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Research Article

ANTIOXIDANT EFFECT OF *SAPTAMRUTHA LAUHA* - IN VIVO STUDY IN WISTAR ALBINO RAT LENS

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ABSTRACT

Oxidative stress affects all structures of the human eye with its special emphasis on the ocular surface, lens and retina contributing to the onset or progression of eye diseases. Age-related ocular pathologies including are largely mediated by the oxidative stress thus highlighting the need to enlighten a preventive measure that can address oxidative stress and slow the progression of age-related ocular pathologies. **Aims and Objective**: *Saptamrutha Lauha* is formulation of seven drugs which could act as *Rasayana* and *Chakshushya* so here they are hoped for an antioxidant network activity. **Methods**: 36 male Wistar albino rats were divided into 6 groups. Single dose of Naphthalene was administered orally for all groups, except the control group on first day. Group I: control, Group II: Silymarin administered orally for 10 days. Group III, IV and V: low, medium, high doses of *Saptamrutha Lauha* respectively administered orally for 10 days. Group VI: naphthalene group, treated with naphthalene alone. After 10th day rats were sacrificed, antioxidant effect was analyzed from serum biochemical parameters and also from antioxidant assays. **Observation and Analysis**: *Saptamrutha Lauha* at all three doses (low dose, intermediate dose, high dose) were found to be effective in antioxidant activity. **Discussion and Conclusion**: The assays done suggests that *Saptamrutha Lauha* is capable of scavenging free radicals evolved in the pathogenesis of Naphthalene induced oxidative stress. Thus, this study provides a scientific basis for usage of *Saptamrutha Lauha* as an antioxidant.

INTRODUCTION

Eye is constantly exposed to both exogenous and endogenous sources of oxidants, which put the cells under a continuous pressure from oxidative stress. The antioxidant defense system is composed of a vast variety of compounds, including both enzymatic and non-enzymatic molecules. In relation to enzymatic antioxidants, primary and secondary enzymatic defenses are distinguished. The main antioxidant enzymes which protect the eye against Reactive oxygen species are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)[1].

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The lens is the structure which is most affected by oxidative damage. It is an avascular structure and is having a constant and spare production of lenticular proteins. It is highly exposed to UV radiation and also shows a marked reduction of antioxidants levels in the lenticular nucleus. Lens constitute as a second antioxidant defense system. The UV radiation that reaching the lens is greatly composed of UVA light, since the cornea absorbs UVC and most UVB but only a small fraction of UVA. The lens contains high levels of GPX and SOD[2]. Superoxide dismutase are the main antioxidant enzymes of the ocular lens. The overall SOD activity will significantly decrease with age and is probably caused by the life-long accumulation of inhibitory modifications. Age-related ocular pathologies including cataracts and age-related macular degeneration are largely mediated by the oxidative stress thus highlighting the need to enlighten a preventive measure that can address oxidative stress and slow the progression of age-related ocular pathologies[3]. *Saptamrutha Lauha* belongs to *Lauha*

Kalpana, herbo-mineral formulation where *Lauha* is a prime ingredient added to other drugs. The word *Sapta* means seven and *Amrutha* means nectar. It implies that *Saptamrutha Lauha* is formulation of seven drugs which could act as *Rasayana.* On analyzing the drugs, most of them possess *Chakshushya* and *Rasayana* property. Also, as they all are supposed to possess antioxidant activity, so here they are hoped for an antioxidant network activity, which is a synergistic combination activity of antioxidants that provide ongoing protection against free radical damage, keeping oxidative stress at bay. The present study is carried out to evaluate the antioxidant potential of *Saptamrutha Lauha* against naphthalene induced oxidative damage by evaluating the status of antioxidant enzymes in in lens tissue after the administration of *Saptamrutha Lauha* for ten days.

AIMS AND OBJECTIVE

To assess the antioxidant effect of orally administered various doses of *Saptamrutha Lauha* in Naphthalene induced oxidative stress in Wistar albino rats lens by biochemical, antioxidant assays.

Hypothesis

Null Hypothesis: There is no antioxidant effect for orally administered various doses of *Saptamrutha Lauha* in the experimental study conducted in oxidative stress induced Wistar Albino rats.

Alternate Hypothesis: There is antioxidant effect for orally administered various doses of *Saptamrutha Lauha* in the experimental study conducted in oxidative stress induced Wistar Albino rats.

Review of Literature

***Saptamrutha Lauha*:** *Saptamrutha Lauha* belongs to *Lauha Kalpana*, herbo-mineral formulation where *Lauha* is a prime ingredient added to other drugs. The word *Sapta* means seven and *Amrutha* means nectar. It implies that *Saptamrutha Lauha* is formulation of seven drugs which could act as *Rasayana.*

Table 1: Drugs of *Saptamrutha Lauha*[4]

S.No	Drugs	Botanical name	Family name
1	*Amalaki*	*Phyllanthus emblica* Gaertn	Euphorbiaceae
2	*Hareetaki*	*Terminalia chebula* Retz	Combretaceae
3	*Vibhitaki*	*Terminalia bellirica* Retz.	Combretaceae
4	*Yastimadhu*	*Glycyrrhiza glabra* Linn.	Fabaceae
5	*Lauha basma*	Calx of iron	-
6	*Sarpi*	Ghee	-
7	*Madhu*	*Apis melifera*	Apidae

Saptamrutha Lauha is also a combination of *Triphala, Yastimadhu, Louhabasma* taken with ghee and honey to fulfil the supplementation of antioxidants. *Triphala*[5] is rich in polyphenols and tannins. Polyphenol contents of *Triphala* are responsible for its antioxidant and radio protective ability. It reduces the oxidative stress by converting reactive oxygen free radicals to non-reactive products. *Glycyrrhiza* (root) has plenty of polyphenol components which acts as a potential source of anti-oxidants[6]. Licochalcones B and D inhibit the microsomal lipid peroxidation. Also, isoflavones like glabridin, hispaglabridin A, and 3′hydroxy-4-*O*-methylglabridin present in *Glycyrrhiza glabra* were found to have potential antioxidant activity. More recently, dehydrostilbene derivatives in *Yastimadhu* like α-dihydro-3, 5, 4- trihydroxy-4, 5-diiodopentenylstilbene have been isolated and reported as free radical scavengers[7]. Iron has a central role in catalyzing free radical scavenging reactions and thus reducing oxidative damage. The iron content of the lens is about 0.18-9.6µg/g wet weight. The main role of ferritin is to store redox iron safely and thereby protecting the cells against oxidative damage caused by iron-catalyzed free radicals[8]. The antioxidant capacity of honey is due to a wide range of compounds including phenolics, peptides, organic acids, enzymes, and Maillard reaction products[9]. The lipid fraction especially the acetone-insoluble fraction of ghee residue had the greatest antioxidant effect. Also, phospholipids, the lipid constituents of ghee residue has the maximum antioxidant property followed by α-tocopherol and vitamin A. As ghee is a fat-rich product, its natural antioxidants and other constituents like phospholipids and protein residues plays major role in preventing the oxidative stress[10].

As a standard antioxidant, silymarin increases the superoxide dismutase activity within the cells. Studies shows silymarin as a potential agent to mitigate cataract formation in vivo or to alleviate the pathological processes in the lenses observed in the in vitro cultures. Silibinin, the active compound is also demonstrated as an agent protecting rabbit lenses against oxidative stress in the in vitro study[11].

Naphthalene is an organic compound with chemical formula $C_{10}H_8$. It is a simplest polycyclic aromatic hydrocarbon with an appearance of white crystalline structure. Naphthalene gives off a pungent

odour, is highly toxic, and is considered potentially carcinogenic. Naphthalene depletes glutathione, inhibits lipid peroxidation, increases DNA fragmentation and thus results in oxidative stress through production of superoxide anion and hydroxyl radicals[12].

MATERIAL AND METHODOLOGY

Study setting

College of Veterinary and Animal Sciences Mannuthy, Trissur. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the College of Veterinary and Animal Sciences, Mannuthy with proposal number IAEC/22/11 and was performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. All the chemicals used in experimental study were of analytical grade and procured from M/s Sigma-Aldrich, India. Thirty-six numbers of male Wistar albino rats weighing 150-200g were procured from Small Animal Breeding Station, Mannuthy. All the animals were kept in well-ventilated polypropylene cages under standard laboratory conditions of 12 h/12 h light/dark cycle at 25°C ± 2°C with 30-70 per cent relative humidity and were acclimatized for 7 days prior to experimentation. They were given standard rat feed and drinking water ad libitum.

Trial drug: *Saptamrutha Lauha* (250mg/kg)

Chemical used to induce oxidative stress: Naphthalene (1100mg/kg)

Standard antioxidant: Silymarin (50mg/kg)

Dose fixation: Human dose* conversion factor (0.018) for rat = x g/ 200g[13]

Rat dose of *Saptamrutha Lauha* is 250mg*0.018= 5mg (approx) for 200g rat

Naphthalene administered at dose of 1100mg/kg rat=220mg for 200g rat Silymarin is administered at dose of 50mg/kg rat =10mg/200g rat.

Grouping

Thirty-six Wistar albino rats of male sex was selected. The rats were weighed, marked with picric acid and grouped to six according to their body weights. The animals were divided into six groups with six animals each.

Group I (Control): Served with regular rodent feed and distilled water only.

Group II (Standard drug): Single oral dose Administration of 1100mg/kg Naphthalene On first day+ Silymarin (50mg/kg) for 10 days. Group III: Single oral dose administration of 1100mg/kg Naphthalene On first day + *Saptamrutha Lauha* in the calculated effective dose (5mg/200g or 25mg/1kg) orally for 10 days.

Group IV: Single oral dose administration of 1100mg/kg Naphthalene on first

Day + *Saptamrutha Lauha* in double the calculated, Effective dose (10 mg/200g or 50mg/1kg) orally for 10 days.

Group V: Single oral dose administration of 1100mg/ kg naphthalene on first day+ *Saptamrutha Lauha* in four times the calculated, effective dose (20mg/200g or 100mg/1kg) orally for 10 days.

Group VI: Single oral dose administration of 1100mg/kg naphthalene on first day.

After 10th day the animals was weighed and sacrificed using diethyl ether in closed chamber. Blood was collected from retro orbital plexus with heparinized capillary tubes under anaesthesia, into sterile centrifuge tubes without anticoagulant. Homogenate of lens tissue was prepared for antioxidant assays.

Collection of samples

Serum: Blood was collected from retro orbital plexus with heparinized capillary tubes under anaesthesia (after 10th day) into sterile centrifuge tubes without anticoagulant. After keeping the sample at room temperature for the clot formation, it was kept in the refrigerator for about half an hour. Serum was separated after centrifugation at 3000rpm for 10 minutes and clear serum obtained was stored at -20^{0}C for the assay of ALT, AST and ALP.

Tissue collection: The animals were euthanized at the end of experiment (day 11) after overnight fasting. The eyeball was harvested quickly, washed in ice cold normal saline and weighed for estimating the relative weight. The next step included weighing and homogenizing the tissue (lens) using a cold 1.15% (w/v) potassium chloride solution in a glass homogenizer and in motor and pestle for the analysis of antioxidant parameters such as catalase, SOD.

Statistical Analysis: The data collected were classified as:

Data regarding blood serum parameters.

Data regarding antioxidant assays.

The mean and standard deviation of various groups were compared with control group and was analyzed by one way ANOVA with multiple comparison (post hoc test) to analyze the level of significance of variation of group means.

OBSERVATION AND ANALYSIS

Table 2: Observations from blood serum parameters

	N	ALP in U/L (Mean ± SD)	SGOT in U/L (Mean ± SD)	SGPT in U/L (Mean ± SD)
Group I	6	130.23 ± 4.3 ^{BC}	161.38 ± 4.16^{BD}	141.68 ± 4.18 ^{ABC}
Group II	6	116.82 ± 8.54 ^{A}	131.7 ± 4.93^{A}	130.58 ± 7.76 ^{D}
Group III	6	123.72 ± 4.53 ^{AB}	150.85 ± 5.69B^{CD}	135.18 ± 4.34 ^{BD}
Group IV	6	129.87 ± 4.41 ^{BC}	147.07 ± 4.19^{C}	138.58 ± 5.3 ^{BD}
Group V	6	134.83 ± 4.7 ^{C}	163.05 ± 4.31^{D}	149.57 ± 5.7 ^{C}
Group VI	6	146.72 ± 5.11 ^{D}	233.47 ± 12.47^{E}	191.05 ± 5.92 ^{E}
p<0.05				

Table 3: Observations from blood serum parameters

	N	Absorbance of Superoxidedismutase Activity (Mean ± SD)	Absorbance of Catalase Activity (Mean ± Sd)
Group I	6	0.866 ± 0.129 ^{A}	0.317 ± 0.044 ^{A}
Group II	6	0.441 ± 0.141 ^{B}	0.266 ± 0.036 ^{B}
Group III	6	1.187 ± 0.061 ^{C}	0.567 ± 0.045 ^{C}
Group IV	6	1.082 ± 0.109 ^{C}	0.629 ± 0.054 ^{D}
Group V	6	0.881 ± 0.05 ^{A}	0.291 ± 0.059 ^{BC}
Group VI	6	1.59 ± 0.249 ^{D}	0.766 ± 0.098 ^{E}
p<0.05			

Thus on analyzing the observations, Average ALP level among Group I, II, III, IV, V and VI were 130.23±4.3, 116.82±8.54, 123.72±4.53, 129.87±4.41, 134.83±4.70 and 146.72±5.11 respectively. The values in test groups were decreased significantly compared to naphthalene group, suggestive of an antioxidant activity in test groups.

Average SGOT level among Group I, II, III, IV, V and VI were 161.38±4.16, 131.7±4.93, 150.85±5.69, 147.07±4.19, 163.05±4.31 and 233.47±12.47 respectively. The values in test groups were decreased significantly compared to naphthalene group, suggestive of an antioxidant activity in test groups.

Average SGPT level among Group I, II, III, IV, V and VI were 141.68±4.18, 130.58±7.76, 135.18±4.34, 138.58± 5.3, 149.57±5.7 and 191.05±5.92 respectively. The values in test groups were decreased significantly compared to naphthalene group, suggestive of an antioxidant activity in test groups.

Absorbance level of SOD activity among Group I, II, III, IV, V and VI were 0.866±0.129, 0.441±0.141, 1.187±0.061, 1.082±0.109, 0.881±0.05 and 1.59±0.249 respectively.

The values in test groups were decreased significantly compared to naphthalene group, suggestive of decrease in absorbance in test groups implying an increase in SOD activity in test groups compared to naphthalene group.

Absorbance level of catalase activity among Group I, II, III, IV, V and VI were 0.317±0.044, 0.266±0.036, 0.567±0.045, 0.629±0.054, 0.291±0.059 and 0.766±0.098 respectively. The values in test groups were decreased significantly compared to naphthalene group, suggestive of decrease in absorbance in test groups implying an increase in CAT activity in test groups compared to naphthalene group.

Fig 1: *Saptamrutha Lauha* at different doses, Naphthalene

Fig 2: Oral administration

Fig 3: Blood drawn from Retro orbital plexus using capillary tubes

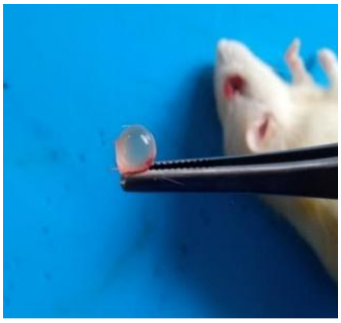


Fig 4: Eye ball extraction

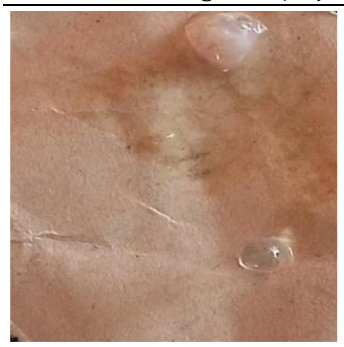


Fig 5: Lens extracted from eye ball

Fig 6: Homogenating lens by maintaining the cold chain

Fig 7: Transferring lens homogenate to tubes

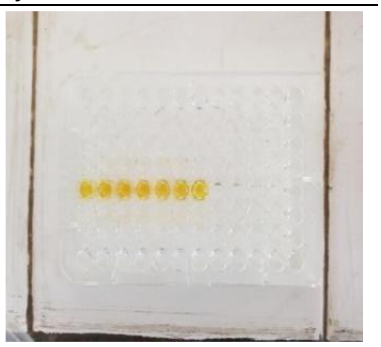
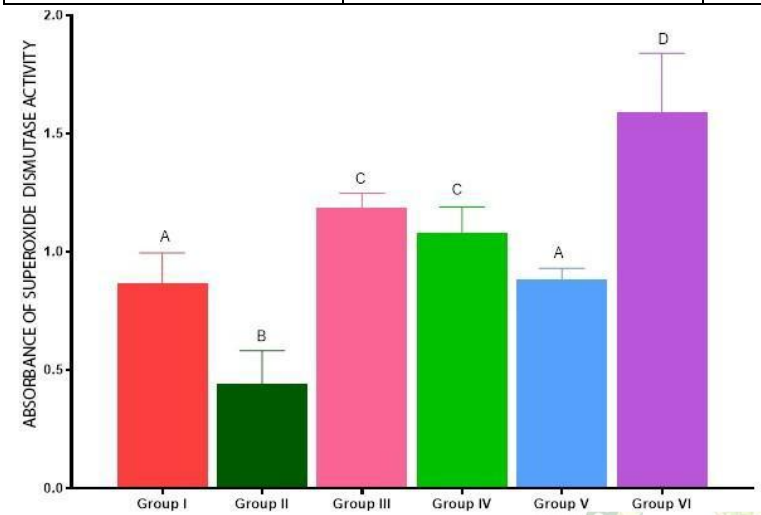
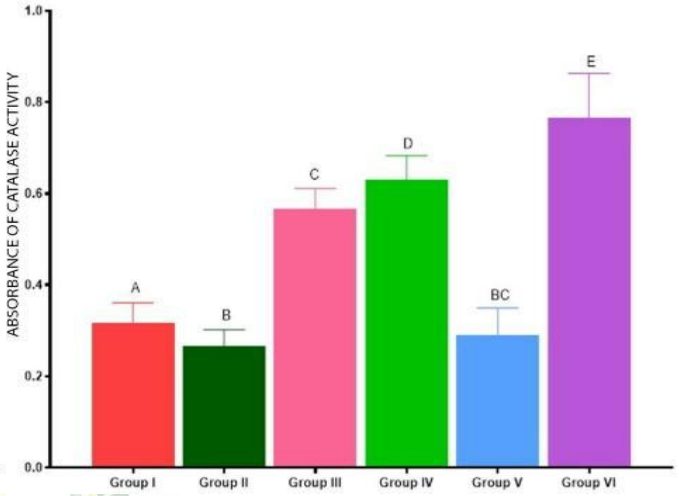


Fig 8: Wells plate (reagents added to homogenate) for Spectrophotometric reading



Graph1: SOD activity



Graph 2: CAT activity

DISCUSSION

Groups provided with Treatment of *Saptamrutha Lauha* in different doses showed a significant reduction in SGPT, SGOT, and ALP levels compared to group treated only with naphthalene. The decrease in levels in group III, IV, V with respect to group VI suggests that the drug *Saptamrutha Lauha* possess an antioxidant effect which overruled the induced oxidative stress.

Groups provided with Treatment of *Saptamrutha Lauha* in different doses showed a significant reduction in absorbance levels compared to group treated only with naphthalene. The decrease in absorbance levels in group III, IV, V with respect to group VI implies an increase in SOD and catalase activity in groups III, IV and V compared to group VI. Suggesting that the drug *Saptamrutha Lauha* possess an antioxidant effect which competed the induced oxidative stress.

The assays done suggests that *Saptamrutha lauha* is capable of scavenging free radicals like hydrogen peroxide, superoxide evolved in the pathogenesis of Naphthalene induced oxidative stress. *Rasayana* can be correlated to antioxidant and immunomodulatory action. On analyzing the combination, each drug possesses *Rasayana* and *Chakshushya* property in common. Thus, by virtue of combined effect of all drugs, the *Kalpana* has a synergestic action. In case of an oxidative stress either by aging or by a toxicity, the *Doshik* balance is interrupted. Age related ocular pathologies including cataract and age-related macular degeneration are largely mediated by oxidative stress. *Vata* is the prime *Dosha* affected. The normal *Gati* of *Vata* becomes altered which further deranges the *Prakrutha Kapha* and *Pitta* by its *Yogavahi* property. The *Kapha Vikruthi* results in interruption of the *Stiratwa, Snigdatwa* and *Sandhibandhana*. These properties are concerned with *Bala* or congruity of a structure. Considering the case of lens, this could result in loss of strength of ion channels, causing lens to be hydrated, further leading to denaturation of proteins, finally resulting in opacification. Considering the case of retina, this would probably cause increased permeability of RPE layer resulting in lipofusin and drusenoid deposition. Also, the *Pitta Vikruthi* results in derangement of *Pachana* and *Ooshma* quality, causing a reduction in ATPase activity and enzymes required for normal metabolism. On considering this, *Triphala, Lauha* and *Madhu* is of *Kashaya Tikta* predominant. These *Rasa* is concerned with *Kapha Pitta Prasamana* and *Kledopashoshanam*. Whereas *Yastimadhu* and *Gritha* in pacifies *Vatapitta*, increases the *Bala* of *Dathus* and improves *Ojus*. Thus, *Saptamrutha Lauha* is a

combination of drugs which balance the *Dosha* and is efficient as a powerful antioxidant.

CONCLUSION

The experimental pharmacology concluded a dose dependent antioxidant effect of *Saptamrutha Lauha* providing scientific support to its Ayurvedic use. Further clinical studies must be conducted based on these criteria in order to substantiate the findings obtained in the experimental work.

REFERENCES

1. Goodman D, Ness S. The Role of Oxidative Stress in the Aging Eye. Life. 2023 Mar; 13(3): 837.
2. Cabrera MP, Chihuailaf RH. Antioxidants and the Integrity of Ocular Tissues. Vet Med Int. 2011; 2011: 1–8.
3. Alvarez Barrios A, Alvarez L, Garcia M, Artime E, Pereiro R, González-Iglesias H. Antioxidant Defenses in the Human Eye: A Focus on Metallothioneins. Antioxidants. 2021 Jan 11; 10: 89.
4. India. Ayurvedic Pharmacopoeia Committee. The Ayurvedic formulary of India [Internet]. Delhi: Controller of Publications; 2003 [cited 2023 Jun 3]. 546 p.
5. Srikumar R, Parthasarathy NJ, Manikandan S, Narayanan GS, Sheeladevi R. Effect of Triphala on oxidative stress and on cell-mediated immune response against noise stress in rats. Mol Cell Biochem. 2006 Feb; 283(1-2): 67-74. doi: 10.1007/s11010-006- 2271-0. PMID: 16444587.
6. Radha, R., and G. Rajaathi. "Phytochemical analysis of Glycyrrhiza glabra." Indian J. Sci 22 (2015): 59-68.
7. Al-Snafi, A.E. (2018). Glycyrrhiza glabra: A phytochemical and pharmacological review. IOSR Journal of Pharmacy, 8(6), 1-17.
8. Upadhyay Madhav, Ayurveda Prakasha, Mishra G.S., Sanskrit - Hindi Commentary, reprint edition 2016, Chaukhamba Bharati Academy, Varanasi, Shlok 3/230-33, Page no 364
9. The Ayurvedic Pharmacopia of India, Department of Ayush, Govt. of India, edition I, Part I, Vol V1, page no: 214-218.
10. Chunekar Krushnchandra. Bhavaprakash Nighantu, Indian Materia Medica, Editor- Dr.Gangasahay Pandey, Chowkhamba bharti academy- Varanasi; Edition-2010. (Ghritavarga-4, 5)
11. Chien MM, Lin HC, Lee LF, Keller JJ, Wang LH. Association Between Silymarin Use and Age-related Macular Degeneration in Patients with Chronic Hepatic Diseases: A Population-based Retrospective Cohort Study [Internet]. In Review; 2021 Nov [cited 2023 Apr 17].
12. Touyz L. Naphthalene mothballs: A silent killer. In 2014. p. 255–63.
13. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016 Mar; 7(2): 27-31. doi: 10.4103/ 0976-0105.177703. PMID: 27057123; PMCID: PMC4804402

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