.0001) attenuated its activity in MSG SpGly group, compared to MSG group [Table 2].

**Relation Between Leptin Levels with Liver and Hippocampal Weights**

There was a significant difference (*P* < 0.0001) in the serum leptin levels between the MSG group (7.7 ± 0.67 ng/ml) and CM group (2.08 ± 0.15 ng/ml). A significant positive correlation was found between serum leptin levels and liver [Figure 1] (*r* = 0.92; *P* < 0.0001) weights in the MSG vs. CM groups. Serum leptin levels showed strongly inverse correlation with hippocampal weights (*r* = −0.96, *P* < 0.0001).

**Acquisition**

Barnes circular maze test, a hippocampus-dependent cognitive task that requires spatial reference memory was followed for establishment of cognitive status.[17] All rats showed decreased latencies and errors across trials. The data

Table 1:

Effects observed in adult (18-month-old) male rats as a consequence of monosodium glutamate administration during neonatal stages

| Parameter                  | Control rats | MSG induced obese rats |
|----------------------------|--------------|------------------------|
| Weight (gm)                | 345±8.6      | 451±13.5**             |
| Nasaonal length (cm)       | 24.67±0.56   | 18.57±0.53**           |
| BMI                        | 0.57±0.014   | 1.2±0.038***           |
| Serum glucose (mg/dl)      | 122.5±8.4    | 313±10.8***            |
| Serum cholesterol (mg/dl)  | 196.1±6.9    | 320.5±6.1**            |
| Serum lepton (ng/ml)       | 2.0±0.15     | 7.7±0.67***            |
| TBARS (nmol of MDA/ml serum)| 1.7±0.18    | 5.3±0.29***            |
| Liver weights (g)          | 6.18±0.12    | 8.33±2.05***           |
| Hippocampi weights (mg)    | 86.5±9.6     | 46.76±0.68**           |
| AChE activity (μ mol)      | 4±0.73       | 32.16±1.53***          |
| substrate/h/mg protein     |              |                       |
| Spatial disorientation score | 1.36±0.21   | 5.53±0.40***           |

Body mass index (BMI) calculated as a ratio of the animal weight (g) and the square of the nasaonal length (cm). *P* < 0.05, **P* < 0.001, and ***P* < 0.0001 compared to age matched control males (CM). Results expressed as mean ± standard error of mean. The differences were analysed by unpaired t-test. Spatial disorientation score is defined by the number of wrong holes searched before identifying the escape tunnel. MSG=Monosodium glutamate, TBARS=Thiobarbituric acid reactive substances, AChE=Acetylcholinesterase

Table 2:

Effects of spirulina, glycyrrhizin, and their combination on the developed 18-month-old MSG-induced obese male rats

| Parameters                     | CM                | CM Sp               | CM Gly              | MSG                | MSG Sp               | MSG Gly              | MSG SpGly            |
|-------------------------------|-------------------|---------------------|---------------------|--------------------|---------------------|---------------------|----------------------|
| Weight (gm)                   | 341.3±3.35        | 336.5±0.13          | 342.16±10.49        | 451.6±6.13**       | 428.16±11.40        | 418.5±26.02          | 409.8±12.3           |
| Nasaonal length (cm)          | 24.67±0.53        | 24.53±0.49          | 18.69±0.51          | 18.56±0.53***      | 18.34±0.51          | 18.68±0.58           | 18.52±0.53           |
| BMI                           | 0.55±0.013        | 0.55±0.015          | 0.56±0.016          | 1.19±0.04**        | 1.12±0.014          | 1.09±0.014           | 1.09±0.01            |
| Serum glucose (mg/dl)         | 122.5±8.4         | 12.16±8.98          | 125±8.85            | 313.33±10.85**     | 176.6±9.88**        | 306.16±9.05          | 184.16±34.65**       |
| Serum cholesterol (mg/dl)     | 196.1±6.9         | 193.50±4.89         | 176.16±7.87         | 320.5±6.18**       | 277.33±7.63**       | 177±5.01**           | 194.66±21.41**       |
| Serum lepton (ng/ml)          | 2.08±0.15         | 2.90±0.34           | 2.14±0.12           | 7.7±0.61**         | 7.6±0.42            | 5.7±0.42             | 3.1±0.26**           |
| TBARS (nmol of MDA/ml serum)  | 1.75±0.18         | 1.9±0.28            | 1.46±0.14           | 5.3±0.29**         | 2.76±0.23**         | 2.8±0.14**           | 2.0±0.16**           |
| Liver weights (gm)            | 6.18±0.12         | 6.11±0.22           | 6.16±0.21           | 8.33±0.25**        | 7.15±0.99**         | 7.13±0.15            | 6.38±0.24**          |
| Hippocampi weights (mg)       | 86.5±1.52         | 84.83±1.60          | 86.1±1.42           | 46.83±0.69**       | 53.6±2.92           | 54.8±3.29            | 58.16±1.30**         |
| AChE activity (μ mol)         | 4±0.73            | 5±0.96              | 5.16±0.60           | 32.16±1.53**       | 17.6±1.40**         | 31.3±1.85            | 10.66±0.27**         |
| Spatial disorientation score  | 1.36±0.21         | 2.56±1.09           | 2.93±0.26           | 5.53±0.40**        | 3.9±0.13            | 3.3±0.26             | 1.5±0.21**           |

Data are presented as mean ± standard error of mean (SEM). *P* < 0.05, **P* < 0.001, and ***P* < 0.0001 compared to MSG males (MSG). #P* < 0.05, ##P* < 0.001, and ###P* < 0.0001 compared to control males (CM). Each value represents means±SEM by repeated measures analysis of variance (ANOVA) followed by tukey’s multiple comparisons test. MSG=Monosodium glutamate, TBARS=Thiobarbituric acid reactive substances, AChE=Acetylcholinesterase, BMI=Body mass index

References:

1. Madhavadas S, Subramanian S. Spirulina with glycyrrhizin improves cognition in rats. Indian J Pharmacol. 2015 Feb;47(1):41.
was analysed using repeated measures ANOVA. Although the latencies to reach the escape hole decreased gradually in all the groups during 5 days of training in Barnes maze test, the mean latency (days 2–5) was significantly ($P < 0.0001$) prolonged in MSG group, as compared to CM group, showing a poorer learning [Table 3]. This was significantly ($P < 0.0001$) improved by the treatment of Sp with Gly combination in MSG SpGly group. There was a significant reduction in number of errors ($P < 0.05$) [Table 4] for MSG vs. MSG Sp and MSG vs. MSG Gly group and a significant reduction of errors ($P < 0.0001$) for MSG vs. MSG SpGly group [Table 4].

**Long-term Retention**

Memory retention was assessed after 5 days and the data were analyzed using repeated-measures ANOVA. There was significant increase in errors ($P < 0.001$) made by MSG group compared to CM group. There was no significant reduction in the number of errors made by MSG Sp and MSG Gly groups. Interestingly, MSG SpGly group showed a significant reduction in errors ($P < 0.001$) compared to MSG group. Area under the curve was calculated for each group to assess the time taken to reach the escape box in Barne’s maze and as revealed in Figure 2, the MSG group exhibited significant alteration in memory retention and had the difficulty in locating the escape box. It is worthwhile to note that administration of SpGly combination had a dramatic effect in minimizing the memory impairment.

**Discussion**

The association of obesity with chronic diseases, such as type 2 diabetes and metabolic syndrome is well-established. Obesity also accelerates cellular processes similar to aging such as oxidative stress and disturbance in homeostatic pathways. Another important obesity-induced aging is the development of resistance to certain hormones like leptin, which further triggers type 2 diabetes and inappropriate fat distribution. What has recently emerged is the association of obesity with cognitive decline[18] and the possible mechanisms have been

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**Table 3: Latency exhibited by the experimental rats during acquisition and retention in Barne’s maze task**

| Latency (sec) | CM | CM Sp | CM Gly | MSG | MSG Sp | MSG Gly | MSG Sp+Gly |
|--------------|----|-------|--------|-----|--------|---------|-----------|
| Day 1        | 50.58±9.55 | 97.00±3.60*** | 59.06±11.77 | 114.40±2.28*** | 107.56±4.84 | 100.16±3.49 | 72.51±5.36** |
| Day 2        | 24.90±1.11 | 64.13±4.02*** | 28.75±1.62 | 108.03±3.78*** | 105.85±5.38 | 90.56±4.79 | 59.78±5.46*** |
| Day 3        | 14.02±1.22 | 19.36±1.08 | 14.48±1.97 | 83.96±15.03*** | 92.58±5.64 | 79.50±3.32 | 38.96±6.26*** |
| Day 4        | 13.75±1.07 | 12.06±1.47 | 14.20±0.94 | 92.11±5.27*** | 82.96±5.36 | 71.36±6.33 | 24.41±3.32*** |
| Day 5        | 12.11±1.61 | 11.25±0.97 | 12.68±1.5 | 86.31±5.06*** | 72.23±3.45 | 71.00±4.68 | 14.46±2.62*** |
| Retention    | 9.33±0.11 | 9.05±0.43 | 20.06±0.58 | 114.3±17.04 | 75.28±14.90 | 77.39±14.90 | 16.67±0.94*** |

Data represented as mean±standard error of mean (SEM) * $P<0.05$, **$P<0.001$, and ***$P<0.0001$ compared to CG males (CM). Each value represents mean±SEM (n=6) by repeated measures analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test.

**Table 4: Errors made by the experimental rats during acquisition and retention in Barne’s maze task**

| Errors | CM | CM Sp | CM Gly | MSG | MSG Sp | MSG Gly | MSG Sp+Gly |
|--------|----|-------|--------|-----|--------|---------|-----------|
| Day 1  | 4.63±0.44 | 7.75±0.73** | 5.83±1.31 | 10.10±0.94** | 8.86±0.43 | 10.18±0.30 | 4.86±0.37*** |
| Day 2  | 4.13±0.65 | 3.95±0.56 | 4.21±0.90 | 8.18±1.52** | 6.45±0.26 | 6.63±0.36 | 4.25±0.23** |
| Day 3  | 2.86±0.26 | 3.46±0.42 | 3.88±0.43 | 7.51±0.51*** | 5.91±0.77 | 5.91±0.77 | 3.98±0.14** |
| Day 4  | 2.01±0.19 | 2.4±0.34 | 2.87±0.27 | 7.08±0.90*** | 6.50±0.80 | 6.66±0.81 | 3.75±0.12** |
| Day 5  | 2.08±0.28 | 2.2±0.23 | 2.4±0.06 | 6.66±0.85*** | 6.13±0.59 | 6.28±0.60 | 2.36±0.13*** |
| Retention | 1.38±0.05 | 2.77±0.38 | 2.94±0.53 | 5.55±0.64** | 5.56±0.75 | 3.88±1.65 | 1.55±0.22* |

Data represented as mean±standard error of mean (SEM) * $P<0.05$, **$P<0.001$, and ***$P<0.0001$ compared to CG males (CM). Each value represents mean±SEM (n=6) by repeated measures analysis of variance (ANOVA) followed by Tukey’s multiple comparisons test.

MSG=Monosodium glutamate
Cholesterol, leptin, TBARS confirming the establishment of increased BMI along with hyperglycemic status, increased biochemical changes were monitored 3 weeks after treatment. Than 2 months (data not shown) and, hence, the cognitive and best after 2–3 weeks of gap between administration of Sp and Gly, have shown that restoration of cognitive functions were at their weight of rats. Preliminary investigations from our laboratory conducted the current animal studies with 0.1 mg of Gly/Kg body weight of rats. Since the acceptable daily intake of Gly is 0.2 mg/Kg,[25] we have explored using in vivo models. The animal studies have shown that high fructose diet induced obesity led to smaller volumes of hippocampus, the brain region critical for learning and memory, with reduced hippocampal synaptic plasticity which may affect memory and cognition.[19,20]

ACh is a key neurotransmitter in hippocampal cholinergic transmission and the magnitude of change in ACh output is positively related with spatial memory performance. AChE, one of the important cholinergic makers, terminates synaptic transmission at cholinergic synapses by rapidly hydrolyzing ACh.[21,22] In the present study, the increased AChE activity in the hippocampus in the MSG group is suggestive of the dysfunction of cholinergic system, which has resulted in alterations in the behavioural parameters. However, the underlying mechanism for the association between dysfunctional cholinergic system and behavioral deficits needs further investigation.

Spirulina maxima, a cyanobacterium, apart from being a rich source of protein and vitamins, contains several therapeutically active compounds with biomodulatory functions.[23,24] The other compound tested in the present study is glycyrrhizin, a triterpenoidsaponin, is naturally extracted from the roots of licorice plants. At low appropriate doses, Gly exhibits anti-diabetic, antioxidant, anti-microbial, anti-viral, anti-cancerous, and anti-inflammatory properties.[25] At high doses, Gly can cause hypokalemia, hypertension, and hyperaldosteronism and thrombocytopenia.[24] Despite the side effects of Gly, its therapeutic potential cannot be ignored. Since the acceptable daily intake of Gly is 0.2 mg/Kg,[25] we have conducted the current animal studies with 0.1 mg of Gly/Kg body weight of rats. Preliminary investigations from our laboratory have shown that restoration of cognitive functions were at their best after 2–3 weeks of gap between administration of Sp and Gly and the extent of restoration did not change further for more than 2 months (data not shown) and, hence, the cognitive and biochemical changes were monitored 3 weeks after treatment.

In our study, as anticipated, MSG-treated animals exhibited increased BMI along with hyperglycemic status, increased cholesterol, leptin, TBARS confirming the establishment of obesity. Reduced hippocampal weights with enhanced AChE activity and significant disorientation in the spatial memory test in MSG-treated rats confirm the cognitive impairment in them. In terms of therapeutic functions, administration of Sp alone exhibited anti-diabetic feature without any major influence on cognitive status. Administration of Gly alone, though had a minimal influence on lowering the elevated serum glucose levels, significantly reduced the cholesterol and leptin levels. On lines similar to the observations made with Sp, Gly alone did not correct the hippocampus associated changes in terms of the hippocampal weight, elevated AChE levels, and the cognitive impairment seen in MSG-treated rats.

It is noteworthy that the combination of Sp and Gly, while reversing the obesity-related serum parameters, restored the cognitive functions with concomitant reduction in AChE levels in the hippocampal extracts. The exact mechanism of cognition enhancing effects of Sp and Gly together, not singly, merits further studies. It is possible that the interaction of Gly with certain compounds from Sp offer a new therapeutic potential for treatment of disorders associated with deficits in learning and memory, including the Alzheimer’s disease.

References

1. Carter S, Caron A, Richard D, Picard F. Role of leptin resistance in the development of obesity in older patients. Clin Interv Aging 2013;8:829-44.
2. Zeki Al Hazzouri A, Stone KL, Haan MN, Yaffe K. Leptin, mild cognitive impairment, and dementia among elderly women. J Gerontol A Biol Sci Med Sci 2013;68:175-80.
3. Ahmad FA, Mahmud S. Acute pancreatitis following orlistat therapy: Report of two cases. JOP 2010;11:61-3.
4. Hwang JH, Lee IT, Jeng KC, Wang MF, Hou RC, Wu SM, et al. Spirulina prevents memory dysfunction, reduces oxidative stress damage and augments antioxidant activity in senescence accelerated mice. J Nutr Sci Vitaminol (Tokyo) 2011;57:186-91.
5. Ming LJ, Yin AC. Therapeutic effects of glycyrhizic acid. Nat Prod Commun 2013;8:415-8.
6. Janouilova U, Zacharia AJ, Kumar P, Bisen PS, Prasad GB. Alleviation of metabolic abnormalities induced by excessive fructose administration in Wistar rats by Spirulina maxima. Indian J Med Res 2012;135:422-8.
7. Fahoon MM, Jernberg JN, Morganti J, Jemperas J, Hudson CE, Klein RL, et al. A Spirulina enhanced diet provides neuroprotection in an α-synuclein model of Parkinson’s disease. PLoS One 2012;7:e45256.
8. Sil R, Ray D, Chakraborti AS. Glycyrrhizin ameliorates insulin resistance, hyperglycemia, dyslipidaemia and oxidative stress in fructose – induced metabolic syndrome – X in rat model. Indian J Exp Biol 2013;51:129-38.
9. Chakravarthi KK, Avadhani R. Beneficial effect of aqueous root extract of Glycyrrhiza glabra on learning and memory using different behavioural models: An experimental study. J Nat Sci Biol Med 2013;4:420-5.
10. Madhavadas S, Kutty BM, Subramanian S. Amyloid β lowering and cognition enhancing effects of gheeerin receptor analog [D-Lys (5)] GHPR-6 in rat model of obesity. Indian J Biochem Biophys 2014;51:257-62.
11. Barnes CA. Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. J Comp Physiol Psychol 1979;93:74-104.
12. Zlalkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953;41:486-92.
13. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem 1969;6:24.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobabituric acid reaction. Anal Biochem 1979;85:351-8.
15. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
16. Ellman GL, Courtney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. Cell 1995;81:905-15.
18. Dahl AK, Hassing LB. Obesity and cognitive aging. Epidemiol Rev 2012.
19. Farr SA, Yamada KA, Butterfield DA, Abdul HM, Xu L, Miller NE, et al. Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 2008;149:2628-36.
20. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 2008;18:1085-8.
21. Pepeu G, Giovannini MG. Cholinesterase inhibitors and memory. Chem Biol Interact 2010;187:403-8.
22. Muthuraju S, Maili P, Patil S, Solanki P, Sharma AK, Singh SB, et al. Role of cholinergic markers on memory function of rats exposed to hypobaric hypoxia. Eur J Pharmacol 2011;672:96-105.

23. Khan Z, Bhadouria P, Bisen PS. Nutritional and therapeutic potential of Spirulina. Curr Pharm Biotechnol 2005;6:373-9.
24. Celik MM, Karakus A, Zeren C, Demir M, Bayarogullari H, Duru M, et al. Licorice induced hypokalemia, edema and thrombocytopenia. Hum Exp Toxicol 2012;31:1295-8.
25. van Gelderen CE, Bijtsma JA, van Dokkum W, Savelkoul TJ. Glycyrrhizic acid: The assessment of a no effect level. Hum Exp Toxicol 2000;19:434-9.

Cite this article as: Madhavadas S, Subramanian S. Combination of Spirulina with glycyrrhizin prevents cognitive dysfunction in aged obese rats. Indian J Pharmacol 2015;47:39-44.

Source of Support: Nil. Conflict of Interest: No.