



Journal of Neuroscience and Behavioral Health

Volume 9 Number 1 July 2017

ISSN 2141-2286



*Academic
Journals*

ABOUT JNBH

The Journal of Neuroscience and Behavioral Health (JNBH) is published monthly (one volume per year) by Academic Journals.

Journal of Neuroscience and Behavioral Health (JNBH) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as metagenics, evolutionary anthropology, fragile X syndrome, immunotherapy etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JNBH are peer-reviewed.

Contact Us

Editorial Office: jnbh@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: <http://www.academicjournals.org/journal/JNBH>

Submit manuscript online <http://ms.academicjournals.me/>

Editors

Prof. Viroj Wiwanitkit, M.D.

*Wiwanitkit House, Bangkhae,
Bangkok Thailand 10160.
Visiting Prof. Tropical Medicine,
Hainan Medical College,
Hainan China.*

Prof. Kenneth Blum

*Institution Department of Psychiatry,
University of Florida college of Medicine,
Gainesville, Fl
USA*

Dr. Abd El-Latif Hesham

*Genetics Department, Faculty of Agriculture,
Assiut University
Egypt*

Prof. Viroj Wiwanitkit

*Wiwanitkit house, bangkhae, Bangkok Thailand
10160
Thailand*

Dr. Pritha Ghosh

*Indian Institute of Chemical Biology
India*

Dr. Israel Fernandez-Cadenas

*Neurovascular Research Laboratory,
Institut de Recerca, Vall d'Hebron Hospital,
Barcelona.
Spain*

Dr. Wanis H Ibrahim

*Qualifications: FRCP (Edin), FRCP (Glasg), FCCP
Hamad General Hospital, Weill-Cornell Medical
College
Qatar*

Prof. Debnath Bhattacharyya

*Hannam University,
Daejeon,
Korea*

Dr. Khaled Abu-Amero

*College of Medicine, King Saud University,
Saudi Arabia*

Dr. Faiyaz Ahmed

*Department of Studies in Food Science and Nutrition
University of Mysore,
India*

Editorial Board

Prof. Rama Devi Mittal

*Sanjay gandhi PGI Lucknow
India
Prof. Kai Li
Suzhou University uzhou,
Jiangsu,
China*

Dr. Aliza Amiel

*Faculty of life Science Bar-Ilan Ramat-Gan,
Israel*

Dr Olufemi Oloyede

*Department of Obstetrics and Gynaecology,
Olabisi Onabanjo University Teaching Hospital
Sagamu,
Ogun State,
Nigeria*

Dr. Vishwanathan Huchtagowder

*Washington University school of medicine
USA*

Dr. Abdelilah S. Gounni

*Faculty of Medicine,
University of Manitoba
Canada*

Prof. Ruixing Yin

*Department of Cardiology, Institute of Cardiovascular
Diseases,
Guangxi Medical University
22 Shuangyong Road,
Nanning 530021,
Guangxi,
China*

Dr. Guangming Han

*Georgia State University
USA*

Dr. C. Emmanuel

*Global Hospitals Group
India*

Dr. Alessio Squassina

*Department of Neuroscience,
University of Cagliari
Italy*

Dr. Jiexiong Feng

*Department of Pediatric Surgery, Tongji Hospital,
Huazhong University of Science and Technology
China*

Dr. Magdy Abd ElRehim Sayed Aly

*Faculty of Science,
Beni Suef University
Egypt*

Dr. Hamid Jafarzadeh

*Mashhad Faculty of Dentistry and Dental Research Center
Iran*

Dr. Youse Rasmi

*Department of Biochemistry,
Faculty of Medicine,
Urmia University of Medical Sciences,
Urmia,
Iran*

Dr Keya Chaudhuri

*Indian Institute of Chemical Biology
India*

Ivan Y. Torshin

*Computational Center of The Russian Academy of Sciences
Russia*

Dr. Wagdy K. B. Khalil

*National Research Centre (NRC)
Egypt*

Vishnu Priya

*Saveetha University
India*

Dr. A.Chandrasekar

*Anthropological Survey of India,
Southern Regional Bogadi 2nd stage,
Mysore-570 026
India*

Dr Raghavendra Babu YP

*Kasturba Medical College, Mangalore
India*

Dr. Shayesteh Jahanfar

*Royal College of Medicine, Perak;
University of Kuala Lumpur
Malaysia*

Prof. Wei Wang

*Capital Medical University, Beijing, China;
Chinese Academy of Sciences, Beijing, China
China*

Journal of Neuroscience and Behavioral Health

Table of Content: Volume 9 Number 1 July 2017

ARTICLE

Cigarette smoking among Jimma University Teaching and referral hospital outpatients attending services at psychiatry clinic Southwest, Ethiopia

13

Yosef Zenebe, Garumma Tolu Feyissa and Wolfgang Krahl

Full Length Research paper

Auditory, visual, kinesthetic-tactile, and multi-sensory modalities: A quantitative study of how preferred modalities create more effective teaching and learning environments

Aaron, J. Miller

Miller Consulting Service, USA.

Received 5 February, 2016; Accepted 6 June, 2016

This study aims to examine how teaching methods and learning styles of instructors play a role in how students learn, and how technology is selected. There are four type of learning styles: auditory, visual, kinesthetic-tactile and multi-sensory. The survey population was evenly distributed, consisting of 100 faculty members and 100 students with a response rate of 25%. The results of the research indicate there is some evidence that: Instructors will have higher perceived teaching effectiveness when using their preferred teaching method; students will prefer a teaching method that is consistent with their learning style(s); students preferences in teaching method, the course subject, and situational factors (that is, class size) are related to instructors' preference in teaching method and use of technological advancements. Overall, this study produced some interesting findings, indicating some significant relationships between teaching methods and learning styles.

Key words: Teaching method, learning style, auditory, visual, kinesthetic-tactile, multi-sensory, computer-based technologies.

INTRODUCTION

Although the traditional mode of college teaching is the lecture method, this method has been criticized for being authoritarian, boring, and predictable due to its emphasis on memorization and tests of specific information (Breishline and Holmes, 2007). Although this teaching style continues to dominate instruction in higher education, many faculty members have used other more interactive teaching styles to supplement or replace the lecture method. A number of factors may influence the

instructor's decision to use certain teaching methods, such as his/her learning style, perception of student preferences, the course subject, and class size or facilities.

College faculty members have a wide range of technologies that can be utilized to better, or in some cases replace, traditional teaching methods. For example, instructors may use lecture-enriching technology, such as PowerPoint presentations or video conferencing,

E-mail: drajmill12@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

to bring guest lectures from distant places into the classroom. Computer-based technologies can also be used to facilitate communication between the instructor and student via electronic mail, web pages, chat rooms, and electronic bulletin boards. Although all of these technology options exist, there is little empirical evidence as to what factors might influence the choice or limit the use of technology in the classroom, such as the instructor's learning style, his/her perception of student technology preferences, the course subject, class size, institutional support, and knowledge of how to use various technologies.

One factor that would likely play a role in an instructor's choice of teaching method is their preference or comfort level with particular methods. Although this may be influenced somewhat by the teaching methods they experienced during their years of education, it is likely that their learning style also plays a role in what they believe are effective teaching methods. A common way of mapping a person's learning style is to determine the modes or senses through which one takes in and processes information. One theory considers three basic modalities: Auditory, Visual, and Kinesthetic-Tactile (KT) (Willis and Hodson, 2000)(Table 4).

Discussions of visual, auditory, and kinesthetic learners are common in educational literature, teacher-preparation programs, and professional development workshops. The theory that students learn more when content is presented in their best modality seems to make sense, seems to be supported by classroom experiences, and offers the hope of maximizing each child's learning by planning different lessons for each type of learner (Hudak & Anderson, 2004). For example, within one kindergarten class, the auditory learner could listen to stories about different holidays around the world, while the visual learner examined pictures of holiday celebrants, and the kinesthetic learner handled costumes and artifacts associated with the holidays (Erikson, 2008). But is the theory correct? And, whether or not the theory is correct, might it not also be true that all of the kindergartners would learn the most about holidays by listening to stories, looking at pictures, and handling costumes?

Before the study tackle the research on using modalities to enhance student learning, let's review a few things that cognitive scientists know about modalities (Williams, 2003). Some memories are stored as visual and auditory representations, but most memories are stored in terms of meaning. Cognitive psychologists have used formal laboratory tasks to investigate the role of modality in memory (Schiedel and Marcia, 2005). An important finding from that research is that memory is usually stored independent of any modality. You typically store memories in terms of meaning — not in terms of whether you saw, heard, or physically interacted with the information. For example, your knowledge that a fire requires oxygen to burn is unlikely to be stored as a visual or an auditory memory (Marcia, 2000). The initial

experience by which you learned this fact may have been visual (watching a flame go out under a glass) or auditory (hearing an explanation), but the resulting representation of that knowledge in your mind is neither visual nor auditory (Richardson, and Davis, 2007).

How did cognitive scientists figure this out? An important clue that memories are stored by their meaning is the types of errors people make on memory tests (Pankratz and Morris, 2000). People who listen to a story will later confidently "recognize" sentences that never appeared in the story — so long as these new sentences are consistent with the story's meaning. The same phenomenon is observed with purely visual stimuli. People rapidly lose the memory of the precise images that make up a picture story (for example, whether a character faced left or right), but they retain the meaning or gist of the story.

The mind is capable of storing memories in a number of different formats, and laboratory research indicates that a single experience usually leads to more than one type of representation (Schunck, 2001). When subjects view a picture story, they do have a visual representation of what the pictures look like, in addition to the meaning-based representation (Bandura, 2006). They usually don't remember the visual representation for long, however, largely because when they see the pictures, they are thinking about what they mean in order to understand the story. If, in contrast, they were asked to remember visual details of the pictures and to ignore the story they tell, they would have a better memory for the visual details and the meaning-based representation would be worse.

Smith (2006) focuses that our minds have these different types of representations for a reason: Different representations are more or less effective for storing different types of information. Visual representations, for example, are poor for storing meaning because they are often consistent with more than one interpretation: A static image of a car driving on a snowy hill could just as well depict a car struggling up the hill or slipping backwards down the hill. And some concepts do not lend themselves well to pictures: How would one depict "genius" or "democracy" in a picture? On the other hand, the particular shade of green of a frozen pea would be stored visually because the information is inherently visual (Hudak and Anderson, 2004).

Stout (2007) highlights that these different memory representations store different types of information, you usually cannot use one representation to substitute for another. This point is illustrated in an experiment by Dodson et al. (2000). They asked subjects to listen to two word lists and to judge whether or not each word on the second list (new words) had appeared on the first list (studied words), as shown below. The interesting twist was that each word on both lists was spoken by either a man (depicted by boldface) or a woman (depicted by italics). If a word had appeared on both lists, it might be

spoken in the same voice ("Window") or in different voices ("Doctor"). The question is whether changing the gender of the voice (and, therefore, the auditory experience) influenced memory for the studied words (Table 1).

Wills and Hodson (2000) found that whether the gender of the voice repeated or switched made no difference at all in remembering the word (75% versus 73% accuracy). That is, subjects were just as likely to remember "Doctor" as "Window." But when subjects judged that a word was on the first list, they also had to say whether a man or woman had said it. For this judgment, subjects were more accurate if the same gender voice spoke the word on the first and the second list (57%) than if the voice switched genders (39%). This experiment indicates that subjects do store auditory information, but it only helps them remember the part of the memory that is auditory — the sound of the voice — and not the word itself, which is stored in terms of its meaning (Wills and Hodson, 2000).

Modality theory may also seem correct because, as the study has discussed, children probably do differ in their abilities with different types of memories. The researcher remember their daughter commenting (out of the blue, as 4-year-olds will) that her preschool teacher said "white" in a way that made the "h" faintly, but distinctly, audible. The researcher was impressed that she had noticed this difference, remembered it, and could reproduce it. So their daughter may have a good auditory memory, and that might help her in certain tasks, such as remembering regional accents, should she decide to be an actress (Hudak and Anderson, 2004). It does not mean that the researcher want her teachers to ensure that she receives primarily auditory input in her coursework, because her superior auditory memory will not help her when she needs to remember meaning. But it is easy to see how one might (mistakenly) believe that complex material would be easier for her to master if presented auditorily (Pankratz and Morris, 2000; Marcia, 2000; Schiedel and Marcia, 2005). Further, as the article *The Content's Best Modality Is Key* indicates, there are various ways in which modality does strengthen instruction (for all kids) — and it's easy to imagine that the effect has to do with a student's modal preference when in fact the effect is due to the content's best modality.

Visual learning defined

A visual learner learns best when information is presented visually. This means that the more the learner is able to see the information, the easier it may be for that learner to learn the information. Some of the things a visual learner might need to use may be: textbooks, worksheets, written notes, maps, flash cards, diagrams, written directions, notes on index cards, notes on the blackboard, information on posters, bulletin boards, written outlines, graphic organizers (a kind of written diagram used for outlining or seeing relationships

between concepts), drawings, and pictures (Williams, 2003).

A visual learner may prefer to study using the materials just listed. The learner may prefer to use a highlighter (a light colored marker) on a page in a book, to highlight important information. If this is not allowed as the learner does not own the book, make photocopies of the pages so that the learner may do this. Another study method is to use flash cards for review. Keep the flashcards neatly organized, by topic, in an index card box (Erikson, 2008).

If the visual learner is presented with an activity that is not highly visual in nature, change the activity to accommodate the learner's needs. For example, let's say the visual learner must remember the information presented at a lecture. A lecture is primarily an auditory presentation (using hearing rather than sight). An example of changing this activity to a visual presentation might be to get permission to tape the lecture (Williams, 2003). Later, the learner would listen to the tape and transcribe the notes into written form. The learner would then be able to study the written notes, which is now a visual presentation (Marcia, 2007). The learning method in this example, has changed from a purely auditory presentation, to an activity requiring visual input. Some other ideas might include seeing if the learner is able to read about the activity before actually listening to the lecture (Marcia, 2000). Explain to the lecturer that you are a visual learner. See if you can get a copy of the notes from the lecturer. Now that you know a little more about your learning style, see if you can match or adapt activities to increase your learning success.

Auditory learning defined

An auditory learner learns best when information is presented auditorily. This means that the more the learner is able to hear the information, the easier it may be for that learner to learn the information (Richardson and Davis, 2007). Some of the things an auditory learner might need to use may be: discussion groups, lectures, tape recorder, cooperative learning (that is, where information is discussed within a group), directions discussed by the teacher before an activity is attempted, listening to books read to the group, books on tape, information put to songs, silly sayings that help you remember information (that is, mnemonic devices), and recited poems of information (Frost and Fukami, 2007).

An auditory learner may prefer to study using the materials just listed. The learner may prefer to listen to study material on tape. He/she may also wish to set information to music. After singing the song that was created, see how much faster an auditory learner is able to retain the information (Management Education, 2002).

If the auditory learner is presented with an activity that is not highly auditory in nature, change the activity to accommodate the learner's needs. For example, let's say the auditory learner must remember the information

presented in a textbook. A textbook is a visual presentation (using sight rather than hearing). An example of changing this activity to an auditory presentation might be, to get someone to make an audio cassette tape of the chapter to be studied (Csikszentmihalyi, 2003). The learner would listen to the tape a number of times. The learner would then be able to study the textbook chapter, which is now an auditory presentation. The learning method in this example, has changed from a purely visual presentation, to an activity requiring auditory input.

Label the tape and keep it in an indexed box (Duncum, 2007). This way, the tape may be easily located for future review. Some other ideas might include teaching the learner to subvocalize. This means to whisper quietly under your breath. If a learner must glean information from written material while working in a quiet group, the learner may whisper under his/her breath, thus adding auditory input to a visual activity (Marcia, 2007). When working alone, the learner may wish to read out loud. Teaching the learner to put information to music, poems, or sayings is another auditory method. Sing or repeat study information out loud (Erikson, 2000). Notice how an auditory learner can remember every word to a song, but may have trouble quietly studying a few spelling words from index cards. If the learner sings those spelling words, you will see a difference in the learner's ability to retain the spelling information, with less effort. Now that you know a little more about your learning style, see if you can match or adapt activities to increase your learning success.

Kinesthetic-tactile learning defined

A kinesthetic-tactile learners learn best when information is presented using touch and movement. This means that the more the learner is able to touch, manipulate the materials used to present the information, or use his/her body movements, the easier it may be for that learner to learn the information (Richardson and Davis, 2007). Some of the things a kinesthetic-tactile learner might need to use may be: a typewriter, computer keyboard, sand in a sand tray, blackboard, letter or word magnets, concept models that may be taken apart, stamp pad letters and numerals, gross motor materials (materials requiring large muscle movement), dioramas, and manipulatives (Carter and Wilson, 2003).

A kinesthetic-tactile learners may prefer to study using the materials just listed. The learner may prefer to study by re-writing his/her notes, typing them on a typewriter, or writing them on a computer. In fact, a kinesthetic-tactile learner may need to write, and re-write his/her school notes, over and over and over again, in order to study. He/she may like to use sign language, physically using hand and finger movements, to help remember concepts. Pretending to write words in the air, or on one's leg is another method (Neal, 2008).

A kinesthetic-tactile learner needs to move, build, investigate, and physically create concepts. Unconventional study methods involving movement may be employed. Bouncing a ball, doing jumping jacks, or jumping rope, while saying ones study information (cadence), are examples of study activities requiring movement (Management Education, 2002). Drumming, tapping one's feet, or marching, while reciting information, are other examples. Kinesthetic-tactile learners will focus on the physical movement to the rhythm to support their learning. Having the learner write the information in large letters on a blackboard, requires large muscle movements. Writing information with your finger, in a tray lightly covered in sand, is another tactile presentation. The idea is to add movement and touch to any learning activity.

If the kinesthetic-tactile learner is presented with an activity that is not highly kinesthetic-tactile in nature, change the activity to accommodate the learner's needs. For example, let's say the kinesthetic-tactile learner must remember the information presented at a lecture (Campbell, 2006). A lecture is primarily an auditory presentation (using hearing rather than touch and movement). An example of changing this activity to a kinesthetic-tactile presentation might be to get permission to tape the lecture (Schunck, 2001). Later, the learner would listen to the tape at home and physically act out the information. The learner needs to be able to add touch and movement to the presentation. Let's say the learner wants to remember specific social studies facts. The learner might play a game of social studies charades, where classroom peers in a study group would have to guess the concept that was being acted out. If the learner is in school, try to find teachers that use hands-on activities during their presentations.

Multi-sensory learning defined

A multi-sensory learner learns best when visual, auditory, and kinesthetic-tactile presentation methods are all employed to learn a particular concept. This means that the more the learner is able to see, hear, touch, manipulate the materials used to present the information, and use his/her body movements, the easier it may be for that learner to learn the information (Bourdieu, 2004).

To determine the types of materials, a multi-sensory learner might use, look at the suggestions for visual learners, auditory learners, and kinesthetic-tactile learners above. Basically, you are combining these three presentation methods, when you employ a multi-sensory method. Look at your visual, auditory, and kinesthetic-tactile scores from the previous page. You will find that you scored almost the same in either two, or three of these learning style categories (Smith, 2006). You don't have one particular learning style preference, but rather a combination of two or three styles. The discussion below

Table 1. Word List Recognition - Dodson and Shimamura (2000) asked subjects if the first list of words also appeared in the second list of words.

LIST 1	LIST 2
Shell	Doctor
Radio	Fleet
Doctor	Midnight
Table	Thread
Window	Reason
-	Window

will focus on a multi-sensory learner that needs all three learning style areas (Stout, 2007). If your scores suggest you only need two learning style areas, adjust your presentations accordingly.

In regards to studying, a multi-sensory learner will need to combine study methods from the visual, auditory, and kinesthetic-tactile areas outlined above. For example, you might look at a learning fact on an index card, while reading the fact out loud, followed by re-writing the fact in large letters on a chalkboard, and tracing your finger through the chalk letters while repeating the words.

If the multi-sensory learner is presented with an activity that is not highly multi-sensory in nature, change the activity to accommodate the learner's needs. For example, let's say the multi-sensory learner must remember the information presented at a lecture. A lecture is primarily an auditory presentation (using hearing). An example of changing this activity to a multi-sensory presentation might be to get permission to tape the lecture (Smith, 2006). Later, the learner would listen to the tape at home, write down notes in a notebook, use a graphic organizer (a kind of written diagram used for outlining or seeing relationships between concepts), and physically act out the information (Richardson and Davis, 2007). Given the above discussion, the following three hypotheses become necessary:

H1: Students will prefer a teaching method that is consistent with their learning style.

H2: Students preferences in teaching method, the course subject, and situational factors (i.e. class size) are related to instructors' preference in teaching method and use of technological advancements

H3: Instructors will have higher perceived teaching effectiveness when using their preferred teaching method.

MATERIALS AND METHODS

Learning style inventory

The population frame for this study comprised of 100 full-time faculty members and 100 students. Using the Directory of Management Faculty (Hasselback, 2001), a stratified sample (by rank) was drawn

from randomly selected institutions. Of the 200 questionnaires mailed, 50 were returned, producing a response rate of 25.0%.

The questionnaire developed for the study contained the Learning Style Inventory following by characteristics of each learning style. The LSI was composed of 24 questions to assess how the participant prefers to learn and process information, using a modified version of Barsch and Haynie's (Wills and Hodson, 2009) learning style inventory.

Items 1 through 24 of the survey measured instructor preferred learning style: Auditory, Kinesthetic-Tactile, and Visual. In order to ascertain whether the measures still retained construct validity, a factor analysis was conducted using a varimax rotation and an orthogonal solution (Tables 2 and 3). Cumulative variance explained by the three factors was 52%. Additionally, internal reliability tests showed Cronbach alphas ranging from 0.51 through 0.61 (Table 5). This factor analysis method was used in order to produce variables representative of each learning style that could then be used in subsequent analysis. Surrogate variables were selected since the scales we used are exploratory with little evidence of reliability or validity (Hair et al., 2002).

RESULTS

This section provides preliminary results of the findings of this study and, at this time, is limited to correlations between the variables in the study. Results show that those instructors who are visual learners tended to prefer "mainly lecture with voluntary student participation" (0.250, $p < 0.005$) and "mainly lecture with some student discussion groups" (0.257, $p < 0.004$).

Results for the other two learning styles did not prove to be significant. Of the other factors examined (perception of student preferences, course subject, or class size and facilities), only class size and facilities was found to significantly impact the use of particular learning methods. Specifically, those instructors who prefer to use "mainly lecture with voluntary student participation" indicated that classroom environmental factors (class size, layout of classroom, etc.) limited their effective use of this method (0.187, $p < 0.037$). Finally, in assessing instructor's perception as to the impact of their preferred method on teaching effectiveness, instructors who utilized "lecture only with no student participation" believe that this preferred teaching method greatly enhances their teaching effectiveness (0.187, $p < 0.038$).

With regard to teaching technologies, results show that those instructors who are auditory learners show a preference for using videos (0.181, $p < 0.044$) and those who are KT learners show a preference for PowerPoint presentations (0.241, $p < 0.007$). Results for visual learners were not significant. Auditory learner instructors showed a preference for technology that was consistent with their learning style. While KT learner instructors showed a technology preference, their preference was not as consistent with their learning style needs.

Although an instructor's assessment of student preferences for various technologies was not found to be significantly related to his/her technology preference, there was some support for H5 with regard to other factors. Instructors who prefer the use of simulations

Table 2. Learning Styles Inventory- This is a 24 question survey designed to determine the best learning style.

S/N	Variable	Often	Sometimes	Seldom
1	I can remember best about a subject by listening to a lecture that includes information, explanations and discussions	-	-	-
2	I prefer to see information written on a chalkboard and supplemented by visual aids and assigned readings	-	-	-
3	I like to write things down or to take notes for visual review	-	-	-
4	I prefer to use posters, models, or actual practice and other activities in class	-	-	-
5	I require explanations of diagrams, graphs, or visual directions	-	-	-
6	I enjoy working with their hands or making things	-	-	-
7	I am skillful with and enjoy developing and making graphs and charts	-	-	-
8	I can tell if sounds match when presented with pairs of sounds	-	-	-
9	I can remember best by writing things down	-	-	-
10	I can easily understand and follow directions on a map	-	-	-
11	I do best in academic subjects by listening to lectures and tapes	-	-	-
12	I play with coins or keys in their pocket	-	-	-
13	I learn to spell better by repeating words out loud than by writing the words on paper	-	-	-
14	I can understand a news article better by reading about it in a newspaper than by listening to a report about it on the radio	-	-	-
15	I chew gum, smoke or snack while studying.	-	-	-
16	I think the best way to remember something is to picture it in your head	-	-	-
17	I learn the spelling of words by "finger spelling" them	-	-	-
18	I would rather listen to a good lecture or speech than read about the same material in a textbook	-	-	-
19	I am good at working and solving jigsaw puzzles and mazes	-	-	-
20	I grip objects in their hands during learning periods	-	-	-
21	I prefer listening to the news on the radio rather than reading the paper	-	-	-
22	I prefer obtaining information about an interesting subject by reading about it	-	-	-
23	I feel very comfortable touching others, hugging, handshaking, etc.	-	-	-
24	I follow oral directions better than written ones	-	-	-

Table 3. Learning styles inventory scoring procedures- Follow the directions above to attribute the correct score.

Visual		Auditory		Tactile	
No.	PTS.	No.	PTS.	No.	PTS.
2	-	1	-	4	-
3	-	5	-	6	-
7	-	8	-	9	-
10	-	11	-	12	-
14	-	13	-	15	-
16	-	18	-	17	-
19	-	21	-	20	-
22	-	24	-	23	-
VPS =	-	APS =	-	TPS =	-
VPS = Visual preference		APS = Audio preference		TPS = Tactile preference	

Learning style inventory (Directions: Place the point value on the line next to the corresponding item below. Add the points in each column to obtain the preference score under each heading; OFTEN = 5 points; SOMETIMES = 3 points; SELDOM = 1 points).

were less likely to see course subject as limiting their use of technology (-0.196. $p < .029$). Those who prefer the use

of experiential exercises believe that classroom environmental factors (class size, layout of classroom,

Table 4. Characteristics of a visual, auditory, and kinesthetic-tactile learning-This is analyzed after completing the learning styles inventory (LSI).

Visual learning characteristics
Mind sometimes strays during verbal activities
Observe rather than acts or talks
Likes to read
Usually a good speller
Memorizes by seeing graphics or pictures
Not too distractible
Finds verbal instruction difficult
Has good handwriting
Remembers faces
Uses advanced planning
Doodles
Quiet by nature
Meticulous, neat in appearance
Notices details
Characteristics of auditory learning
Talks to self aloud
Enjoys talking
Easily distracted
Has difficulty with written directions
Likes to be read to
Memorizes sequentially
Enjoys music
Whispers to self while reading
Distracted by noise
Hums or sings
Outgoing by nature
Enjoys listening activities
Characteristics of kinesthetic-tactile learning
Likes physical rewards
In motion most of the time
Likes to touch people when talking
Taps pencil or foot when studying
Enjoys doing activities
Reading not a priority
Poor speller
Likes to solve problems by physically working through them
Will try new things
Outgoing by nature; expresses emotions by physical means
Uses hands while talking
Dresses for comfort

availability of technologies) limit their use of this technology (0.195, $p < 0.03$). Those instructors who feel pressured by their institution to use certain technologies were those who preferred computer simulations (0.185, $p < .04$) or email and web pages (0.277, $p < 0.002$). Finally,

in assessing instructor's perception as to the impact of their preferred technology on teaching effectiveness, no significant results were found, providing no support for H6. It is interesting to note, however, that those who perceived their preferred teaching method as enhancing

Table 5. Factor analysis of learning styles- Scored were assessed in the areas of auditory learning, visual learning, and kinesthetic-tactile learning. With regard to teaching technologies, results show that those instructors who are auditory learners show a preference for using videos (0.181, $p < .044$) and those who are KT learners show a preference for PowerPoint presentations (0.241, $p < .007$). Results for visual learners were not significant.

Variable	Factor 1 kinesthetic-tactile (KT)	Factor 2 auditory	Factor 3 visual
The researcher enjoys working with his or her hands or making things	0.79	-	-
The researcher is good at working and solving jigsaw puzzles and mazes	0.64	-	-
The researcher is skillful and enjoys developing and making graphs and charts	0.58	-	-
The researcher prefers to use posters, models, or actual practice and other activities to help me learn.	0.57	-	-
The researcher grips objects in their hands during learning periods	0.41	-	-
The researcher feels very comfortable touching others, hugging, handshaking, etc	0.37	-	-
The researcher would rather listen to a good lecture or speech than read about the same material in a book or journal	-	0.80	-
The researcher retains academically related material best by listening to presentations and tapes	-	0.66	-
The researcher prefers listening to the news on the radio rather than reading about it in the newspaper	-	0.63	-
The researcher follows oral directions better than written ones	-	0.51	-
The researcher remembers best by writing things down several times	-	-	0.72
The researcher requires explanations of diagrams, graphs, or visual directions	-	-	0.62
The researcher likes to write things down or to take notes for visual review	-	-	0.59
The researcher prefers to see information on a chalkboard and supplemented by visual aids and assigned readings	-	-	0.46

their teaching effectiveness also believed that their preferred technology enhanced their teaching effectiveness (0.415, $p < .000$).

DISCUSSION AND CONCLUSION

The results of this study indicate that selected factors are related to an instructor's preferred teaching method and preferred use of technology for instructional support. Although it was expected that instructors' learning style would be consistent with preferences in teaching methods, this was not the case. Significant results for instructors who are visual learners yielded findings more consistent with what one expect for auditory learners (for example, lectures with student participation or discussion

groups). It is possible that instructors, recognizing their own learning style, choose to utilize teaching methods that they believe will cater to other types of learners. These findings require further investigation. Not surprising, classroom environmental factors (class size, class layout) do appear to limit those who wish to use "lecture with voluntary student participation." Large classes, which are becoming increasingly common on many university campuses, do not allow for such participation on the part of students. It also appears that a number of faculty members believe that using a "lecture only with no student participation" greatly enhances their teaching effectiveness, supporting the idea that some faculty members continue to feel very comfortable with the traditional lecture method of teaching. Learning style was also found to be related to one's preferred choice in

technology use. For example, instructors who are auditory learners showed a preference for using videos that is consistent with the needs of that particular learning style. Instructors with the KT learning style indicated a preference for PowerPoint presentations, which is less consistent with their learning needs. This finding requires further investigation. It was interesting to note that, with the use of simulations becoming more popular, the course subject did not appear to be limiting factor for those instructors who prefer to use such technology. This may also be influenced by the fact that there are now more computer simulations available for different courses (for example, International Business, Strategic Management, Human Resource Management), as opposed to being limited to one or two course subjects.

Classroom environmental factors also were found to restrict the type of technology used in courses, particularly the use of experiential exercises. Of the environmental factors listed, it is likely that class size would play the biggest role in using this technology. Institutional pressure also influenced the use of technology. Those who indicated that they experienced institutional pressure to use technology were those who preferred computer simulations or email and web pages. It is difficult to determine if the pressure experienced to use technology was for specific types of technology that may or may not be consistent with one's preferences. For example, an instructor may experience institutional pressure to use on-line technology but their preference is to use simulations.

Overall, this study produced some interesting findings, indicating some significant relationships between teaching methods, learning styles, use of technology, and factors that might influence either of these.

Conflict of Interests

The author has not declared any conflict of interests.

REFERENCES

- Bandura A (2002). Social foundations of thought and action. *The health psychology reader*. pp. 94-106.
- Bourdieu P (2004). *Distinction: A social critique of the judgment of taste*, translated by Richard Nice. Cambridge, MA: Harvard University Press.
- Breishline MJ, Holmes CB (2007). Student preference for various teaching styles. *J. Instr. Psychol.* 24:95-99.
- Campbell A (2006). Subjective measure of well-being. *Br. Psychol.* 31:87-124.
- Carter DJ, Wilson R (2003). *Minorities in higher education: 2002 eleventh annual status report*. Washington, DC: British Council on Education.
- Csikszentmihalyi M (2003). *The evolving self: A psychology for the third millennium*. New York: Harper Perennial.
- Dodson CS, Slotnick SD, Klein SA, Shimamura AP (2000). An analysis of signal detection and threshold models of source memory. *J. Exp. Psychol.* 26(6):1499-1517.
- Duncum P (2007). Art education for new times. *Stud. Art Educ.* 38(2):68-128.
- Erikson EH (2008). *Identity, youth, and crisis*. New York: Norton.
- Erikson EH (2000). *Identity and the life cycle (2nd Ed.)*. New York: Norton.
- Frost PJ, Fukami CV (2007). Teaching effectiveness in the organizational sciences: Recognizing and enhancing the scholarship of teaching. *Acad. Manag. J.* 40(6):1271-1281.
- Hair JF, Anderson RE, Tatham RL, Black WC (2002). *Multivariate Data Analysis with Readings (3rd edition)*. New York: Macmillan.
- Hasselback JR (2001). *Directory of Management Faculty*. Boston: McGraw-Hill.
- Hudak MA, Anderson DE (2004). Teaching styles and student ratings. *Teaching Psychol.* 11:177-184.
- Management education at risk (2002). Report of the AACSB Management Education Task Force to the AACSB International Board of Directors (August).
- Marcia J (2000). Ego, identity development. In: J. Andelson (Ed.), *The handbook of adolescent psychology*. New York: Wiley.
- Marcia J (2007). The identity status approach to the study of ego identity development. In: *Self and Identity: Perspectives across the life span*, Hones T, Yardley K (Eds.), London: Routledge & Kagan Paul.
- Neal E (2008). Using technology in teaching: We need to exercise healthy skepticism. *Chronicle of Higher Education*.
- Richardson TR, Kring JP, Davis SF (2007). Student characteristics and learning or grade orientation influence preferred teaching style. *Coll. Stud. J.* 31:347-355.
- Pankratz DB, Morris VB (2000). *The future of the arts: Public policy and arts research*. New York: Praeger.
- Schiedel D, Marcia J (2005). Ego integrity, intimacy, sex-role orientation, and gender. *Dev. Psychol.* 21:149-160.
- Schunck DH (2001). Self-efficacy and academic motivation. *Educ. Psychol.* 26:207-232.
- Smith RA (2006). *Excellence in art education: Ideas and initiatives*. Reston, VA: National Education Association.
- Stout CJ (2007). Multicultural reasoning and the appreciation of art. *Stud. Art Educ.* 38(2):96-111.
- Williams L (2003). *Teaching to the two-sided mind*. New York: Simon and Schuster.
- Wills M, Hodson VK (2000). *Discover your child's learning style*. Rocklin, CA: Prima Publishing.

Full Length Research Paper

Neuroprotective effect of Silibinin against middle cerebral artery occlusion induced focal cerebral ischemia and brain injury in Wistar rats

Shivani Chauhan*¹, Lubhan Singh¹, Kedarinath Talishetty² and Vipul Kumar¹

¹Department of Pharmaceutical Technology, Meerut Institute of Engineering and Technology, Meerut, UP, India.

²Department of Pharmacology, Krupanidhi College of Pharmacy, Bangalore, Karnataka, India.

Received 23 May, 2015; Accepted 27 July, 2015

Silibinin (Silybin) as an active constituent derived from milk thistle (*Silybum marianum*) has been shown to have antioxidant and anti-apoptotic properties. In traditional system of medicine, *Silybum marianum* has been used for treating various kinds of ailments including liver disease and cancer; however, clinical studies are largely heterogeneous and contradictory. The present study was designed to investigate whether Silibinin protects neuronal injury against middle cerebral artery occlusion induced oxidative stress associated damages in focal cerebral ischemia in Wistar rats. Rats weighing 250 to 300 g were pretreated with Silibinin 100 mg/kg and 200 mg/kg body weight, suspended in 0.5% of gum acacia) once daily for seven days. On 8th day, they underwent for middle cerebral artery 2 h suture occlusion by nylon suture. After 120 min of middle cerebral artery occlusion (MCAO) and 22 h of reperfusion, behavioral tests were assessed in terms of neurological deficits. Animals were sacrificed and infarct volume in Triphenyltetrazolium chloride (TTC) stained brain sections was measured. Further, various oxidative biomarkers were estimated in brain homogenates of rats. Pretreatment with Silibinin at doses of 100 and 200 mg/kg significantly improved the neurobehavioural alterations and reduced the infarct volume whereas 200 mg/kg Silibinin significantly increases the Glutathione (GSH) level and the others parameters with respect to control values. These results clearly indicate the neuroprotective effect of Silibinin against middle cerebral artery occlusion associated with oxidative damage induced brain injury due to its antioxidant and anti-apoptotic property.

Key words: Neuroprotective, MCAO, Silibinin, oxidative stress, infarct volume, cerebral ischemia.

INTRODUCTION

Ischemic stroke and neuronal death is the second major cause of death worldwide (Van derWorp and van Gijn, 2007). Cerebral ischemia produces oxygen and glucose deficiency due to reduction and complete blockade of

blood that flows to regions of brain (Zemke et al., 2004). This occlusion, thus damaging physiological activity of brain leads to oxidative stress and further neuronal damage. Ischemic reperfusion injury can causes cellular

*Corresponding author. E-mail: shivanichauhan232@gmail.com

damage by cascade of events such as release of free radicals and cytokines, induction of inflammation, apoptosis, and excitotoxicity that affect structure and function of brain (Kuroda and Siesjo, 1997). It has suggested that oxidative stress, excitotoxicity, inflammation and apoptosis are the major mechanisms that should be involved in the pathogenesis of ischemia/reperfusion injury (Bondy 1995; Lipton 1999). The ischemic mediated neuronal damage might be a result of mitochondrial dysfunctioning of resident cell that disrupts the organized redox balance, and therefore initializing the cascade of damaging events which supports the necrotic and apoptotic cell death pathway (Takemura et al., 1993; Courtois et al., 1998). It has been evidently concluded that oxidative stress is the major mechanism of brain damage in occlusion and reperfusion. That's why it has been concluded that occlusion of middle cerebral artery (MCA) by using nylon suture is a widely suggested experimental model of cerebral ischemia for neuroprotective drug development as it closely resembles stroke injury seen in human patients (Carmichael, 2005; Durukan and Tatlisumak, 2007).

Chain (2001) evidently concluded that oxidative stress is the major mechanism of brain damage in occlusion and reperfusion. In recent years, herbal medicines has been gaining much interest, as these appears to be safe and effective treatment for combating neurodegenerative diseases associated with oxidative damage. It has been reported that the formulations and plant extracts which have antioxidant properties, protect the neurons against ischemic and reperfusion injury (Zhu et al., 2004; Shukla et al., 2006). Among herbal medicines, Silibinin categorized under the group of flavonolignans, that is extracted from milk thistle plant *Silybum marianum*. Silibinin is a potent hepatoprotective used every day in clinical practice for treating liver disorders; it could prevent lipid peroxidation, and also increase antioxidative enzyme levels. It has already been shown at the neuroprotective effect of Silibinin in diabetic mice by Tota et al. (2011).

MATERIALS AND METHODS

Chemicals

1-Chloro-2, 4-dinitrobenzene (CDNB), reduced glutathione (GSH), 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), 2, 3, 5-triphenyltetrazolium chloride (TTC), thiobarbituric acid (TBA), nitroblue tetrazolium (NBT) and trichloroacetic acid (TCA) were purchased from SD fine chemicals, Mumbai, India. All the other chemicals used were of analytical grade.

Animals

Adult male Wistar rats were obtained from Animal house of Meerut Institute of Engineering and Technology, Meerut India. The rats weighing 250±10 g were used at the start of the experiment. Rats

were housed under standard laboratory conditions, maintained at an ambient temperature of 25±2°C and relative humidity of 45 to 55% with 12 h light: 12 h dark cycle and had free access of food and water. The food was withdrawn for 12 h before the surgical procedure. All the procedures performed were in accordance with Control and Supervision of Experiments on Animals (CPCSEA) guidelines, under Ministry of Animal Welfare Division, Government of India, and New Delhi.

Middle cerebral artery occlusion (MCAO) to induce focal cerebral ischemia

MCAO was carried out according to the procedure described by Koizumi et al. (1986). Rats were anesthetized with Ketamine and Diazepam (70 mg/kg and 5 mg/kg, i.p. respectively) and placed in dorsal recumbency. A longitudinal incision of 1 cm in length was made in the midline of the ventral cervical skin. The right common carotid artery at the level of internal and external carotid artery bifurcation were exposed and carefully isolated. A nylon monofilament (40 mm in length and 0.24 mm in diameter), whose tip was rounded by exposing to flame was inserted from the lumen of the common carotid artery to that of the right ICA to occlude the origin of the right middle cerebral artery (MCA). The right MCA was occluded for 60 min, and thereafter the filament was withdrawn and allowed to be reperfused with blood. 24 h after reperfusion, rats were decapitated. Animals were maintained in a warm condition using a hot blower throughout the surgical procedure.

Post-operative care

After 4 to 5 h of surgery, recovery of anesthesia took place. The animals were kept in a maintained temperature at 25±3°C in individual cages until they gained full consciousness, and then they were kept together in a group of 3 animals per cage. Food with ad lithium water was kept inside the cage for 24 h, so that the animal could easily access it.

Experimental design

Animals were divided into five groups. The first group was sham operated (n=7) (animals were subjected to surgical procedure, but did not occluded MCA except for exposure of ICA and ECA), received 0.5% gum acacia, 10 ml/kg orally; second was MCAO group only (n=12); third was standard group (n= 12) received aspirin before MCAO; fourth was MCAO group pretreated with Silibinin (100 mg/kg in 0.5% gum acacia, orally) (n=12); and fifth was MCAO group pretreated with Silibinin (200 mg/kg in 0.5% gum acacia, orally) (n=12). Vehicle or drugs were fed once daily for 7 consecutive days prior to experimental procedure. After completion of reperfusion period, animals were assessed for neurobehavioural activity, and then sacrificed for biochemical estimations.

Dose selection and drug administration

A dose of 100 and 200 mg/kg of Silibinin was selected on the basis of previous literature of survey (Tota et al., 2011; Lu et al., 2009). These doses have also shown the maximal protection in various types of brain diseases. Rats were pretreated systematically with 100 and 200 mg/kg Silibinin orally suspended in 0.5% gum acacia, once daily for seven consecutive days. On day 8th, MCAO was performed for 120 min followed by 22 h reperfusion (Longa et al., 1989).

Neurological function assessment

After 22 h of reperfusion, neurological test was carried out by an examiner to the experimental groups before the rats were sacrificed. The deficits were scored on a modified scoring system described by Longa et al. (1989), as; 0 = rats moved around in the cage and explored the environment; 1= rats moved in the cage but did not approach to all the sides and hesitated to move; 2= rats barely moved in the cage and showed postural abnormalities curved towards the paretic side; 3= rats unable to move at all with their posture curved towards the paretic side.

Locomotor activity

The spontaneous locomotor activity was recorded by using actophotometer equipped with infrared sensitive photocells. Before locomotor task, animals were placed individually in the actophotometer cage for 2 min for habituation. Thereafter, locomotor activity was recorded for a period of 5 min. The ambulatory activity was expressed in terms of total photo beam counts per 5 min as described by Kulkarni (1999).

Biochemical estimations

The animals were sacrificed under deep ether anesthesia, perfused transcardially with ice-cold saline and the brains were dissected out. A 10% brain homogenate was prepared with ice-cold phosphate buffered saline and was centrifuged at 10,000 rpm at -4°C for 15 min, and the supernatant obtained was used for biochemical estimations.

Estimation of reduced glutathione (GSH)

GSH was measured by the method of Ellman (1959). Equal quantity of brain homogenate was mixed with 10% TCA and centrifuged to separate proteins. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of DTNB and 0.4 ml double-distilled water was added. Mixture was vortexed and the absorbance was recorded at 412 nm. The concentration of GSH was expressed as nmol/mg of protein.

Estimation of tissue levels of malondialdehyde (MDA)

The minced brain was homogenized in a buffer containing 30 mM Tris-HCl and 2.5 mM CaCl₂ (pH 7.6). To separate cellular debris the homogenate was centrifuged at 750 gyrations. The supernatant was accurately divided into two portions and centrifuged at 8200 gyrations to obtain mitochondrial fraction. One fraction was utilized for determination of MDA (Yagi, 1982), and the other one was employed for protein estimation (Lowry et al., 1951; Kakkar et al., 1984). The concentration of MDA in brain homogenates was expressed in terms of nM MDA/mg protein.

Estimation of superoxide dismutase (SOD)

SOD activity in cerebral tissues of all the rats were estimated by using the technique of Kakkar et al. (1984), based on inhibition of the formation of nicotinamide adenine dinucleotide, PMS and NBT formazan. Briefly, to 10 µl of homogenate was added 90 µl of 30 mM sodium tetrapyrophosphate buffer (pH 8.3), 30 µl of 0.3 mM NBT, 10 µl of 0.96 mM PMS and 40 µl of double-distilled water. Reaction was initiated by addition of 20 µl 0.72 mM NADH.

Absorbance was measured at 560 nm. A single unit of enzyme was expressed as 50% inhibition of NBT reduction/min/mg protein. Results were expressed as unit of SOD/min/mg protein.

Estimation of catalase (CAT)

CAT was assayed using the method of Aebi (1984). Briefly, the assay mixture of 1.5 ml contained 980 µl of 50 mM sodium phosphate buffer pH 7.0 and 20 µl of homogenate (10 to 15 µg protein). Reaction was started by addition of 500 µl of 30 mM hydrogen peroxide. The decrease in absorbance was then observed for 60 s at every 15 s interval at 240 nm. CAT activity was expressed as U/mg protein.

Estimation of nitrite (NO)

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined by a colorimetric assay with Greiss reagent according to Green et al. (1982). The absorbance was measured at 540 nm using UV spectrophotometer. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve.

Estimation of protein

Protein was determined by the method of Lowry et al. (1951) using BSA as a standard. Protein was measured in all brain samples for GSH, MDA, SOD, CAT and nitrite. BSA (1 mg/ml) was used as standard and measured in the range of 0.01 to 0.1 mg/ml.

Measurement of infarct volume

Rats were sacrificed and their brains were quickly removed and sectioned coronally into slices each with a 2 mm thickness. The brain slices were then immersed in 2% triphenyltetrazolium chloride (TTC) for 30 min at 37°C and then fixed with formalin. Infarcted areas were identified as regions lacking brick red staining of normal brain tissues. It was expressed as mm³.

Statistically analysis

The values were expressed as mean ±S.E.M. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey's post hoc test. P values <0.05 were considered as significant.

RESULTS

Effect of Silibinin on percent survival of animals after MCAO

After MCAO for 120 min and 22 h reperfusion, approx 50% of animals survived in the vehicle treated group. Pretreatment with Silibinin at 100 and 200 mg/kg increased the survival rate significantly to 66.6 and 83.3% respectively. There was no mortality in sham operated rats treated with vehicle (Table 1).

Table 1. Effect of Silibinin on percent survival after MCAO in rats.

Treatment	No. of animals survived/ used	Survival (%)
Sham operated	7/7	100
MCAO	6/12	50
Standard (Aspirin + MCAO)	11/12	91.66
Silibinin (100 mg/kg) + MCAO	8/12	66.66
Silibinin (200 mg/kg) + MCAO	10/12	83.33

Table 2. Effect of Silibinin on behavioral parameters after MCAO in rats.

Treatment (no of animals used)	Neurological score	Locomotor activity (counts/5 min)
Sham operated (n = 7)	0	130.83±5.73
MCAO (n = 12)	2.83±0.30	27.83±4.28 ^{d, ***}
Standard (Aspirin + MCAO) (n = 12)	0.33±0.21 ^{b, ***}	98.5±5.34 ^{b, ***, e **}
Silibinin (100 mg/kg) + MCAO (n = 12)	1.66±0.2 ^{a, *}	47.33±4.37 ^{c, ***, f ***}
Silibinin (200 mg/kg) + MCAO (n= 12)	1.16±0.30 ^{a, **}	87.16±5.85 ^{a, ***, f ***}

Values are expressed as mean ± S.E.M. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post test. ^a = p < 0.05 MCAO vs. Silibinin treated group. ^b = p < 0.05 MCAO vs. standard, ^c = p < 0.05 standard vs. Silibinin treated group, ^d = p < 0.05 sham operated vs. MCAO, ^e = p < 0.05 sham operated vs. standard, ^f = p < 0.05 sham operated vs. Silibinin treated group.

Effect of Silibinin on neurological deficit

The spontaneous motor activity showed no neurological deficits in sham operated rats, while in MCAO group followed by reperfusion for 22 h caused marked change in the behavior of the animals. In spontaneous motor activity test, MCAO animals spend most of the time in the center of the cage with posture curved toward the paretic side. The animals in the vehicle treated group subjected to ischemic reperfusion injury exhibited severe neurological deficit (score: 2.83±0.30), and showed circling towards the contralateral side and had a reduced mobility when compared to the sham operated animals. The rats in the Silibinin 100 and 200 mg/kg treated animals subjected to MCAO showed significant (p < 0.05) improvement in the behavior when compared to the ischemic rats (score: 1.66±0.21 and 1.16±0.30 respectively) (Table 2).

Effect of Silibinin on locomotor activity

In comparison to the sham operated rats, the locomotor activity of the MCAO group rats were significantly decreased (p < 0.05). A dose dependent increase in locomotor activity was observed in Silibinin pretreated groups. Pretreatment with Silibinin 100 mg/kg in ischemic rats showed an increase in locomotor counts, whereas Silibinin 200 mg/kg pretreated ischemic rats exhibited a significant increase (p < 0.05) in the locomotor counts when compared to vehicle treated rats (Table 2).

Effect of Silibinin on tissue Glutathione (GSH) in MCAO rats

The brain glutathione levels were estimated in all the groups. Level of reduced glutathione in MCAO rats were significantly reduced as compared to sham operated rats whereas in all pretreated groups glutathione were significantly increased when compared with MCAO group (Table 3).

Effect of Silibinin on tissue Lipid Peroxidation (LPO) or MDA in MCAO rats

The MDA level measured after 24 h of middle cerebral artery occlusion were found to be significantly increased in the MCAO rats than in normal rats. High dose of Silibinin produced significantly reduction in MDA levels when compared to that of MCAO group (Table 3).

Effect of Silibinin on tissue superoxide dismutase (SOD) in MCAO rats

The level of SOD after 22 h of reperfusion in MCAO occluded group were significantly reduced as compared to the normal rats. In both Silibinin pretreated group, levels of SOD were significantly increased as compared to the MCAO group (Table 3).

Table 3. Effect of Silibinin on brain oxidative and nitrative stress parameters after MCAO in rats.

Treatment (no of animals used)	LPO (nmoles of MDA/mg protein)	CAT (U/mg protein)	SOD (U/min/mg protein)	GSH (nmoles/mg protein)	Nitrite (nmoles/mg protein)
Sham control (n = 7)	2.817±0.13	16.95±1.35	12.06±0.33	3.00±0.080	62.41±0.22
MCAO (n = 12)	4.656±0.38 ^{a,*}	8.51±0.50 ^{a,***}	6.02±0.40 ^{a,***}	1.00±0.069 ^{a,***}	111.71±0.38 ^{a,***}
Standard + MCAO (n = 12)	2.419±0.50 ^{b,*}	13.26±0.20 ^{a,**,b,***}	9.69±0.30 ^{a,***,b,***}	2.35±0.20 ^{a,*;b,***}	55.72±0.12 ^{a,***,b,***}
Silibinin (100 mg/kg) + MCAO (n = 12)	3.966±0.85	9.77±0.32 ^{a,***,c,**}	7.29±0.12 ^{a,***,b,*;c,***}	1.37±0.05 ^{a,***,c,***}	64.23±0.21 ^{a,***,b,***,c,***}
Silibinin (200 mg/kg) + MCAO (n = 12)	2.638±0.10 ^{b,*}	12.46±0.22 ^{a,***,b,**}	9.09±0.20 ^{a,***,b,***}	2.12±0.17 ^{a,***,b,***}	53.46±0.18 ^{a,***,b,***,c,***}

Values are the means SEM. ^ap < 0.05 as compared to sham operated; ^bp < 0.05 as compared to MCAO group; and ^cp < 0.05 as compared to pretreated group with standard + MCAO (repeated measures one-way ANOVA followed by Tukey's test for multiple comparisons).

Effect of Silibinin on tissue catalase (CAT) in MCAO rats

The levels of catalase were reduced in MCAO occluded group as compared to normal rats. All Silibinin pretreated rats showed elevation in the levels as compared to the MCAO group (Table 3).

Effect of Silibinin on tissue nitrite (NO) level in MCAO rats

The NO level measured after 24 h of middle cerebral artery occlusion were found to be significantly increased in the MCAO rats than in normal rats. High dose of Silibinin produced significantly reduction in NO levels when compared to that of MCAO group (Table 3).

Effect of Silibinin on infarct volume in MCAO rats

TTC dye staining of brain slices of all Silibinin pretreated animals showed significant improvement in the infarct volume, aspirin, and Silibinin 200 mg/kg pretreated animals showed a highly significant reduction in infarct volume as compared to MCAO group (Figure 1).

DISCUSSION

In the present study critically evaluated the most abundant naturally occurring flavonoid Silibinin isolated from *S. marianum* as a potential new prophylactic anti-oxidative and anti-apoptotic target in cerebral stroke. MCAO is a classical and well-characterized experimental model of cerebral ischemia. The cytotoxic response occurs within minutes after the onset of cerebral ischemia, which then encompasses proinflammatory response, apoptosis, oxidative stress and neurological damage. Characteristically, ischemic/reperfusion induced brain injury is associated with biochemical, behavioral and histopathological alterations which is seen to be well ameliorated with the pretreatment of Silibinin. Herein, the study observed that Silibinin prevents cerebral ischemia/reperfusion injury by ameliorating oxidative damage. The development of pathogenesis of cerebral ischemia, which is associated with an increased production of free radicals, specifically hydroxyl radical, superoxide, higher lipid peroxidation and lower enzymatic antioxidant defenses (Halliwell 1992; Dringen et al., 2000). The above damages can be prevented by detoxification of free radicals. Beneficial effects of various antioxidants and free radical scavengers

in ischemic investigate the pathology of cerebral ischemia. However, the success behind these models lies only when they can reproduce the end results. Silibinin at dose of 200 mg/kg BW treatment (p.o.) showed significant reduction in brain infarct volume in MCAO induced focal cerebral ischemia model of stroke in rats; and improves brain impairment due to its antioxidant effect. Silibinin at dose of 100 mg/kg also decreases oxidative stress induced lipid peroxidation reaction and increased glutathione peroxidase with increased activity of SOD.

Conclusion

Conclusively, the present study demonstrates that Silibinin possess Neuroprotective effect against ischemia-reperfusion induced brain oxidative damage in rats.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

The authors thank the Department of

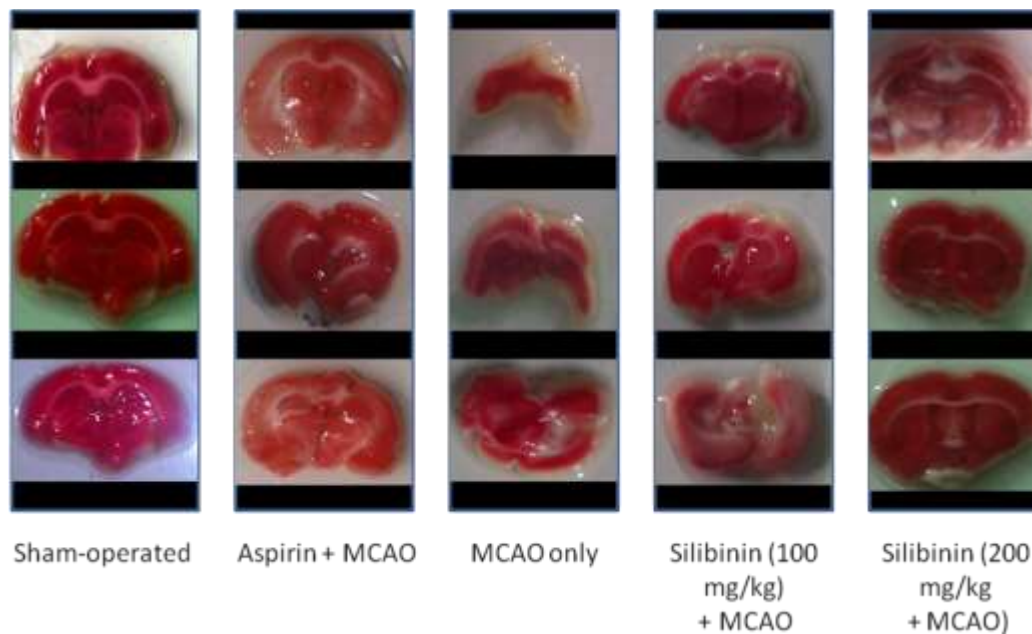


Figure 1. Effect of Silibinin on ischemic rat brains. The infarct volume is significantly reduced in Silibinin (200 mg/kg) group and aspirin group when compared with MCAO only.

Pharmaceutical Technology, MIET Meerut, India.

REFERENCES

- Aebi H (1984). Catalase *in-vitro*. Methods of Enzymology, Academic Press 113:121-126.
- Bondy SC (1995). The relation of oxidative stress and hyperexcitation to neurological disease. Proc. Soc. Exp. Biol. Med. 208:337-345.
- Carmichael ST (2005). Rodent models of focal stroke: size, mechanism, and purpose. NeuroRx. 2:396-409.
- Chan PH (2001). Reactive oxygen radicals in signaling and damage in the ischemic brain. J. Cereb. Blood Flow Metab. 21:2-14.
- Courtois M, Maupoil V, Fantini E, Durot I, Javouhey-Donzel A, Athias P (1998). Correlation between direct ESR spectroscopic measurements and electromechanical and biochemical assessments of exogenous free radical injury in isolated rat cardiac myocytes. Free Radic. Biol. Med. 24:121-131.
- Dringen R, Gutterer JM, Hirrlinger J (2000). Glutathione metabolism in brain metabolic interaction between astrocytes and neurons in the defense against reactive oxygen species. Eur. J. Biochem. 267:4912-4916.
- Durukan A, Tatlisumak T (2007). Acute ischemic stroke: Overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol. Biochem. Behav. 87:179-197.
- Ellman GL (1959). Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82:70-77.
- Green LC, Wagner DA, Glogowski J, Skipper L, Wishnok JS, Tannenbaum SR (1982). Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal. Biochem. 126:131-138.
- Halliwell B (1992). Reactive oxygen species and the central nervous system. J. Neurochem. 59 (5):1609-1623.
- Kakkar P, Das B, Viswanathan PN (1984). A modified spectrophotometric assay of superoxide dismutase. Indian J. Biochem. Biophys. 21:130-132.
- Koizumi J, Yoshida Y, Nakazawa T, Ooneda G (1986). Experimental studies of ischemic brain edema. 1. A new experimental model of cerebral embolism in rats in which recirculation can be introduced in the ischemic area. Jpn. J. Stroke 8:1-8.
- Kulkarni SK (1999). Hand Book of Experimental Pharmacology. Vallabh Prakashan, New Delhi.
- Kuroda S, BK Siesjo (1997). Reperfusion damage following focal ischemia: Pathophysiology and therapeutic windows. Clin. Neurosci. 4:199-212.
- Lipton P (1999). Ischemic cell death in brain neurons. Physiol. Rev. 79:1431-1568.
- Longa EZ, Weinstein PR, Carlson S, Cummins R (1989). Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20:84-91.
- Lowry OH, Rosebrough NJ, Far AL, Randall RJ (1951). Protein measurement with folin-phenol reagent. J. Biol. Chem. 193:265-275.
- Lu P, Mamiya T, Mouri A, Nagai T (2009). Silibinin prevents amyloid b peptide-induced memory impairment and oxidative stress in mice. Br. J. Pharmacol. 157:1270-1277.
- Shukla PK, Khanna VK, Ali MM, Maurya R, Khan MY, Srimal RC (2006). Neuroprotective effect of *Acorus calamus* against middle cerebral artery occlusion-induced ischaemia in rat. Hum. Exp. Toxicol. 25:187-194.
- Takemura G, Onodera T, Millard RW, Ashraf M (1993). Demonstration of hydroxyl radical and its role in hydrogen peroxide-induced myocardial injury: hydroxyl radical dependent and independent mechanisms. Free Radic. Biol. Med. 15:13-25.
- Tota SK, Kamat PK, Shukla R (2011). Improvement of brain energy metabolism and cholinergic functions contributes to the beneficial effects of Silibinin against streptozotocin induced memory impairment. Behav. Brain Res. 221:207-15.
- Van derWorp HB, van Gijn J (2007). Clinical practice. Acute ischemic stroke. New Engl. J. Med. 357:572-579.
- Yagi K (1982). Assay for serum lipid peroxide level and its clinical significance. In: Yagi, K. (Ed.), Lipid Peroxides in Biology and Medicine. Academic Press, New York, P 232.
- Zemke D, Smith JL, Reeves MJ, Majid A (2004). Ischemia and ischemic tolerance in the brain: An overview, Neurotoxicology 25:895-904.
- Zhu YZ, Huang SH, Tan BK, Sun J, Whiteman M, Zhu YC (2004). Antioxidants in Chinese herbal medicines: A biochemical perspective. Nat. Prod. Rep. 21:478-489.



Journal of Neuroscience and Behavioral Health

Related Journals Published by Academic Journals

- *Journal of Diabetes and Endocrinology*
- *Journal of Medical Genetics and Genomics*
- *Journal of Public Health and Epidemiology*
- *Medical Case Studies*
- *Medical Practice and Reviews*
- *Journal of Infectious Diseases and Immunity*

academicJournals