

Homoeopathic medicine *Mercurius solubilis* treatment improves cerebral blood flow and memory in experimentally induced Dementia in rats

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Abstract

Background: *Mercurius solubilis* (*Merc. sol*) is a known, accepted homoeopathic medicine for the treatment of various mouth, throat, eye, and ear infections. Previous studies suggested that *Merc. sol* has anti-inflammatory properties which could be beneficial in memory impairment. **Objective:** The present study was designed to investigate the effect of *Merc. sol* on learning and memory and cerebral blood flow (CBF) in the rat model of impaired learning and memory function induced by intracerebroventricularly (ICV) administered *streptozotocin* (STZ). **Materials and Methods:** The different potencies of *Merc. sol* (6, 30, 200, and 1M) were given for 17 days in memory-impaired rats, induced by ICV administration of STZ (3 mg/kg). The Morris water maze test was used to evaluate the learning and memory function on the 14th, 15th, and 16th day. The laser doppler flow meter was used to measure CBF on the 17th day. **Results:** There was a significant reduction in CBF along with learning and memory functions in STZ (ICV)-treated rats, which were significantly attenuated by the treatment of *Merc. sol* at all potencies (6, 30, 200, and 1M). **Conclusion:** Our results demonstrated the effectiveness of *Merc. sol* in improving memory function and CBF. Thus, it could be used as a therapeutic agent in dementia.

Keywords: Cerebral blood flow, Cognitive dysfunction, *Mercurius solubilis*, *Streptozotocin*

INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is associated with oxidative stress, inflammation, and cerebral endothelial dysfunction. Previous reports have shown that memory impairment is associated with poor cerebral blood flow (CBF), which may be involved in the progression of dementia, and these reports gave strength to the thought that a relationship exists between the memory and CBF^[1] as increased CBF provides protection for cognitive disorders.^[2] Inflammation has been implicated as a common cause of various neurodegenerative diseases,^[3,4] and evidence suggests that neuroinflammation and sustained increases in inflammatory cytokines in the central nervous system are closely correlated with cognitive dysfunction in the progression of AD.^[4,5] Previous studies have shown that anti-inflammatory drugs such as *silibinin* and *ibuprofen* ameliorate memory impairment by reducing oxidative stress and inflammation in mice brain.^[6-8]

Previous reports have proposed that the herbal medicines or products can be considered as a therapeutic approach to treat Alzheimer or other dementia-related disorders, showing cognitive impairment and neuroinflammation.^[9] *Mercurius solubilis* (*Merc. sol*) is a commonly used homoeopathic remedy for mouth-throat infections, catarrh, eye, ear infections, and fever. In homoeopathy, *Merc. sol* is also known as quicksilver or black oxide of mercury. In a previous study, *Merc. sol* has been found altering reactive oxygen species, reactive nitrogen species, and cytokine secretion, which improve wound healing and homoeostasis.^[10] Homoeopathic physicians also

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How to cite this article: Hanif K, Kumar M, Singh N, Shukla R. Homoeopathic medicine *Mercurius solubilis* treatment improves cerebral blood flow and memory in experimentally induced Dementia in rats. Indian J Res Homoeopathy 2017;11:58-63.

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10.4103/ijrh.ijrh_74_16

use *Merc. sol* for the treatment of various acute and chronic inflammatory conditions. A study by Vangoori *et al.*, 2013 also showed that *Merc. sol* has anti-inflammatory property in acute and chronic experimental animal models of inflammation.^[11]

Intracerebroventricular (ICV) injection of *streptozotocin* (STZ) in rats leads to oxidative stress, inflammation, and impaired energy metabolism, resulting in progressive deficits in learning and memory and reduced CBF, which resembles various pathological characteristics of AD.^[12] As earlier studies have shown that *Merc. sol* possesses anti-oxidant and anti-inflammatory properties, therefore, the present study was designed to explore the effect of various potencies of *Merc. sol* (6C, 30C, 200C, and 1M) on the learning and memory and CBF on rat model of memory impairments induced by STZ.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (230–250g) procured from the National Laboratory Animal Centre of CSIR–Central Drug Research Institute, Lucknow, India, were used in all experiments. They were allowed free access to food and water and maintained at 12 h day/night cycle (room temperature 24°C–27°C and humidity 60%–62%). The Institutional Animal Ethical Committee (IAEC, IAEC/2011/08 Renew 01 dated May 16, 2012) and Committee for the Purpose of Control and Supervision of Experiments on Animals - India guidelines were followed throughout the experiments.

Homoeopathic drug procurement

The *Merc. sol* was procured from Hahnemann Publishing Company Pvt. Ltd., Kolkata. We selected *Merc. sol* for this study as per the recommendations of the Scientific Advisory Committee, Central Council for Research in Homoeopathy, New Delhi. Authenticated samples of homoeopathic medicines in 6, 30, 200, and 1M potencies and absolute alcohol as vehicle were used for the experimental purpose.

Intracerebroventricular injection of *Streptozotocin*

STZ (3 mg/kg) was dissolved in artificial CSF (aCSF; 147 mM NaCl, 2.9 mM KCl, 1.6 mM MgCl₂, 1.7 mM CaCl₂, and 2.2 mM dextrose). The rats were anesthetized with *chloral hydrate* (300 mg/kg, i.p) and 10 µl volume of STZ was injected into each lateral cerebral ventricle (ICV) on days 1 and 3

using the coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, 3.6 mm ventral using Hamilton microsyringe.^[13,14]

Treatment protocol

Merc. sol potencies of 6C, 30C, 200C, and 1M were prepared in a dilution of 1:10 with distilled water and administered orally. The rats were divided into six groups and each group had six rats [Table 1].

Evaluation of learning and memory

The learning and memory function was evaluated by Morris water maze test in rats.^[15,16] This consisted of a video capturing system, with a large circular black vessel of 120 cm diameter, 50 cm heights, filled to depth of 30 cm with water at 26 ± 2°C; containing four virtual quadrant points as north, east, south, and west. A round platform (black, 8 cm diameter) was placed below the surface (1 cm) of water in the middle of the northeast quadrant in the pool. The starting point was in the southwest quadrant in all the trials. Latency time to reach the platform was measured and recorded on accomplishment of each trial. The cutoff time was set to 120 s for rats to find the hidden platform and stay on it for 30 s. For evaluation of learning and memory, rats chronically treated with different potencies of *Merc. sol* were subjected to Morris water maze trial from 14th to 16th day. Latency time to reach the platform was recorded in each trial and significant decrease in latency time was considered as successful learning.

Measurement of cerebral blood flow

CBF was measured using a laser Doppler flowmetry (LDF 100, Biopac, USA) which is equipped with a tiny microfiber laser probe of 2 cm length and 0.5 mm diameter.^[1,17] To measure CBF, the point was made by drilling using stereotaxic instrument (1 mm posterior and 6 mm lateral to bregma) on the skull of anesthetized rats (*chloral hydrate*, 300 mg/kg, i.p). A laser probe was placed on the point on the skull of rats. Then probe was attached to LDF for 45 min. Blood flow meter qualitatively measures blood flow in arbitrary blood perfusion units (BPUs).

Statistical analysis

The results were expressed as mean ± standard error of the mean. The two-way of analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test was used to analyze Morris water maze results, and one-way ANOVA followed by Bonferroni's comparison test was used for CBF (BPU). The

Table 1: Treatment schedule for *Mercurius solubilis* treatment in *streptozotocin*-treated rats

Group	STZ treatment (ICV route, 10 µl)	Treatment (oral route, 100 µl)	Duration of treatment
Sham	*	*	Rats were kept for 17 days
STZ treated	On the 1 st and 3 rd day	*	Rats were kept for 17 days
STZ + vehicle	On the 1 st and 3 rd day	Absolute alcohol from 1 st to 17 th day	Rats were kept for 17 days
STZ + <i>Merc. Sol</i> 6	On the 1 st and 3 rd day	<i>Merc. Sol</i> 6 from 1 st to 17 th day	Rats were kept for 17 days
STZ + <i>Merc. Sol</i> 30	On the 1 st and 3 rd day	<i>Merc. Sol</i> 30 from 1 st to 17 th day	Rats were kept for 17 days
STZ + <i>Merc. Sol</i> 200	On the 1 st and 3 rd day	<i>Merc. Sol</i> 200 from 1 st to 17 th day	Rats were kept for 17 days
STZ + <i>Merc. Sol</i> 1M	On the 1 st and 3 rd day	<i>Merc. Sol</i> 1M from 1 st to 17 th day	Rats were kept for 17 days

Merc. Sol: *Mercurius solubilis*; STZ: *Streptozotocin*; ICV: Intracerebroventricularly; *no treatment

$P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significance.

RESULTS

Effect of *Mercurius solubilis* on impaired memory function in *Streptozotocin*-treated Rats

There was no significant decrease in the latency time of first- and second-retention trial as compared to acquisition trial in sham group. Impairment in learning and memory function was found in the STZ-treated group as evident by no change in the latency time of acquisition trial and first- and second-retention trials. The treatment for 17 days with *Merc. sol* 6 ($F [2, 15] = 14.9, P < 0.001$), *Merc. sol* 30 ($F [2, 9] = 26.15, P < 0.001$), *Merc. sol* 200 ($F [2, 12] = 18.7, P < 0.001$), and *Merc. sol* 1M ($F [2, 9] = 23.32, P < 0.001$) reduced latency time of first- and second-retention trials as compared to acquisition trial, respectively, in STZ-treated rats [Figure 1a]. There was no significant effect of alcohol which was used as a vehicle on latency time of STZ-treated rats.

The results obtained from latency time were also confirmed by the path length results. The STZ-treated rats showed memory impairment as evident by longer path length in acquisition trial and first- and second-retention trials in comparison of sham rats. The treatment with *Merc. sol* 6 ($F [2, 15] = 6.6, P < 0.05$), *Merc. sol* 30 ($F [2, 9] = 20.59, P < 0.001$), *Merc. sol* 200 ($F [2, 12] = 15.0, P < 0.001$), and *Merc. sol* 1M ($F [2, 9] = 17.8, P < 0.001$) treated rats have shown shorter path length in the first- and second-retention trials as compared to acquisition trial in STZ-treated rats [Figure 1b and c].

Effect of *Mercurius solubilis* on cerebral blood flow in *Streptozotocin*-treated Rats

A significant decrease was observed in CBF in STZ-treated rats (328.3 ± 5.2 BPU, $P < 0.001$) as compared to the

sham rats (493.6 ± 19.7 BPU). There was a significant improvement observed in CBF in *Merc. sol* 6 (400.1 ± 5.9 BPU, $P < 0.001$), *Merc. sol* 30 (1061 ± 11.0 BPU, $P < 0.001$), *Merc. sol* 200 (531.3 ± 9.3 BPU, $P < 0.001$), and *Merc. sol* 1M (557.1 ± 4.3 BPU, $P < 0.001$) as compared to STZ-treated rats [Figure 2], being the result obtained after *Merc. sol* 30 treatment 100% above the control parameters.

DISCUSSION

The present study examined the effect of *Merc. sol* on memory impairment and CBF in STZ-administered rats. Pretreatment with different potencies of *Merc. sol* in STZ-administered rats improved learning and memory functions along with CBF in the rat brain, suggesting the protective role of *Merc. sol* in memory impairment.

In the present study, there was no significant decrease in latency time and path length in STZ-treated rats, indicating memory impairment which was also supported by previous reports.^[16,17] In cognitive disorders such as AD, memory impairment has been linked to the inflammation in brain tissue. Previous studies have shown that reduction in inflammatory cytokines or inflammation is beneficial in the treatment of dementia.^[18,19] Administration of different potencies of *Merc. sol* (6, 30, 200, and 1M) attenuated STZ-induced memory deficit as evidenced by reduced latency time and path length as compared to control. Studies by Vangoori et al., 2013 and S Shadfar et al., 2015 have shown that *Merc. sol* has anti-inflammatory properties which may be one of the reasons behind the improvement in memory function in *Merc. Sol*-treated rats as compared to STZ-treated rats.^[11,19]

Decreased CBF is also responsible for the impairment in the memory function as several reports showed the disturbed CBF in the patients of AD and other cognitive diseases.

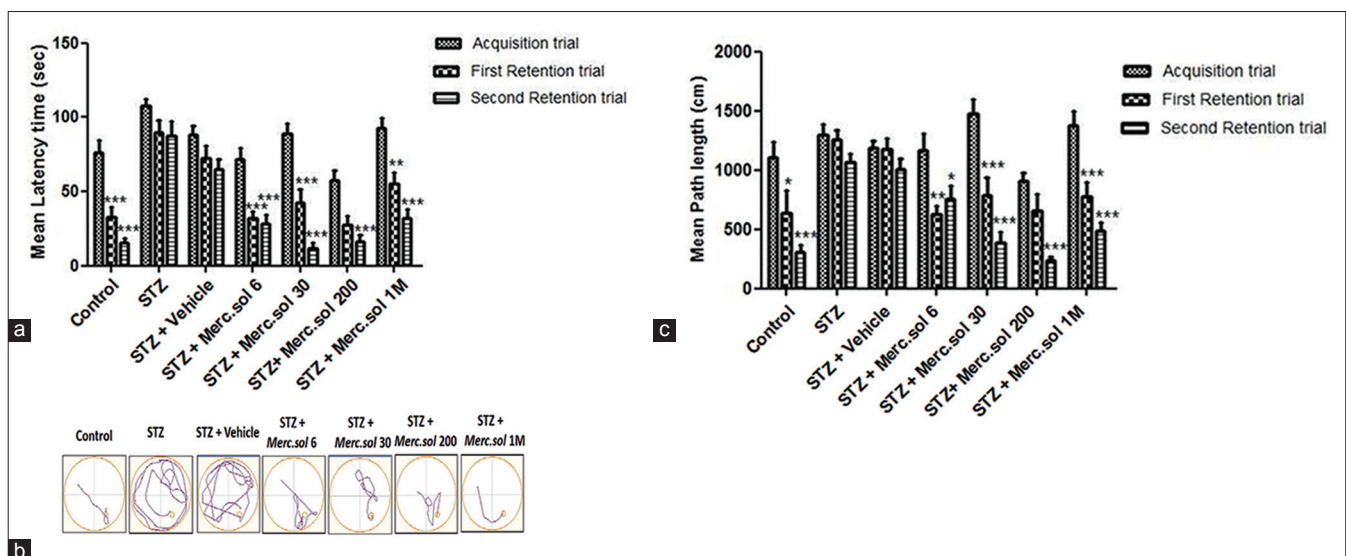


Figure 1: Effect of *Mercurius solubilis* on (a) latency time (s), (b and c) mean path length (cm) in memory impaired (dementic) Sprague-Dawley rats. Memory deficit was induced by intracerebroventricular administration of *streptozotocin* (3 mg/kg) and treated with different potencies of *Mercurius solubilis* (6, 30, 200, and 1M) for 17 days in rats. Latency time and path length were evaluated by Morris water maze test on 14th, 15th, and 16th day (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$ vs. acquisition trial)

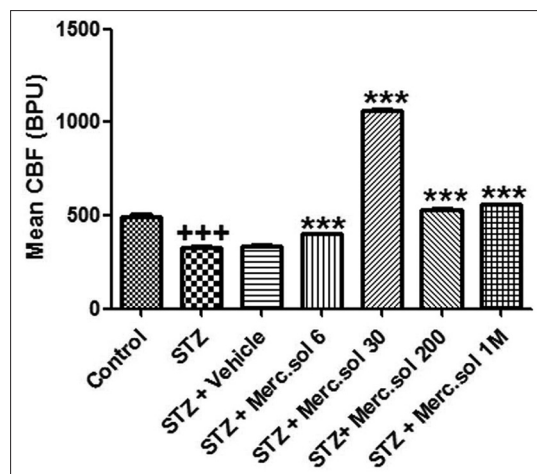


Figure 2: Effect of *Mercurius solubilis* on cerebral blood flow in memory impaired Sprague–Dawley rats. Memory deficit was induced by intracerebroventricular administration of streptozotocin (3 mg/kg) and treated with different potencies of *Mercurius solubilis* (6, 30, 200 and 1M) for 17 days in rats. Cerebral blood flow was measured by laser Doppler flowmetry on the 17th day (*** $P < 0.001$ vs. streptozotocin and +++ $P < 0.001$ vs. sham group)

A significant reduction in CBF was found in STZ-injected rats along with impaired memory function in present work. This finding is in conformity with our earlier work, in which STZ treatment impaired learning and memory function along with decreased CBF.^[17] In the present study, all the potencies of *Merc. sol* (6C, 30C, 200C, and 1M) significantly reversed the decline in CBF caused by STZ. However, the result obtained after *Merc. sol* 30 treatment was 100% above the normal parameters found in untouched control.

The anti-inflammatory property of *Merc. sol* can be the reason behind the improvement in CBF in STZ-treated rats although more specific research protocols are needed to highlight this hypothesis.

CONCLUSION

The present study supported findings that disturbed cerebral circulation is associated with impaired memory functions as evidenced by decreased CBF following STZ.^[17,20] *Merc. sol* treatment offered protection in memory impairment along with an increase in CBF, which may be one of the reasons for its beneficial effect. This neuroprotective protective effect may be due to its anti-inflammatory properties as suggested by previous reports.^[11,21] Therefore, the mechanism underlying protective effect of *Merc. sol* is required to explore to treat cognitive disorder.

Financial support and sponsorship

We would like to appreciate the financial support from the Central Council for Research in Homeopathy (CCRH), New Delhi.

Conflict of interest

None declared.

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Das homöopathische Arzneimittel *Mercurius solubilis* verbessert den zerebralen Blutfluss und das Gedächtnis bei Versuchsratten mit Demenz

Auszug

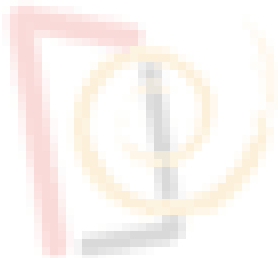
Hintergrund: *Mercurius solubilis* (*Merc. sol*) ist eine häufig angewendete homöopathische Arznei zur Behandlung verschiedener Mund-, Rachen-, Augen- und Ohrinfektionen. Frühere Studien haben nahe gelegt, dass *Merc. sol* entzündungshemmende Eigenschaften hat, was bei Gedächtnisstörungen von Vorteil sein könnte.

Ziel: Die vorliegende Studie wurde entwickelt, um die Wirkung von *Merc. sol.* auf das Lernen, das Gedächtnis und den zerebralen Blutfluss (CBF) im Rattenmodell mit beeinträchtigter Lern- und Gedächtnisfunktion, induziert durch intraventrikulär (ICV) verabreichtes Streptozotocin (STZ), zu untersuchen.

Materialien und Methoden: Die verschiedenen Potenzen von *Merc. sol.* (6, 30, 200 und 1M) wurden 17 Tage lang bei Gedächtnisstörungen, induziert durch ICV-Verabreichung von STZ (3 mg/kg), gegeben. Der Morris-Wasserlabyrinth-Test wurde durchgeführt, um die Lern- und Gedächtnisfunktion am 14., 15. und 16. Tag zu bewerten. Der Laser-Doppler-Durchflussmesser wurde verwendet, um CBF am 17. Tag zu messen.

Ergebnisse: Durch die Behandlung von *Merc. sol.* in allen Potenzen (6, 30, 200 und 1M) gab es eine signifikante Verringerung der Beeinträchtigung des CBF, der Lern- und Gedächtnisfunktionen bei STZ (ICV)-behandelten Ratten.

Fazit: Unsere Ergebnisse zeigten die Wirksamkeit von *Merc. sol.* in der Verbesserung der Gedächtnisfunktion und CBF. So könnte es als therapeutisches Mittel bei Demenz verwendet werden.



Homeopatía *Mercurius solubilis* mejora el flujo sanguíneo cerebral y la memoria en ratas deménticas experimentales

RESUMEN

Fundamento: *Mercurius solubilis* (*Merc. sol*) es un medicamento homeopático conocido y aceptado en el tratamiento de diferentes infecciones orales, faríngeas, oculares y óticas. Los estudios previos han indicado que *Merc. sol* posee propiedades antiinflamatorias que pueden ser beneficiosas en los trastornos de la memoria.

Objetivo: El presente estudio se diseñó para investigar el efecto de *Merc. sol* en el aprendizaje y la memoria, así como en el flujo sanguíneo cerebral (FSC) en un modelo de ratas con trastornos de la función de aprendizaje y memoria, inducidos por estreptozotocina (STZ) administrada por vía intracerebroventricular (ICV).

Materiales y métodos: Se administraron diferentes potencias de *Merc. sol* (6, 30, 200 y 1M) durante 17 días a ratas con trastornos de la memoria, inducidos por STZ administrada por vía ICV (3 mg/kg). Se utilizó la prueba del laberinto de agua de Morris para evaluar la función de aprendizaje y memoria en los días 14, 15 y 16. Para medir el FSC, se utilizó un flujómetro de láser Doppler de láser en el día 17.

Resultados: En las ratas tratadas con STZ (ICV), se constató una reducción significativa del FSC junto con trastornos de las funciones de aprendizaje y memoria. Todas estas ratas fueron atenuadas de manera significativa con el tratamiento con *Merc. sol* en todas las potencias (6, 30, 200 y 1M).

Conclusiones: Nuestros resultados demuestran la eficacia de *Merc. sol* en mejorar la función de la memoria y el FSC. Por lo tanto, puede utilizarse como agente terapéutico en la demencia.

बहोम्योपैथिक औषधि मरक्युरियस सोलुबिलिस द्वारा प्रयोगात्मक विक्षिप्त चूहों में मस्तिष्क रक्त प्रवाह और स्मृति संबंधी सुधार

सार

पृष्ठभूमि: मरक्युरियस सोलुबिलिस एक ज्ञात और स्वीकृत होम्योपैथिक औषधि है जिसका उपयोग मुंह, गले, आंख और कान के विभिन्न संक्रमणों के उपचार के लिए किया जाता है। पूर्व अध्ययनों से यह पता चलता है कि मरक्युरियस सोलुबिलिस में सूजन विरोधी गुण है जो स्मृति हानि के उपचार में फायदेमंद हो सकता है।

उद्देश्य: वर्तमान अध्ययन का उद्देश्य इंद्रासेरिब्रो वेनट्रिकुलरली (आईसीवी) प्रशासित स्ट्रेपटोजोटोसिन (एसटीजेड) द्वारा प्रेरित, क्षीण अधिगम और स्मृति वाले चूहों के माडल में अधिगम, स्मृति और मस्तिष्क रक्त प्रवाह (सीबीएफ) पर मरक्युरियस सोलुबिलिस के प्रभाव की जांच करना था।

सामग्री और विधि: एसटीजेड (3 मिली ग्राम/कि.ग्रा) के आईसीवी प्रशासन द्वारा प्रेरित, स्मृति हानि वाले चूहों को 17 दिनों के लिए मरक्युरियस सोलुबिलिस की विभिन्न पोर्टेंसिज़ (6सी, 30सी, 200सी और 1 एम) दी गई। 14वें, 15वें और 16वें दिन, अधिगम और स्मृति के मूल्यांकन हेतु मॉरिस वाटर मेज़ परीक्षण का इस्तेमाल किया गया। 17 वें दिन, सीबीएफ के मापन हेतु लेजर डॉपलर प्लो मीटर का इस्तेमाल किया गया।

परिणाम: एसटीजेड (आईसीवी) उपचारित चूहों में क्षीण अधिगम और स्मृति के साथ-साथ सीबीएफ में महत्वपूर्ण कमी पाई गई, जो मरक्युरियस सोलुबिलिस की सभी पोर्टेंसिज़ (6सी, 30सी, 200सी और 1 एम) द्वारा उपचारित थे।

निष्कर्ष: मरक्युरियस सोलुबिलिस का सीबीएफ और स्मृति सुधार के लिये चिकित्सीय एजेंट के रूप में उपयोग किया जा सकता है।

