EFFICACY OF GLYCYRRHIZA GLABRA LINN. (YASTIMADHU) IN LEARNING, MEMORY AND COGNITIVE ACTIVITY –CURRENT FINDINGS AND FUTURE AVENUES

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ABSTRACT

Glycyrrhiza glabra Linn, a novel drug with several well known medicinal properties, has been reviewed here for current findings with regards to its efficacy in improving learning, memory and cognitive activity. A methodical review has been conducted on pharmacological, clinical study articles and dissertation works published from 2004 to July 2008, using PubMed and manual search of bibliographies as source. The pharmacological researches indicate that Glycyrrhiza glabra Linn shows improvement in memory, learning and spatial memory retention enhancement effects in vivo via multiple mechanisms of action, a clinical study on the synthetic derivative of glycyrrhetinic acid shows promising result in improving cognitive functions, enhancement of Medha (Intellect and Memory) was also observed in 2 dissertation works based on the properties of the drug. The present review is on some of the relevant studies on Glycyrrhiza glabra Linn wherein the methods employed, results obtained and mechanism of action of the drug have been explained. The review also emphasizes on the future thrust areas of research upon this drug on the same background.

Keywords: Glycyrrhiza glabra Linn, Learning, Memory, Cognitive activity, Medha
INTRODUCTION

Plenty of plants in traditional medicine are being used in age-related brain disorders for improvement of memory and cognitive function.\(^1\) Glycyrrhiza glabra Linn is one among them and is known as “Yastimadhu” in the traditional Indian system of medicine “Ayurveda”. This traditionally much valued plant is one among the four herbs mentioned in ‘Charaka Samhitha’ – An Ancient Indian Medical treatise, as “Medhya Rasayana” (drug promoting intellect and memory).\(^4\)

Even though Yastimadhu has been praised as an effective Medhya drug and mentioned as second best Medhya rasayana,\(^4\) very minimal works have been carried out in the field of Ayurvedic research owing to the above activity and lots are to be still done. With an aim to re-establish and interpret the Medhya property of Yastimadhu, a methodical collection of works done pertaining to the above said properties on pharmacological and clinical background has been done here.

MATERIALS

Glycyrrhiza glabra Linn of Fabaceae family (Photo slides 1 & 2) is a perennial plant, grows up to more than 1 m in height, erect, with highly developed stoloniferous roots. Leaves compound, 9–17 alternate imparipinnate leaflets, oblong to elliptical-lanceolate, acute or obtuse; racemes loose, shorter than the leaves or a little longer. Flowers 1 cm long, flat pods oblong to linear, 1–3 cm long by 6mm wide, more or less densely echinate glandular, many seeded or abbreviated 2- or 3-seeded.\(^5,6\) The commercial name of the dried rhizome and root of the plant is liquorice.\(^7\)

Roots and Rhizome are the officinal part, which are nearly cylindrical pieces, up to 1m long and 5–20 mm in diameter; externally, the bark is brownish grey to dark brown, longitudinally wrinkled, occasionally bearing small dark buds in rhizomes or small circular or transverse rootlet-scars in roots. The peeled root is yellow, smooth, fibrous, finely striated; fracture fibrous in the bark and splintery in the wood; internally, bright yellow. A distinct cambium ring separates the yellowish grey bark from the finely radiate yellow wood; central pith, only in rhizomes.\(^5,8,9\)

Photo slides 1 & 2: Plant and roots of Glycyrrhiza glabra Linn

Source of Photographs:
The main compounds accountable to bring about the pharmacological effects of G. glabra are Saponins, Flavonoids, Alkaloids, Tannins, Steroids, etc. The major constituents are triterpene saponins. Glycyrrhizin is generally regarded as the active principle of G. glabra and is responsible for its sweetness, which is 50 times more than that of sucrose. 

Glycyrrhizin (glycyrrhizic acid, glycyrrhizinic acid) is the major component (2–9%); minor components occur in proportions that vary depending on the species and geographical location. [11-13] Glycyrrhizin occurs as a mixture of potassium and calcium salts. [14] It is a monodesmoside, which on hydrolysis releases two molecules of D-glucuronic acid and the aglycone glycyrrhetic (glycyrrhetinic) acid (enoxolone). [15]

Properties: [16]
Rasa (Taste) : Madhura (Sweet)
Guna (Quality) : Guru (Heavy), Snigdha (oily)
Virya (Potency) : Sheetha (Cold)
Vipaka : Madhura (Sweet)
Karma (Action) : Balya (Immune booster), Cakshushya (Vision promotor), Varnya (Complexion promoter), Vatapittajit (Reduces vitiated Vata and pitta humors), Raktaprasadana (enhancing the qualities of blood)
Prabhava (Special Action) : Medhya (drug promoting intellect and memory)

Pharmacokinetics: Glycyrrhizic acid, the main constituent of licorice after oral administration, is hydrolyzed to glycyrrhetic acid by intestinal bacteria possessing a specialized β-glucuronidase. [9, 17] After oral administration, glycyrrhetic acid is rapidly absorbed and transported via carrier molecules to the liver. In the liver it is metabolized to glucuronide and sulfate conjugates, which are subsequently rehydrolyzed to glycyrrhetic acid. Glycyrrhetic acid is then reabsorbed, resulting in a significant delay in terminal clearance from plasma. [18] After oral administration of 100 mg glycyrrhizin (Fig 1.) in healthy volunteers, no glycyrrhizin was found in the plasma but glycyrrhetic acid was found at < 200 ng/ml. In the 24-hour period after oral administration, glycyrrhizin was found in the urine, suggesting that it is partly absorbed as an intact molecule. [15]

Dosage: Unless and otherwise prescribed, average daily dose of crude plant material is 5–15 g, corresponding to 200–800 mg of glycyrrhizin and should not be used for longer than 4–6 weeks without medical advice. [19] Doses of other preparations should be calculated accordingly. [19]

Adverse reactions: No adverse reactions have been associated with the drug when used within the recommended dosage and treatment period. Prolonged use (6 weeks) of excessive doses (>50 g/day) can lead to pseudoaldosteronism, which includes potassium depletion, sodium retention, oedema, hypertension, and weight gain. [14, 20, 21] In rare cases, myoglobinuria and myopathy can occur. [22]

Toxicity: The acute toxicities of licorice extract and glycyrrhizins salts are low with oral LD₅₀s generally greater than 4 gm glycyrrhizinate/kg b.w in mice and rats. Other in vivo studies reported no teratogenic effects when glycyrrhizin salts were administered maternally to mice, rats, hamsters, or rabbits during gestation at doses as high as 1000 mg/kg/day. However, dominant lethal testing in male rats suggests that an intake of 4000–5000 mg glycyrrhizin/kg/day could lead to mutagenic effects in offspring. Microbial tests found that licorice extract and glycyrrhizates were nongenotoxic and had some anti-genotoxic properties. There is abundant evidence from both case reports and clinical studies that the habitual consumption of glycyrrhizin results in adverse effects marked by the development of pseudohypercorticosteroidism. [23]
Current Pharmacological findings on Learning, Memory:

(1) Memory strengthening activity of *Glycyrrhiza glabra* Linn in exteroceptive and interoceptive behavioral models [24]:- Aqueous extract of *G. glabra* was administered for 7 successive days in separate groups of mice in three doses (75, 150, and 300 mg/kg p.o.). An exteroceptive and interoceptive behavioral models of memory was used. The dose of 150mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice. Furthermore, this dose reversed the amnesia induced by diazepam (1 mg/kg i.p.), scopolamine (0.4 mg/kg i.p.), and ethanol (1 g/kg i.p.). Anti-inflammatory and antioxidant properties of liquorice may be contributing favorably to the memory enhancement effect. Since scopolamine-induced amnesia was reversed by liquorice, it is possible that the beneficial effect on learning and memory may be because of facilitation of cholinergic transmission in brain.

(2) Memory enhancing activity of *Glycyrrhiza glabra* Linn in mice [25]:- With the same methodology as the above, ‘Elevated plus-maze’ and ‘passive avoidance paradigm’ were employed to test learning and memory. The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice.

(3) The central cholinergic pathways play a prominent role in the learning and memory processes. Acetyl cholinesterase is an enzyme that inactivates acetylcholine. In a study undertaken to evaluate the “Comparative brain cholinesterase-inhibiting activity of *Glycyrrhiza glabra* Linn, *Myristica fragrans*, Ascorbic acid, and Metrifonate in mice,” [24] Aqueous extract of *G. glabra* (150 mg/kg p.o. for 7 successive days), n-hexane extract of *Myristica fragrans* seeds (5mg/kg p.o. for 3 successive days), ascorbic acid (60 mg/kg i.p. for 3 successive days), and metrifonate (50 mg/kg i.p.) were administered to young male Swiss albino mice. Acetyl cholinesterase enzyme was estimated in brains of mice. *Glycyrrhiza glabra, Myristica fragrans, Ascorbic acid, and Metrifonate* significantly decreased acetyl cholinesterase activity as compared to their respective vehicle-treated control groups.

(4) Spatial memory retention in rats [27]:- Aqueous extract (GE) of *G. glabra* (Fabaceae) was administered systemically to rats and the time course of the effects on the spatial memory retention in the Morris water maze was studied. The dose of *glycyrrhizin* (GL), i.e., 0.5, 2.5 and 5 mg/ml in daily water intake of GE was administered to three groups of rats. The first, second and third groups received GE for 1, 2 and 4 weeks, respectively (each group included 3 subgroups). After terminating the treatments, all animals were trained for four days; each day included one block and each block contained four trials. Test trials were conducted 48 h after the completion of the training period. GE treatment decreased both escape latency and travelled distance, suggesting significant spatial memory retention enhancement by GE.

(5) Effect of glabridin on learning and memory in mice [28]:- *Glabridin* (Fig 2) was isolated from the roots of *G. glabra* and its effects on cognitive functions and cholinesterase activity were investigated in mice. *Glabridin* (1, 2 and 4 mg kg^-1^, P. O.) and *piracetam* (400 mg kg^-1^, i. p.), a clinically used nootropic agent, were administered daily for 3 successive days to different groups of mice. The higher doses (2 and 4 mg kg^-1^, P. O.) of *glabridin* and *piracetam* significantly antagonized the amnesia induced by scopolamine (0.5 mg kg^-1^, I. P.) in elevated plus maze test and passive avoidance test. Furthermore, both *glabridin* (2 and 4 mg kg^-1^, P. O.) and *metrifonate* (50 mg kg^-1^, I. P. used as a standard drug), both remarkably reduced the brain cholinesterase activity in mice compared to the control group.
Fig 1: Glycyrrhizin

Fig 2: Glabridin

Fig 3: Carbenoxolon

Clinical studies on cognitive activity of Glycyrrhiza glabra Linn:

(1) Two randomized, double-blind, placebo-controlled crossover studies\textsuperscript{[29]} were conducted on Carbenoxolone (Fig 3) a synthetic derivative of glycyrrhetinic acid upon healthy volunteers and type 2 diabetic. 11β-HSD inhibitor carbenoxolone was administered 100 mg, orally, three times per day for 4 weeks with placebo in ten healthy volunteers. During each phase, subjects also received amiloride (10 mg/day) to prevent renal mineralocorticoid excess.\textsuperscript{[30]} The two phases were separated by an 8-week washout period.

Twelve subjects with stable type 2 diabetes were enrolled. In this study the subjects were administered with 11β-HSD inhibitor carbenoxolone 100 mg, orally, three times per day for 6 weeks with placebo. During each phase, subjects also received amiloride (10 mg/day) to prevent renal mineralocorticoid excess. The two phases were separated by a 12-week washout period. Cognitive ability was assessed with the help of various tools in a similar manner for both healthy volunteers and type 2 diabetes subjects. Nonverbal reasoning was evaluated with Raven's Standard Progressive Matrices by using the number correct in 20 min.\textsuperscript{[31]} Verbal fluency, thought to assess executive function and semantic memory, was assessed with the Controlled Word Association Test.\textsuperscript{[32]} Verbal memory was evaluated with a test of list-learning, the Rey Auditory-Verbal Learning Test (AVLT)\textsuperscript{[32]} and paragraph recall, with the Logical Memory (immediate and 30-min-delayed) subtest of the Wechsler Memory Scale-Revised.\textsuperscript{[33]} Visuo-spatial memory was evaluated with the Visual Reproduction (immediate and 30-min-delayed) subtest of the Wechsler Memory Scale-Revised.\textsuperscript{[33]} Attention and processing speed were evaluated with the Digit-Symbol Substitution Test from the Wechsler Adult
Intelligence Scale-Revised. [34] Prior general cognitive ability was assessed with the National Adult Reading Test. [35] Mood was assessed with the University of Wales Institute of Science and Technology (UWIST)-Mood Adjective Checklist (MACL) [36] and the Hospital Anxiety and Depression Scale (HADS). [37] The result showed improved verbal fluency ($P < 0.01$) after 4 weeks in 10 healthy elderly men (aged 55-75 year) and improved verbal memory ($P < 0.01$) after 6 weeks in 12 patients with type 2 diabetes (52–70 year).

(2) In a dissertation work, “A study on the effect of Medhya Rasayana in school going children,” [38] 11 school going children were administered with one tablet of 500 mg containing equal quantity of Guduchi (Tinospora cordifolia), Yastimadhu (G. glabra), Shankhapushpi (Convolvulus pluricaulis) and Mandukparni (Hydrocotyle asiatica) twice daily with milk for a period of two months. The above drugs showed highly significant ($P < 0.001$) increase in Medha (Intellect) by 28.29%. The effect on Smriti (Memory) in treated group at the end of treatment was highly significant ($P < 0.001$) by 30.36%. The Seguin Form Board test battery was used for assessment which gave a specific reading associated with Visuo-Motor and Visuo-perceptual areas. The mode of action of these compounds was explained based on the pharmacodynamics of the drugs present in the compound. The ushna virya drug Guduchi could be helpful mainly in Grahana (Grasping power) functions. The sheetha virya drugs Yastimadhu, Shankhapushpi and Mandukparni could be helpful mainly in Dharana (Retention power) functions.

(3) In a dissertation work, “The Conceptual and Applied Study of Medha and Manas in Ayurveda,” [39] a placebo controlled clinical study; Volunteers were selected between the age of 16–25 years irrespective of their sex and religion. In Group A, Yastimadhu (G. glabra) granules were administered in the dose of 4 gm twice a day with milk as Anupana (Adjuvant with medication) for 30 days duration. In Group B, Granules of roasted Suji were administered at the dose of 4 gm twice a day with milk as Anupana and for 30 days duration+ Sattvavajaya Cikitsa (Psychological and Behavioural therapy). P.G.I. Memory scale of National Psychological Corporation, Agra had been adopted for the assessment of Medha before and after treatment. In group A, 17 volunteers completed the treatment while in group B, 15 volunteers completed the treatment. Group A showed a statistically highly significant improvement in P.G.I. memory scale and long term memory recall. Again the mode of action has been attributed to the pharmacodynamics of the drug Yastimadhu.

DISCUSSION

‘Ayurveda’ the science of life has in store a vast knowledge of medicinal plants, metals and minerals, the medicinal efficacy of which are probably assessed through immense clinical trials and errors without any aide of modern equipments. It is amazing to the best of our knowledge, after exploring various pharmacological and clinical studies done on this novel drug Yastimadhu that these findings were in point of fact indicated thousands of years back in various treatises of Ayurveda. The current findings strongly uphold the rich knowledge of traditional systems of medicine and give a scientific revalidation of mode of action of the drug which is indeed the need of the hour.

Current findings advocated that the aqueous extract of G. glabra at 150 mg/kg for 7 days can significantly improve learning and memory of mice as noted on ‘elevated plus maze’ and ‘passive avoidance paradigm’ tests. The findings also suggested that this dose reversed amnesia induced by diazepam, scopolamine and ethanol in mice. The same result was also observed after administration of glabridin (aqueous extract of G. glabra) an active isoflavone which remarkably reduced the brain cholinesterase activity (which plays a prominent role in learning and memory) in mice at 1, 2, and 4 mg/kg [25] Aqueous extract
of G. glabra and glycyrrhizin showed significant results on spatial memory retention of rats in Morris water maze. Improvement in verbal fluency in healthy adults and verbal memory in type 2 diabetics was seen after administration of carbenoxolone (a synthetic derivative of glycyrrhetinic acid). The study suggested that 11β-HSD1 inhibition may be a new approach to prevent/ameliorate cognitive decline. By analysing the above mentioned few clinical studies it can be understood that G. glabra and its contents is definitely having a significant potential in enhancing learning and memory via multiple mode of action.

Medha can be understood by Grahana Shakhti (Grasping power) [40] for which proper functioning of smriti and buddhi is a vital essentiality. The above pharmacological studies give us a clear reflection about the ability of Yastimadhu to influence smriti and medha shakthi. The clinical studies re-ascertain this action of the drug in healthy and diseased human volunteers too.

Medha (Intellect and Memory) has been said as the function of Pitta. Balanced state of Pitta is responsible for proper functioning of Medha. The dominant rasa (Taste) of Yastimadhu is Madhura (Sweet). Madhura rasa is said to be ‘Sadindriya prasadaniya’ (Nourishing five sense organs and Manas-Mind) and therefore has a direct effect over the site of these Indriyas (Sense organs) i.e. Sirah (Head). Madhura rasa, Sita Virya and Madhura Vipaka of Yastimadhu pacifies Pitta. By Guru, Snigdha Guna and Madhura Vipaka it controls the Chala Guna of Vata. Vata is the controller and stimulator of Mind. As Medha is closely related to Manas the factors affecting Manas will affect Medha too.

Various drugs and combinations promoting Medha and Smriti have been mentioned in Ayurveda. But choosing an appropriate drug for the necessity is left to Yukthi (logic) of the physician. On observing the results and mode of actions of G. glabra in pharmacological studies, we can interpret that it can be a drug of choice in patients suffering from Smriti hrasa (reduced memory) and Smriti nasha (dementia). The drug also shows its therapeutic efficacy in improving the Medha of school going children, suggesting that it could be an effective medicine in children suffering from learning disabilities and scholastic backwardness.
Table 1: Memory-improving effects of *Glycyrrhiza glabra* Linn \(^{29, 47}\)

<table>
<thead>
<tr>
<th>Test</th>
<th>Test materials /dose</th>
<th>Test model</th>
<th>Endpoint/ biomarkers</th>
<th>Mechanisms</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In vivo</strong></td>
<td>Extracts 75–300 mg/kg, 7d diet 0.5 or 1%, 6w</td>
<td>Diazepam treated mice</td>
<td>Elevated plus-maze test</td>
<td>Cholinergic</td>
<td>Improves memory</td>
<td>[23]</td>
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<tr>
<td></td>
<td></td>
<td>Scopolamine treated mice</td>
<td>passive avoidance test</td>
<td></td>
<td></td>
<td>[22]</td>
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<tr>
<td></td>
<td></td>
<td><em>Aβ</em> 25–35 treated mice</td>
<td>Morris water-maze test</td>
<td>Quench oxidative stress</td>
<td>Inhibit AChE</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glibridin 1–4mg/kg, 3d</td>
<td>Innate Mice</td>
<td>ChE</td>
<td>Inhibit ChE</td>
<td>[24]</td>
</tr>
<tr>
<td><strong>Oral</strong></td>
<td>Carbenoxolone (Synthetic derivative of glycyrrheticic acid)</td>
<td>Healthy volunteers &amp; Type2 Diabetes patients</td>
<td>Various cognitive tests</td>
<td>11β-HSD inhibition</td>
<td>Improves cognitive functions</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td><em>Medhya rasayana</em> Tablets</td>
<td>Healthy volunteers (School going childrens)</td>
<td>Seguin Form Board test battery</td>
<td>Pharmacodynamics of the drug</td>
<td>Improves Medha</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td><em>Yastimadhu</em> Granules</td>
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<td>Pharmacodynamics of the drug</td>
<td>Improves Medha</td>
<td>[39]</td>
</tr>
</tbody>
</table>

#Acetylcholinesterase (AChE); cholinesterase (ChE); thiobarbituric acid-reactive substances (TBARS); \(\beta\) amyloid (*Aβ*); 11β-Hydroxysteroid dehydrogenase (11β-HSD).

**Future avenues**

Further studies are desirable to explore the role of drug transporters in restricting or permitting the brain penetration of various active ingredients of *G. glabra*.

Bioavailability of glucose at brain level helps in its nourishment and proper functioning. A study on *Glucose, Learning and Memory* \(^{41, 42}\) by Psychology Professor Paul E. Gold found that as rats went through a maze, concentrations of *glucose* declined in the animal’s hippocampus, a key brain area involved in learning and memory – even more dramatically so in older brains. The study suggested that presence of optimal level of *glucose* in brain plays a vital role in learning and memory. The roots of *G. glabra* contain *glycyrrhizin*, which is a *saponin* that is 50 times sweeter than sucrose. \(^{10}\) Hence a suitable clinical study to evaluate the *glucose* level in brain after administration of *glycyrrhizin* and its role in learning and memory in healthy and diseased subjects would be an important milestone.

*Glibridin* (Fig.2), *liquiritigenin* (Fig 4) and *Isoliquiritigenin* (Fig 5), the lipophilic compounds present in *G. glabra* with comparatively low-molecular weight (200 ~ 700) are likely to be absorbed into blood and distributed to brain according to Lipinski rule of 5. \(^{43}\) *Liquiritigenin*, a plant-derived highly selective estrogen receptor \(\beta\) agonist has been identified to alleviate the cognitive recession in the elder. \(^{44}\) *Glabrene* also could be beneficial to increase memory due to estrogen-like activities, like isoliquiritigenin, liquiritigenin,
Evaluating the role of these contents of *G. glabra* in improving cognitive functions in healthy and diseased subjects would be a significant avenue in future researches.

**CONCLUSION**

*Glycyrrhiza glabra* Linn (*Yastimadhu*) appears to be a promising herbal drug for cognitive improvement and it will be worthwhile to explore the potential of this noble drug with suitable clinical studies in the management of Impaired memory disorders like Dementia, Alzheimer’s and Learning disabilities of school going children, etc.

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