Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45** Issue **2** **Year 2024** Page **744:749***

Evaluation Of Anti-Cataract Activity Of The Siddha Formulation Muthu Parpam Using Isolated Goat Lens Model

Chitra V[1*], Kehren R. Rayma[2], Mahalakshmi V[3], Muthukumar NJ[4], Akila M[5], Thayalini K[6], Meenakumari R[7]

*[1]*Assistant professor, Department of Siddhar Yoga Maruthuvam , National Institute of Siddha, Tambaram, Sanatorium, Chennai -47.*

[2]BSMS CRRI, Velumailu Siddha Medical College and Hospital, Sriperumbudur -602105.

[3]HOD (i.c) Associate professor, Department of Siddhar Yoga Maruthuvam, National Institute of Siddha, Tambaram, Sanatorium, Chennai – 47.

[4]HOD, Professor, Department of Varma Maruthuvam, National Institute of Siddha, Tambaram, Sanatorium, Chennai – 47.

[5]PG Scholar, Department of Siddhar Yoga Maruthuvam, National Institute of Siddha, Tambaram, Sanatorium, Chennai – 47.

[6]PG Scholar, Department of Siddhar Yoga Maruthuvam, National Institute of Siddha, Tambaram, Sanatorium, Chennai – 47.

[7]Professor, Director, National Institute of Siddha, Tambaram, Sanatorium, Chennai – 47.

***Corresponding Author :** Dr. V. Chitra MD(Siddha),*

**Assistant professor, Department of Siddhar Yoga Maruthuvam , National Institute of Siddha, Tambaram, Sanatorium, Chennai 47. Email: vchitramdmscvarma@gmail.com, Contact number: 8838466338*

Abstract

Siddha system recommends a number of herbal and herbomineral ophthalmic formulations as both internal and external therapeutic measures. *Muthu parpam* (MPM) is one such therapeutic measure that has been indicated in several eye formulations as an ingredient. While Muthuchippi parpam (Calcined shell of pearl oyster)) has been previously evaluated for its antioxidant profile, till date no study has been performed on anticataract activity of Siddha formulation Muthu parpam (Calcined Pearl). In the present study, the Aldose reductase inhibitory activity of *Muthu parpam* (MPM) were studied along with their effect on sugar-induced cataractogenic changes in sheep lenses in vitro. Goat eye lenses were divided into 4 groups; Group I served as normal control, Group II as cataract control, Group III (100µg/ml of MPM) and Group IV(200 µg/ml of MPM). Group II, III and IV were incubated in 55 mM glucose in artificial aqueous humor to induce lens opacification. Freshly prepared aldoreductase enzyme was allowed to react with different volume of test drug at 100, 200,300, 400 and 500µg/and the formation of NADP was observed and the results were expressed as percentage inhibition and were calculated as follows. A dose dependent action of the drug was observed in two groups (III & IV) incubated with 100 µg and 200 µg of the test drug with a visibility scoring of 13.8 ± 3.34 and 22.2 ± 6.14 respectively. It was observed from the results of the present investigation that the Siddha formulation MPM shown significant

	inhibition of aldose reductase enzyme with the maximum inhibition of about 45.41± 1.761 % and the corresponding IC50 is 558.1 ± 29.79 µg /ml.
CC License CC-BY-NC-SA 4.0	***Keyword: Cataract, Muthu parpam (Calcinated pearl), Eye disease, Siddha, Traditional medicine.***

INTRODUCTION

A cataract is a condition where clouding forms in the crystalline or lens capsule of the eyes, causing the lens to become opaque and preventing light from passing through. The illness is complex and mostly caused by the accumulation of large protein clumps within and around the lens. Women were more likely than males to have an untreated cataract, and its frequency grew with age.[1] According to a recent World Health Organisation (WHO) report, cataracts are the primary cause of preventable blindness, accounting for about 50% of cases worldwide. If appropriate action is not done promptly, this number will double over the next 20 years [2]. Age, smoking, ultraviolet (UV) radiation, gender, and diabetes mellitus are among the frequent risk factors for cataract development. The number of people with cataracts is expected to rise as life expectancies rise globally, particularly in low-income countries where access to cataract surgery is limited. This calls for the search for affordable pharmaceutical alternatives to treat the condition.[2]

In less developed countries where cataract is linked to severe dehydration from diarrhoea caused by cholera or inadequate nutrition, public health approaches to prevent cataract may be feasible. With the possible exception of lowering radiation exposure and quitting smoking, the majority of risk factors are unavoidable, hence there is little room for further prevention in industrialised nations. Alternative methods of preventing cataracts include anti-cataract medications like multivitamin supplements and N-acetylcarnosine eyedrops.

The only therapy that has been shown to work is surgery combined with intraocular lens implantation and phocoemulsification. This comes with a large price tag and is not easily accessible, particularly in underdeveloped nations where cataract prevalence is highest. Therefore, receiving medical treatment for cataracts is a widely wanted alternative.[3] Modern research states that calcium salts can be used therapeutically to prevent and cure hyperkalemia, urticaria, osteoporosis, cardiac arrest, and calcium shortage. Siddha medical system uses pearl and oyster shell to treat various fevers, disorders of the brain or neurological system, corneal opacity, iritis, and other ailments including night blindness and urinary discomforts. It's also supposed to improve energy, nutrition, and strength including diabetic neuropathy and hyperglycemia .[4]

The field of Siddha medicine known as Opthalmological Diseases has been expertly specialised by Siddhars. They have divided eye conditions into 96 categories, and *Kankasam* falls within the same category as cataracts. The following may demonstrate that they used copper-metal *"Salagai"* and *"Muppattai"* instruments to run the *Kankasam* (Cataract).[5]

“Mūṭar kuttum cattirantāṉ muṭṭai tūrpōla muḷaiyāmē
vāṭā nīḷam nal viralāl vaikkōl tāḷiṉparumaṉatāy
Nāṭāy nīyu mitaṉuṭaiya navaccit tiraṉi laiyaṟintu
Kōṭā vaṇṇam nellaḷavu kuṟittut tāṉuṅ kuttiviṭavē”

"Kuttuṅ kācam muttup pōl kūṭip puriyum kuṉṟāmal
caṟṟum kaḷimpoṉ ṟillāmal tāmpirac calukai mup paṭṭam
Muṟṟum āṟu viralnīḷam muṉṉum piṉṉum viralnīkki
māṟṟu nilaiyai vaittaṉaittu vaittuk kaṭṭi kuṟaitīrē"

- (*akattiyar nayaṉaviti)* 67-68

There are about 16 types of Kan Kasam (Cataract) mentioned in ancient text namely-

1. *Vatha Kasam*(Cataract due to Vatha)
2. *Anthi Kasam* (Cataract which makes vision clear in evening twilight)
3. *Varatchi Kasam* (Membraneous cataract)
4. *Pitha Kasam* (Cataract attended with bilious humour of the eye)
5. *Olu Kasam* (Another variety)
6. *Vala Kasam* (Infantile or Juvenile cataract)
7. *Vizhi Kasam* (Cataract affecting the eyeball)

8. *Kuvalai Kasam* (Cataract affecting the socket)
9. *Mandha Kasam* (Cataract causing dullness of vision)
10. *Neerozhi Kasam* (Fluid Cataract)
11. *Silethuma Kasam* (Cataract due to phlegmatic causes)
12. *Neela Kasam* (Cataract in which the lens is turned blue)
13. *Kumari Kasam* (Immature cataract marked by dullness of vision due to the affection of the lens)
14. *Ratha Kasam* (Blood Cataract)
15. *Piravi Kasam* (Congenital Cataract)
16.*Karungkasam* (Black Cataract)[6]

Among the trihumours, Vatham (Air component) imbalance manifests as dry and shifty eyes. *Pitham* (Fire component) imbalances cause yellowing and light sensitivity in the eyes. Excessive *kapham* (Fluid component) can cause oiliness, watery secretions, and dullness in the eyes. Eyes will get red and irritated when any one of the three doshas is disturbed.[7]
A cataract, coloquially known as a "flower in the eye," is a degeneration of the lens that appears white or blue white. It should not be confused with the cornea's opacity. If treatment is delayed, it might spread to the entire eye, which would compromise vision.[6] Siddha literature compares the medical benefits of the less expensive *Muthuchippi parpam* (Calcinated pearl) to those of the more costly *Muthu parpam.*

Role of antioxidants in the prevention of free radical generation:

Free radicals and oxidants are produced either from normal cell metabolism inside the body or from external factors. When the amount of free radicals are increased to a point where they cannot be destroyed timely, it results in a phenomenon called oxidative stress. Increased oxidative stress in the body plays a major role in several chronic degenerative diseases like autoimmune diseases, aging, rheumatoid arthritis, cataract, cardiovascular, neurodegenerative conditions and cancer.
Antioxidants work by safeguarding the cell membranes from damage by free radicals. There are over 4000 plus antioxidants found in the medicinal plants like flavonoids, polyphenols, soflavones, catechins, anthocyanins, flavoproteins. proanthocyanidins, alpha-tocopherol, ascorbate, carotenoids, and zinc. Several mechanisms are involved to compensate the oxidative stress in the body, among which antioxidants from plants are known to exhibit. They act by inhibiting lipid peroxification, increasing superoxide dismutase activity in various tissue sites reducing oxidative stress and stimulation of DNA-repair exerted by sulphur-containing compounds. Shielding of sensitive structures by some polyphenols, free radical scavenging activity by polyphenols, quenching of singlet oxygen and radicals by carotenoids, induction of oxidation and of conjugation (protective) enzymes by dithiothiones indoles and isothiocyanates, inhibition of ornithine decarboxylase by polyphenols and carotenoids are studied. In addition to these experimental evidence suggests that it also possess anti-cancer properties of polyphenols scavenging effects on activated mutagens and carcinogens.[8][9]

MPM and its ingredients

Ingredients
Muthu (Pearl)
Notchi leaves (*Vitex negundo* leaves)
Nilapanai kizhanghu (Rhizome of *Curculigo orchoides*)
Procedure-
Muthu (Pearl) was purified as per Siddha literature and the fresh leaf extract of *Vitex negundo*, and Curculigo *orchoides* rhizome extract were used to triturate the purified Muthu. The pulverised mixture is then put through the Pudam process, which involves utilising cow dung cakes and earthenware pots with ribbon covered with clay as per standard Siddha text.[4]

METHODOLOGY

Lens culture
Fresh goat eyeballs were obtained from slaughterhouse immediately after slaughter and transported to the laboratory at temp 0-4°C. The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl, KCl , MgCl 2 , NaHCO, NaH (PO) 2, CaCl and Glucose 5.5 mM) at room temperature

and pH 7.8 for 72 h. Penicillin 32mg % and streptomycin 250 mg % were added to the culture media to prevent bacterial contamination.

Grouping

Goat eye lenses were divided into 4 groups; Group I served as normal control, Group II as cataract control, Group III and Group IV were incubated with test drug (100µg/ml and 200 µg/ml). Group II, III and IV were incubated in 55 mM glucose in artificial aqueous humor to induce lens opacification.

Square Visibility score

Photographic evaluation

The lenses were incubated at 37°C for 72 h. At the end of the incubation period, the lenses were visually evaluated for the development of opacification by placing them on a graph paper. The number of squares clearly visible through the lens was counted as a measure of lens opacity.

Aldose Reductase inhibitory activity

The reaction mixture comprised of freshly prepared aldoreductase enzyme, different volume of test drug at 100, 200,300, 400 and 500µg/ml in 0.067 M buffer (pH 6.2), 0.125 mM NADPH, 400 mM lithium sulfate, and 40 mM xylose. The reaction was performed in a 96-well plate. The formation of NADP was observed as decrease in absorbance at 340 nm recorded for 3 min. The assay was performed in triplicates. The results were expressed as percentage inhibition and were calculated as follows:

% Inhibition = ([Absorbance of control – absorbance of Test /absorbance of control) × 100

Table 1: Effect of test drug on opacity scoring on number of squares counted on glucose-induced cataract in isolated goat lens

Group	Treatment	Number of Squares Counted (Mean ± SEM)
I	Control	36.2 ± 5.80
II	Negative Control – Only Glucose	5 ± 2.12
III	Treatment- MPM 100 µg/ml	13.8 ± 3.34
IV	Treatment- MPM 200µg/ml	22.2 ± 6.14

Data expressed as mean± SEM, n=5

Figure-1. Effect of test drug MPM on opacity scoring on number of squares counted on glucose-induced cataract in isolated goat lens

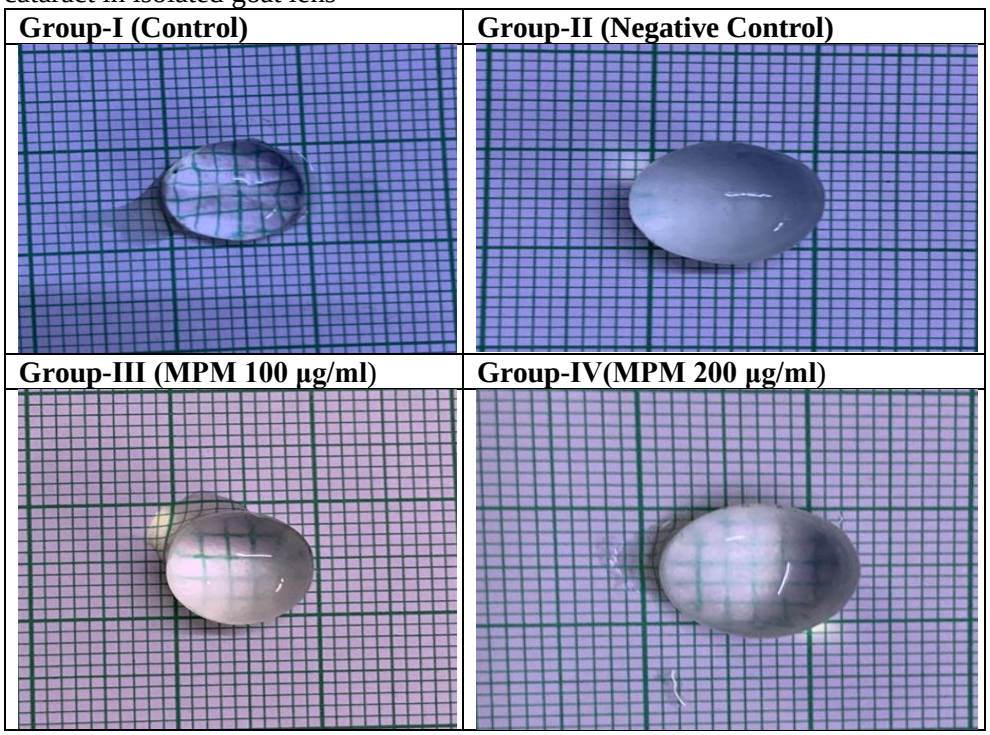

Table-2 : Percentage inhibition of test drug MPM on Aldose reductase enzyme Inhibition assay

Dose in µg/ml	% Inhibition by test drug MPM
100 µg/ml	14.7 ± 2.7
200 µg/ml	24.99 ± 2.61
300 µg/ml	29.04 ± 2.527
400 µg/ml	39.05 ± 2.2
500 µg/ml	45.41 ± 1.761

Data are expressed as Mean ± SD (n=3)

Table-3. IC50 Values for Aldose reductase enzyme inhibition by test drug MPM

Test Drug	IC50 Value of Aldose reductase enzyme inhibition ± SD (µg/ml)
MPM	558.1 ± 29.79

Data are given as Mean ± SD (n=3)

RESULTS AND DISCUSSION

It was observed from the result of the present investigation as shown in table- 1 and figure-1, that group I lens maintain the normal transparency with high visibility score of 36.2 ± 5.80. Group II lens treated with glucose 5.5 mm has shown complete cataractogenesis with absolute loss of transparency and the corresponding visibility score was found to 5 ± 2.12 which was significantly low when compare to group I. Improved visibility were observed in the lens belongs to group III incubated with 100 µg of the test drug with the score of 13.8 ± 3.34. Similarly group IV lens incubated with 200 µg has shown significantly higher level of visibility score (22.2 ± 6.14). The siddha formulation MPM shown significant inhibition of aldose reductase enzyme with the maximum inhibition of about 45.41% ± 1.761 % and the corresponding IC50 is 558.1 ± 29.79 µg /ml (Table-3).

Pinctada margaritifera also called black lipped Indian pearl oyster, rich in nutrients, minerals and antioxidants used as a medicinal formulation in siddha to treat several conditions. =The major contents of Muthuchippi includes phosphate, Calcium Carbonate, Magnesium, Sulphate of calcium, Oxide of iron, Aluminum and Silica. Studies have evidenced the efficacy of Muthuchippi Parpam (Calcinated shell of Pearl oyster) for bilious diseases, abdominal tumors, Hyperkalemia, otitis media, otorrhoea, asthma, diabetes, eye diseases like iritis, night blindness and improves opacity of cornea, hemorrhoids and other neuro muscular diseases. Also the presence of tannins and flavones, proves the formulation to be potent antioxidants and bioactive nano sized particle may be the reason for its therapeutic efficacy.[10] MPM is also studied to have Anti-osteoporotic and anti-inflammatory activity. [10][11]

Evaluating the ingredients of *MPM*, the presence of glutathions, phenols, falvanoids and vitamin C supports to the the antioxidant activity of the formulation.[12] Luteolin is a bioactive flavonoid, isolated and characterized from the leaves of *Vitex negundo an ingredient of MPM has been proven to reduce* selenite induced oxidative stress and inhibit cataractogenesis in lens of Sprague-Dawley strain rats by maintaining antioxidant status, reducing ROS generation and lipid peroxidation in the lens .[13] A study by Nagesh et al, reported antioxidant activity of Curculigo orchioides that showed dose dependent 1, l-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity invitro. [14]. In another study, free radical scavenging activity of MPM with 100 µg/ml concentration was determined by 1, 1-diphenyl- -picrylhydrazil (DPPH) using the method of Blois when compared to Gallic acid. The potential decrease in the concentration of ABTS radical was due to the scavenging ability of Gallic acid standard and MPM showed 90.72% and 69.18% respectively. Total phenolic content using Folin - Ciocalteu's method when compared to the standard BHA and Muthuchippi parpam 89.19% and 63.01% respectively. In the same study, the aqueous extract of Muthuchippi parpam found to possess significant antioxidant activity and remarkable antifungal activity.[14]Several bioactive peptides and proteins are derived from the whole, mantle, and gill tissues of oyster's exhibit antimicrobial, antioxidant, antihypertensive, anticancer, anticoagulant, and anti-aging properties. [15]

Upon considering the various antioxidant profiles of ingredients of Muthuchippi parpam (Calcined shell of Pearl Oyster) and its ingredients, till date this is the first study to test the anticataract effect of Muthu Parpam (Calcined Pearl) by measuring the opacity scoring on glucose-induced cataract in isolated goat lens. A significant difference was found in the visibility score between the groups treated with the siddha formulation MPM versus the group not treated. A dose dependent action of the drug was observed in two groups (III & IV) incubated with 100 µg and 200 µg of the test drug with a visibility scoring of 13.8 ± 3.34 and 22.2 ± 6.14 respectively. The observations made other than the visibility score was inhibition of aldose reductase enzyme with the maximum inhibition of about 45.41± 1.761 and the corresponding IC50 is 558.1 ± 29.79 µg /ml. The

direct inhibition of aldose reductase enzyme prevents the damage of nerve cells and protects the eye. Also improvement in the visibility scoring between the groups evidenced the antioxidant, cytoprotective and anti-cataract property of the formulation. The results of this study has evidenced the antioxidant activity of the MPM supporting its traditional claims.

Conclusion

From the result of the present in-vitro study it was concluded that the test drug MPM possess convincing anti-cataract property in isolated goat lens preparation MPM and has shown significant inhibition of aldose reductase enzyme with the maximum inhibition of about 45.41± 1.761 % and the corresponding IC50 is 558.1 ± 29.79 µg /ml. Aligning with the previous evidences, also with the therapeutic efficacy evidenced in the current study on MPM can be considered as to confirm MPM in preventing and treating cataractogenesis. Further animal studies and clinical research is required on the same to generate more evidence to the current study.

References

1. Sumithra, M., Choudhury, S., Dhivya, L. S., Nithiyanand, M., & Zachariah, J. (2019). Protective role of Borreria hispida against galactosamine induced diabetic cataract. *Research Journal of Pharmacy and Technology*, *12*(3), 1235-1238.
2. Heruye, Segewkal H., Leonce N. Maffofou Nkenyi, Neetu U. Singh, Dariush Yalzadeh, Kalu K. Ngele, Ya-Fatou Njie-Mbye, Sunny E. Ohia, and Catherine A. Opere. "Current trends in the pharmacotherapy of cataracts." *Pharmaceuticals* 13, no. 1 (2020): 15.
3. Toh, T. Y., Morton, J., Coxon, J., & Elder, M. J. (2007). Medical treatment of cataract. *Clinical & Experimental Ophthalmology*, *35*(7), 664-671.
4. The Siddha Formulary of India, author. *Part I, First Edition, Formulary - Chapter No. 21. Curanams.* New Delhi: Published by Department of Health, Ministry of Health and Family Welfare, Govt. of India; 1992.
5. .Suresh, A., and G. Veluchamy. "Surgery in Siddha System." *Heritage of the Tamils: Siddha Medicine* (1983): 461-471.
6. Sambasivam Pillai, T. V. "Tamil-English Dictionary of Medicine." *Chemistry, Botany and Alien Sciences (Based on Indian Medical Science)* 5 (1994).
7. Parasuraman, Subramani, and Pandurangan Perumal. "Siddha, an indigenous medical system of peninsular India." *Herbal medicine in India: Indigenous knowledge, practice, innovation and its value* (2020): 9-21.
8. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008 Jun;4(2):89-96.
9. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: a review. Asian Pac J Trop Biomed. 2014 Jan; 4 (1):78-84. Doi: 10.1016/S2221-1691(14)60213-6. Velu Kanthal, *N. S., Chithra, V., Rahul, V. .A, Usha, S., & gladys, R. J. (2017). Scientific evaluation of siddha formulation muthuchippi parpam-a review. International Journal of Ayurveda and Pharma Research, 5(1). Retrieved from S. Balamurugan1, R. Madhavan2, Antimicrobial Activity of Muthuchippi Parpam. International Journal of Science and Research
10. H, Vetha & Karthikeyan.Karu, & Sofia, H.Nalini & Manickavasakam.K,. (2018). Antioxidant activity of adathodai chooranam by in vitro approach.
11. B.N. Rooban, V. Sasikala, V. Gayathri Devi, V. Sahasranamam, Annie Abraham,Prevention of selenite induced oxidative stress and cataractogenesis by luteolin isolated from Vitex negundo,Chemico-Biological Interactions,196 (1–2) :2012,Pages 30-38
12. Nagesh, K. S., Nayaka, M. A., Dharmesh, S. M., Shanthamma, C., & Pullaiah, T. (2008). In vitro propagation and antioxidant activity of Curculigo orchioides. *Journal of Tropical Medicinal Plants*, *9*(2).
13. R. Ganesan *et al*. Evaluation of antioxidant and antimicrobial activities of *muthuchippi parpam* - a siddha drug, *Asian Journal of Research in Biological and Pharmaceutical Sciences*, 4(1), 2016, 21-25.
14. Selvakumari Ulagesan, Sathish Krishnan, Taek-Jeong Nam, Youn-Hee Choi. A Review of Bioactive Compounds in Oyster Shell and Tissues. Front. Bioeng. Biotechnol., 06 June 2022 Sec. Nanobiotechnology. Volume 10 - 2022 | https://doi.org/10.3389/fbioe.2022.913839
15. Ganeshpurkar A, Bhadoriya SS, Pardhi P, Jain AP, Rai G. In vitro prevention of cataract by Oyster Mushroom Pleurotus florida extract on isolated goat eye lens. Indian J Pharmacol. 2011;43:667–70.