Pharmacological Evaluation of Ziziphus Mauritiana L. Leaves Extracts for Anti-Amnetic Activity

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Abstract: *This study investigates the anti - amnetic properties of Ziziphus mauritiana L. leaves extracts. The research includes a phytochemical investigation, acute oral toxicity study, in - vitro antioxidant activity assessment, and pre - clinical pharmacological evaluation for anti - amnetic activity. The findings of this study could contribute to the development of new therapeutic agents for amnesia.*

Keywords: Ziziphus mauritiana L., Anti - amnetic activity, Phytochemical investigation, Antioxidant activity, Pharmacological evaluation

1. Introduction

Amnesia is when a person can no longer memorize or recall information that is stored in memory. It is very rare, despite being a popular theme for movies and books. Being a little forgetful is completely different to having amnesia. Amnesia refers to a large - scale loss of memories that should not have been forgotten. These may include important milestones in life, memorable events, key people in our lives and vital facts we have been told or taught.

Types of Amnesia:

There are many different types of amnesia. Below is a list of the most common ones:
1) **Anterograde amnesia**:
The person cannot remember new information. Things that happened recently and information that should be stored into short - term memory disappear. This usually results from a brain trauma, when a blow to the head causes brain damage, for example. The person will remember data and events that happened before the injury.

2) **Retrograde amnesia**:
In some ways the opposite of anterograde amnesia, the person cannot remember events that occurred before their trauma, but they remember what happened after it. Rarely, both retrograde and anterograde amnesia can occur together.

3) **Transient global amnesia**:
It is a temporary loss of all memory and in severe cases, difficulty forming new memories. This is very rare and more likely in older adults with vascular (blood vessel) disease.

4) **Traumatic amnesia**:
Memory loss results from a hard blow to the head, for instance, in a car accident. The person may experience a brief loss of consciousness or a coma. The amnesia is usually temporary, but how long it lasts normally depends on how severe the injury is. Amnesia can be an important indicator of concus.

Plant Profile

***Ziziphus mauritiana* L**: *Ziziphus mauritiana* L. belongs to the family Rhumnaceae, occupies an important place among the indigenous fruits of India. It is known by different names, Ber, Chinese date, Chinese apple, ber duck, Chinese jujube, da zao, date seed, date chionese, dattenoire, fructus jujubae, fructus ju jujube, hei zao, hongb zao, hong zao, jujube rouge, jujube, chinois jujebe plum, etc. its fruits and leaves are considered sacred and uses of offering to the is it a spiny, evergreen shrub or small tree up to 15m with truck 40cm or more in diameter, spreading crown: stipular spines and many drooping branches. the fruit is of variable shape and size it can be oval, obovate, oblong or round and can be 1 - 2.5 in long depending on the variety. the flesh is white and crisp. when slightly underripe. This fruit is a bit juicy and has a pleasant aroma. the fruit skin is smooth, glossy, thin, but tight. The spices is beliverd to have originated in indo - Malaysia region of south - east asia it is now widely naturalised throughtly the old world tropics from southern Africa through out the middle east to the Indian subcontinent and china, indomalyaya, and into Australiasis and the pacific island it can from dence stands and become invasive in some areas, including and Australia and has become a serious environment weed in northern Australia. It is a fast - growing tree with a medium lifespan, that can quickly reach up to 10 - 40 ft (3to 12m) The utility of ber is mentioned in the Indian ancient system of medicine. Every part of the tree such as roots, bark, flower, fruits, seed and even its latex are important in several traditional system of medicine. *Ziziphus mauritiana* L. traditionally used to treat jaundice, constipation, chronic diarrhea, dysentry, fever, asthma. It is also used to treat anemia, healing of wound, high blood pressure, ulcer, mental illness, Hepatoprotective and anticancer activity in cell culture studies. This may act against cell division and cause the death of cancer cell the

roots have been used to treat coughs and headaches, while the bark has been used on boils and for dysentery. The leaves are antipyretic, ehile the fruit has been used to assist digesion and to treat tuberculosis the seeds help to cure eye disease and are helpful in leukorrhea and as an astiringent tonic to relieve thirst, and brain the seed also help to a sedative and hyponotic effect which is helpful in insomnia pain, physical weakness, and rheumatic symptomology (p. oudhia, personal, communication).

Medicinal Uses:
Ziziphus mauritiana L. has been used as herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India. *Ziziphus mauritiana* L. is traditionally used to treat antipyretic, analgesic, antibacterial, sedative, sedative antioxidant, GIT proactive, anti - diabetic, cardiovascular, antifungal and anti - inflamatory. It is also used to treat anemia, healing of wound, high blood pressure, amorous dreams, chronic dysentery cyclopeptide alkanoids polysaccharides, flavoids, saponins and terpenoid have been isolated from this genus. The use of ziziphus species therapeutics to treat chronic inflammatory diseases are widespred and on the rise. The chronic inflammatory reasonse are also the cause of artherosclerosis, obesity, and biological activities. Unfortuanaly, ziziphus mauriatiana worldwide from different region with anti - inflammatory properties have not been document in a single review paper. Therefore, it is crucial to established ethnobotanical knowledge and application of ziziphus species against chronic inflammatory disease. The current article exhausting reviews phytochemical profile, pharmacological studies, taxocological effect and ethanobotanical uses of genus ziziphus in chronic anti - inflammatory disease. Furthermore a genus ziziphus bioactive compound discussed. This review would be a valuable resoirce for contempory researcher in the field to understand the promising role of the ziziphus genus in chronic inflammatory disorders species and document their.

Ayurveda prescribes the fruit of the herb for heart, stomach, intestinal tonic, chronic constipation and dysentery; some forms of indigestion, typhoid, debility, cholera, hemorrhoids, intermittent fever, hypochondria, Diarrhea, healthy mind and Brain Typhoid Troubles during pregnancy. Sweet drink prepared from the pulp of the fruits a produce smoothing effect on the patients who have just recovered from bacillary.

Aim and Objective

"Phytochemical and Pharmacological evaluation of *Ziziphus mauritiana* L. leaves extracts for Anti - amnetic activity"

With an objective of –
Phytochemical investigation and standardization of *Ziziphus mauritiana* L. leaves extracts. .
1) Acute oral toxicity study of extracts.
2) *In - vitro* antioxidant activity of extracts
3) Pre - clinical Pharmacological evaluation of plants extracts for **Anti - amnetic activity.** .

Plan of Work:

1) **Collection, Identification and authentication of plant material.**
2) **Pharmacognostic evaluation of plant material**
3) **Processing of crude drug.**
4) **Extraction of plant material -**
   a) Selection of extraction method
   b) Selection of solvent
5) **Physiochemical screening**
   a) Physical screening of plant material for -
   • Extractive value and ash content
   • Acid soluble and water insoluble ash
   • Loss on drying
   b) Phytochemical qualitative analysis of extract for -
   • Carbohydrates, tannin, alkaloids, terpenoids, lipide, proteins etc.
   • TLC fingerprinting
   c) Quantitative analysis of extract for Total phenolic &Flavonoid content.
6) **Pharmacological screening of plant extracts for -**
   a) *In - vitro* antioxidant activity
   b) Safe dose calculation
   c) Anti - amnetic activity.
7) **Interpretation of data and its presentation**

2. Literature Survey

Literature survey has been done as follow:
• Desai Nilesh et al. (2012) investigated analgesic activity from the serial extracts of the leaves of *Ziziphus mauritiana* L. The extract produced marked analgesic activity by reduction in the early and late phases of paw licking in mice of the *Ziziphus mauritiana* L.
• Gagetia G. C. et al. (2005) carried out anticancer effect of Hydroalcholic extract of*Ziziphus mauritiana* leaves in the animal model of Ehrlich ascites carcinoma and proposed that induction of apoptosis may be due the presence of skimmianine in extract.
• Bhandurga A. P. et al. (2012) aqueous and ethonolic extract of dried seeds of *Vigna radiate*Linn has been shown anti - amnesisc effect of scopolamine induced memory deficit in mice using the Radial arm maze and Morris water maze models.
• Jeong E. J. et al. (2008) *Scrophularia buergeriana* has shown significantly enhance in cognitive activities against scopolamine induce amnesia in the Morris water maze test in the mice. This activity was observed due to E - harpagoside and MCA - Hg, an iridoid glycosides isolated from SB. E - harpagoside or MCA - Hg significantly decreased TBARS levels, which was accompanied by an increasing in the activities or contents of glutathione reductase, SOD and reduced GSH.
• Patil R. H et al. (2009) carried out antifungal activity of ethonolic extract of the *Ziziphus mauritiana*leaves including antidiarrheal activity and antiamnetic activity.
• Parlera M. et al. (2012) The ethonolic extracts of roots of N. jatamansi (200mg/kg) has been shown significantly improved learning and memory in young mice and also reversed the amnesia induced scopolamine by facilitation of cholinergic transmission in the brain.

- Rana B. K. et al. (1997) Evaluated anti - amnetic activity of essential oils isolated from the leaves of *ziziphus mauritiana* using spore germination assay the oil the exhibited variable efficacy against different fungal isolated and 100% inhibition of spore germination of all the fungi tested was observed at 500ppm. They proposed that essential oil from *ziziphus mauritiana* fruit may interfere with the Ca2+ dip colonic acid metabolism pathway and possibly inhibit the spore information.

- Ghangale G. R et al. (2008) Evaluated aqueous extract of *Ziziphus mauritiana*for anti - amnetic activity leaves by using rat paw edema model and proposed that*Ziziphus mauritiana* leaves possess anti - inflammatory activities.

- Kim D. H. et al. (2007) *Salvia miltriorrhiza* has been able to significantly ameliorate the scopolamine induced amnesia in passive avoidance test. This activity was observed due to Tanshinone, a major diterpenoids found in the roots of saliva miltriorrhiza Bunge. Tanshinone has significantly shown the anti - emnetic effect due to the enhancement of cholinergic signaling in the mice brain.

- Arul et al. (2005) Carried out anti - emnetic, analgesic, antipyretic properties of serial extract of leaves of ziziphus mauritiana, and presented that most of the extract caused a significant inhibition of the carrageenan induced paw edema and cotton - pellet granuloma in rats. The extracts also produced marked analgesic activity by reduction the early phase late phase of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts.

- Venkatesh P. et al. (2005) carried out Radioprotective effect of *Ziziphus mauritiana* leaves extracts by exposing to different dose of gamma - radiation in mice and found that oral administration of extracts resulted in an increase in radiation tolerance by 1.6 Gy.

- Yusuf S. et al. (2009) The extracts of *Ziziphus mauritiana* leaves impaired spatial recognition of rodents, the activity of which was greatly produced by the portion extracted with ethyl acetate. Anti - amnetic activity measured by the Y - maze test is dependent on hippocampal learning and memory function and is related to the NMDA receptor/ Ca2+ influx signaling pathway. It is possible that, compound contained in the ethyl acetate portion of the extract may inhibit this hippocampal NMDA receptor/Ca2+ signaling pathway.

- Joshi H. et al. (2007) *Phyllanthus amarus* has shown to produce a dose dependent significant improvement in memory scores of young and older mice in Elevated Plus maze and passive avoidance. PA has also reversed successfully the amnesia induced by scopolamine by decreasing brain AChE activity.

- Orhan I. et. al. (2009) An ethonolic extract of *T. Populnea* reversed the scopolamine induced amnesia through reduced brain cholinesterase activity.

- Kanwal A. et al. (2010) Pre - treatment with aqueous extracts of *V. negunon* has shown a significant decrease in the phenomenon of scopolamine induced amnesia by increasing in leering about memory through effect and decreasing AChE activity.

- Upadhya S. et al. (2004) Evaluated aqueous extract of *Ziziphus mauritiana* leaves of hypoglycemic and antioxidant effect by using alloxon induced diabetes in male albino rats and proposed AML may be useful in the long term management of diabetes.

- Suderma K. et al. (2009) Carried out antidiabetes of Ziziphus mauritiana of Leaves on Alloxane induced diabetes and reported that used extract was enough capable to reduced oxidative stress by scavenging lipid peroxidation and enhancing certain antioxidant levels which causes lowering of elevated of blood glucose level.

- Patkar Atul et al. (2012) Carried out antihistaminic activity from the roots of Ziziphus mauritiana L. . The result concludes that the ziziphus mauritiana show anti - emnetic effect.

3. Materials and Methods

Plant collection:
The fresh leaves of *Ziziphus mauritiana*will be used for extraction. Stem will be collected from local region and will be authenticated by taxonomist.

Pharmacognostic evaluation of plant material:
Ziziphus mauritiana Leaves will be subjected to gross morphological and organoleptic evaluation. Further plant material will be subjected to microscopic evaluation as TS and macroscopic evaluation for powder characteristics.

Processing of crude drug:
Dried and crushed stem will be used for extraction.

Preparation of extract:
Successive solvent extract of *Ziziphus mauritiana* leaves is planned to use for the study.

Physiochemical screening:
Physiochemical screening of powdered drug will be done for checking Extractive value and ash content, Acid and water soluble ash, Loss on drying and phytochemical qualitative analysis of extract will be done for confirmation of carbohydrates, tannin, alkaloids, terpenoids, lipid, proteins etc. followed by TLC fingerprinting. Further quantitative analysis of plant extracts will be carried out for total phenolic and flavonoid contents.

Pharmacological screening of plant extracts for –

1) *In - vitro* **antioxidant activity:**
DPPH Radical Scavenging Assay
The free radical scavenging activity of the extract will be measured by 1, 1 - diphenyl - 2 - picrylhydrazyl (DPPH) assay as per standard reference

2) **Safe dose calculation:**
Safe dose will be calculated as per OECD guidelines or literature survey will be carried out for referencing.

3) *In - vivo* **Anti - amnetic activity:**
The scopolamine test:
Experiments will carry out on adult albino rats. A total of 20 albino rats (200 - 300gm) will use in these experiments. Amnesia will induce by scopolamine injection (3 mg/kg, i. p) 30 min before the behavioral experiments. Rats will divide into seven groups.
(n = 6);

Group1 - normal control,
Group 2 - disease control (scopolamine hydro bromide 3 mg/kg, i. p.),
Group 3 - standard treatment (scopolamine + standard drug 3 mg/kg, i. p.)
Group 4, 5, 6 and 7 - scopolamine + respective test dose.

Group 4, 5, 6 and 7 rats will dose every 24 h interval with respective drugs for 14 consecutive days. The acquisition trail for Morris water maze, elevated plus maze will carry out on the 14th days and

scopolamine (3 mg/kg i. p.) will administration on the 14th days after the acquisition trail to all groups expect normal control group, which provoke the cognitive impairment in rats.

Assessment of Activity

1) Spatial learning in the radial arm maze:
- The apparatus is a wooden elevated eight - arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm.
- The maze will well illuminate and numerous cues will present, food pellets (reward) will Placed at the end of the arms.
- During the test, rats are placed at the end of their body Weights maintained at 85% of their free feeding weight to motivate the rat to run the maze.
- The animals will train on daily basis in the maze to collect the food pellets.
- The session will terminate after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors.
- The Number of errors (Entries to non - baited arms) will be counted during the session.

2) Spatial learning in the water maze:
- Different strains of rats are generally used (long Evans, Wistar, Sprague - Dawley). The apparatus is a circular water tank filled to a depth of 20 cm with 25°C water (Brioni et al.1990: Morris 1984).
- Four points equally distributed along the perimeter of the tank serve as Starting locations. The tank will divide in four equal quadrants and a small platform (19 cm height) will located in the center of one of the quadrants.
- The platform remains in the same position during the training days. The rat will be released into water and allowed 60 - 90 s to find platform.
- Animals usually receive 2 - 4 trials per day for 4 - 5 days until they escape onto the platform, well trained rats escape in less than 10 s.
- The latency to reach the escape platform will measured during the training days. A free swim trial will generally perform after training days where escape platform will remove and animal will allow to swim for 30 s., with the help of video system, the latency to reach the previous position of platform, the number of annulus crossing as well as the time the rat spent in the training quadrant will measured.

3) Elevated plus maze test:
- The plus Maze consists of two open arms, 50 - 10 - 40cm, and two enclosed arms, 50 - 10 - 40 cm, with an open roof, arranged so that the two arms are opposite to each other.
- The maze elevated to height of 50 cm. The rats (200 - 500 g body weight) are housed in pairs for 10 days prior to testing in the apparatus. During this time the rats are handled by investigator on alternate days to reduce stress.
- Group consists of 6 rats for each dose. Thirty min after i. p. administration of the test drug or the standard, the rat is placed in the center of the maze, facing one of the enclosed arms.
- During 5 min test period the following measures are taken: the number of entries and time spent in the open and enclosed arms; the total number of arms entries.
- The procedure is conducted preferably in a sound attenuated room, with observations made from an adjacent room via a remote - control camera.

Significance: The findings of this study could contribute to the development of new therapeutic agents for amnesia.

4. Interpretation of data

1) Data collected from Pharmacognostic evaluation of plant extracts will be presented in tabular form and microscopic observation will be presented as figures of TS & for powder characteristics.
2) Qualitative evaluation of plant extracts for the content of phytoconstituents will be presented as either present (+ve) or absent (-ve) and presence of phytoconstituents will be confirmed by TLC by comparing with RF value of standard. Results of total phenolic and flavonoid content will be correlated to TLC studies and later will be also correlated to observed pharmacological activity.
3) The assessment of *in - vitro* Antioxidant activity will be observed through % scavenging activity against DPPH of extracts against free radicals.
4) Safe dose will be calculated as 1/10 dose of LD_{50} value of plant extracts.
5) The assessment of Antiamnetic activity will be observed through change in levels of various parameters listed in the methodology.

All the data related to efficacy of leaves extracts will be subjected to statistical analysis like student's T - test, ANOVA etc. to find level of significance between extracts and between extract to control data. Such data will also be presented as chart diagram.

References

[1] D. Gupta. Et al Pharmacological Review of *Ziziphus mauritiana* L. Fruits, International Journal of Pharmaceuticals Sciences and Research, 2011, Volume 2 (8), pp.231 - 236.
[2] Dhananjay Hasanda et al. Biological Activities of crude extracts and chemical Constituents of Bael, *Ziziphus mauritiana* L., Indian Journal of Experimental Biology, 2009, Volume 47, pp.849 - 861.

[3] Desai Nilesh V et al A review on *Ziziphus mauritiana* leaves A Potential Medicinal Tree, International Research Journal of Pharmacy, 2012 Volume 3, (8).

[4] Dinesh Kumar Sekar et al. A Review on Pharmacological Phytochemical Properties of *Ziziphus mauritiana* L., Asian Journal of plant Science and Research, 2011 Volume 1 (2) pp.8 - 17.

[5] H. Gerhard Vogel, Drug Discovery & Evolution, Pharmacological assays, 2002 Second edition, pp.430 - 437.

[6] Kumari Anupama et al A Review Article Of Ziziphus mauritiana L., International Ayurvedic Medicinal Journal, 2020 Volume 8 (2).

[7] K. Suresh et al Antimicrobial Activity of Ziziphus mauritiana Leaves. against Clinical Pathogens, Journal of Phytology, 2009, Volume 1 (5), pp.323 - 327.

[8] Pushpendra K. Patel et al A Review on its Medicinal Properties, International Journal Of Pharmaceutical and phytopharmacological Research, 2012 Volume 1 (5) pp.332 - 341

[9] Pojala Kumar et al Effect of Ziziphus mauritiana Levaes. Methanolic extracts of leaf and fruit pulp on learning and Memory Enhancing in albino rats, International Journal of Basic & Clinical Pharmacology, 2020, Volume 9 (3), pp.403.

[10] R. Bhavani et al Antidiabetic activity medicinal plant Ziziphus mauritiana L. On alloxan induced diabetic rats, International Research Journal Pharmaceutical and Biosciences, 2014, Volume 1 (1), pp.36 - 44.

[11] V. Ramamurthy et al Hepatoprotective Study on *Ziziphus mauritiana* Extract against staphylococcus aureous Intoxicated Albino Rats, American Journal of Phytomedicine and Clinical Therapeutics, 2015, Volume 3 (2), pp.120 - 128.

[12] Vikas B Taged et al. Phytochemical study and GC - MS Analysis of Bael fruit Pulp, Research Journal of Life Sciences, Bioinformatics, Pharmaceuticals and Chemical.2018 Volume 4 (5) pp.779.

[13] Rahmatullah M. et al. A Review *Ziziphus mauritiana* L. A Potential Source for the Treatment of Diabetes Associated Alzheimer's Disease, Open Access Journal of Pharmaceutical Research, 2019, Volume 3 (4) pp.186.