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Belaguli Govardhan
PG Scholar, Dept of Manovigyan evum Manasa Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

Adiga Manjunatha
Associate Professor, Dept of Manovigyan evum Manasa Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

Shetty Suhas Kumar
Professor and HOD, Dept of Manovigyan evum Manasa Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

Correspondence
Belaguli Govardhan
PG Scholar, Dept of Manovigyan evum Manasa Roga, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan, Karnataka, India

Standardisation of *Lashuna taila*: An Ayurvedic oil based medicine

Belaguli Govardhan, Adiga Manjunatha and Shetty Suhas Kumar

Abstract
Herbalism or phytomedicine is a trending branch of science. Ayurveda and herbal formulations play an important role in world medical science. Due to their safety, low cost, easy availability they are opted as alternate for modern medicines. The quality, safety and efficacy are the commonest issues raised by the common people and experts in medical field. Standardisation and validation are the only scientific way to answer those issues. One such attempt in standardising an Ayurveda medicated oil formulation quoted in classical texts and deriving the standard parameters is done through this study. The study found to be promising in the evolution of a standardized drug with rich in antioxidants and unsaturated fatty acids.

Keywords: lashunataila, allium sativum linn, *Sesamum indicum* linn, antioxidants, ayurveda, atatwabhinivesha, OCD

1. Introduction
The usage of herbalmedicines has increased in the present era on humans for various diseased conditions as they are safe, costs less and easily available. Issues regarding their quality, safety, and efficacy have been raised up by the people who consumes it and by the evidence based sciences. Almost all Ayurveda formulations claim themselves as highly effective. The exact effectiveness is a question and challenge in clinical trials. In order to fulfil this challenge, the drugs have to undergo standardisation and thus a scientific, valid answer is got through it, increasing faith in health seekers. The current analytical study was taken up in order to fulfil such attempt and genuine standard parameters were derived.
Lashuna taila (LT) is a classical formulation mentioned in Ayurveda text [1] in the diseased condition called *Atatwabhinivesha,* likely to be an Ayurveda description for OCD.

The following table gives a brief introduction about constituents of LT

Drug	Parts used	Botanical name	Family name
Lashuna (garlic)	Bulbs	*Allium Sativum linn*	Amaryllidaceae
Tilataila (sesame oil)	Seed oil extract	*Sesamum indicum linn*	Pedaliaceae
Go ksheera (cow's milk)	Natural form	-	-

2. Materials and Methods
2.1 Pharmacognosy
The drug *lashuna* is said to be having

<table>
<tr><td>Qualities [2]</td><td colspan="2">• Rasa (taste):Amlavarjita pancharasa (has all 5 kinds of taste except sour), while Katu(acrid/pungent) is main taste
• Guna (qualities) :Snigdha (unctous), teekshna (sharp), guru (heavy), sara (flowing), picchhila (slimy)
• Veerya (potency) :Ushna (hot)
• Vipaka (metabolic end product) : Katu</td></tr>
<tr><td>Chemical constituents [3]</td><td colspan="2">Alliin, Allicine, S- allyl cysteine, Diallyl disulphide, Ajoene, Saponins, Fructans, Salenium, Allyl methyl trisulfinate, vitamins like nicotinic acid, ascorbic acid, Vit A, thiamine, riboflavin, niacin, biotin, folic acid</td></tr>
<tr><td>Action</td><td>Deepana-pachana (carminative-digestive), rasayana (rejuvenative), vatanulomana (pushes accumulated and vitiated vata-downwards), medhya(nootropic / cognitive enhancer) [4]</td><td>Anti-oxidant, anti-stress and anti-depressant activity, neuro protective activity [5]</td></tr>
</table>

The drug *tila taila* has

Qualities [6]	• *Rasa :Madhura* (sweet) and *Anurasa* (subtle rasa) are *kashaya* (astringent) & *tikta* (bitter) • *Guna :Snigdha, guru,vyavayi (separates, resolves into components), vishada(expands, opens), sara, vikasi*(enters into channels*)* • *Veerya: Ushna* • *Vipaka: Madhura*	
Chemical constituents [7]	Sesamin, Sesamol, Sesamolin, Sesamolin glucosides, Lignans, Linoleate,oleic acid, Tocopherol	
Action	*Vatahara* (pacifies *vata*), *balya* (strength), *vikasi, deepana, medhya* [8]	Anti oxidant, Maintains HDL, Lowers LDL, Synthesis of Vit E, Fatty acid metabolism [9]

Cow's milk has

Qualities [10]	• *Rasa:Madhura* • *Guna: Guru, snigdha* • *Veerya: Sheeta* (cold) • *Vipaka: Madhura*
Chemical constituents [11]	Lactose, Lectithin, Tocopherols, Casein, Globulins, vitamins, enzymes, salts of citric acid, phosphoric acid, hydrochloric acid
Action	*Kaphakarak* (increases normal *Kapha*), *medhya, Ayu, ojo – dhatuvardhaka* (immune booster), *deepana, rasayana, Manasaroga* (effective in neurological/psychiatric disorders), *brihmana* (nourishes whole body) [12]

2.2 Raw materials

Raw materials *lashuna* bulbs, processed edible *tila taila*, milk were obtained as per requirment from Hassan local market, Karnataka.The materials were authenticated from experts of department of *Dravyaguna*, Sri Dharmasthala Manjunatheshwara college of *Ayurveda* and hospital, Hassan.

Taila preparation

Taila was prepared from collected raw materials according to guidelines mentioned in *Ayurvedic* formulation of India [13]. Total 7 liters of *taila* was prepared at the ratio of 1:4:16 part and is mentioned in the following table

Lashuna bulb	1.75 kg	1 part
Processed edible *tilataila*	1.75 litres	4 part
Milk	7 litres	16 part

First *lashuna* bulbs were peeled and were crushed manually using porcelain mortar-pestle and were weighed (Fig 1). The bulbs were added to the process until we obtained desired weight of 1.75 kg. On the other hand cow's milk of 7 litres was boiled and crushed *lashuna* bulb was added to it slowly and boiled again. Care was taken on milk for not being spoilt.

The processed edible *tilataila*of 1.75 litres was later added to the boiling preparation. The whole formulation was boiled continuously in low flame for about 2 days until we got the ideal sign of *taila* mentioned in classics.[14]The formulation was later cooled and filtered and was bottled in 100 ml of sterile bottles (Fig 2) and was labelled accordingly.

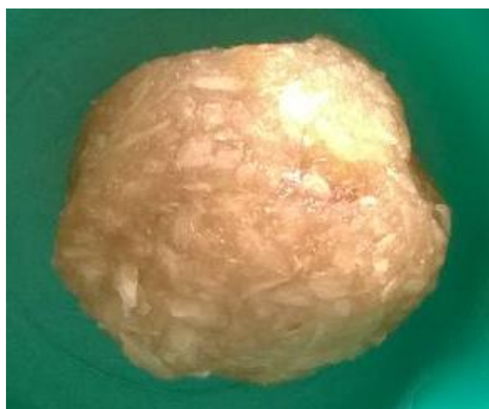


Fig 1: Crushed lashuna bulbs

Fig 2: Bottled LT

The bottles were kept available for proposed research work in dispensary section of Sri Dharmasthala Manjunatheshwara college of *Ayurveda* and hospital, Hassan and was dispensed free of cost using coupon method.

2.3 Methodology

3 bottles i.e, 300 ml of prepared LT was sent for analytical study to S.D.M. centre for research in *Ayurveda* and allied sciences, Kuthpady, Udupi, Karnataka which is an AYUSH Centre for Excellence and Recognized SIROs by DSIR.

Sample was coded as: 17101602

Investigations performed were: Refractive index, Specific gravity, Acid value, Saponification value, Iodine value, Determination of Unsaponifiable matter, Peroxide value, viscosity, Rancidity test, HPTLC.

Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundry line intersects the

separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.33217 at 28˚C. The difference between the reading and1.33217 gives the error of the instrument. If the reading is less than 1.33217, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of LT is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

Specific gravity
Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Acid value
Weighed 2- 10g of oil in a conical flask. Added 50 ml of acid free alcohol-ether mixture (25+25ml) previously neutralised with the 0.1M potassium hydroxide solution and shaken well. Added One ml of Phenolphthalein solution and titrated against 0.1M Potassium hydroxide solution. End point is the appearance of pale pink colour. Repeated the experiment twice to get concordant values.

Saponification value
Weighed 2g of the Oil / Fat into a 250 ml RB flask fitted with a reflux condenser. Added 25ml of 0.5M alcoholic potash. Refluxed on a water bath for 30 minutes. Cooled and added 1 ml of Phenolphthalein solution and titrated immediately with 0.5 M Hydrochloric acid (a ml). Repeated the operation omitting the substance being examined (blank) (b ml). Repeated the experiment twice to get concordant values.

Iodine value
The LT was accurately weighed in a dry iodine flask. Dissolved with 10ml of CCl_4, 20ml of iodine monochloride solution was added. Stopper was inserted, which was previously moistened with solution of potassium iodide and flask was kept in a dark place at a temperature of about 17^0 C for 30 min. 15ml of potassium iodide and 100ml of water was added and shaken well. This was titrated with 0.1N Sodium thiosulphate, starch was used as indicator. The number of ml of 0.1N sodium thiosulphate required (a) was noted. The experiment was repeated with the same quantities of reagents in the same manner omitting the substance. The number of ml of 0.1N sodium thiosulphate required (b) was noted. The experiment was repeated twice to get concordant values.

Determination of Unsaponifiable matter
Weighed 5g of the LT was weighed into the flask. added 50ml alcoholic KOH into the sample. Boiled gently but steadly under reflux condenser for one hour. The condensor was washed with 10ml of ethyl alcohol and the mixture was collected and transferred to a separating funnel. The transfer was completed by washing the sample with ethyl alcohol and cold water. Altogether, 50ml of water was added to the separating funnel followed by an addition of 50ml petroleum ether. The stopper was inserted and shaken vigorously for 1 minute and allowed it to settle until both the layers were clear. The lower layer containing the soap solution was transferred to another separating funnel and repeated the ether extraction six times more using 50ml of petroleum ether for each extraction. All the extracts were collected in a separating funnel. The combined extracts were washed in the funnel 3 times with 25ml of aqueous alcohol and shaked vigorously. And drawing off the alcohol-water layer after each washing. The ether layer was again washed repeatedly with 25ml of water until the water no longer turns pink on addition of a few drops of Phenolphthalein indicator solution. The ether layer was transferred to a tarred flask containing few pieces of pumice stone and evaporated to dryness on a water bath. Placed the flask in an air oven at 85°c for about 1 hour to remove the last traces of ether. A few ml of acetone was added and evaporated to dryness on a water bath. Cooled in a desicator to remove last traces of moisture and then weighed.

Rancidity test
1ml of melted fat was mixed with 1ml of conc. Hcl and 1ml of 1% solution of phloroglucinol in diethyl ether and then mixed thoroughly with the fatacid mixture. A pink color indicates that the fat is slightly oxidized while a red color indicates that the fat is definitely oxidized.

Peroxide value
5g of the LT was weighed accurately into a conical flask, added 30 ml of mixture of 3volumes of glacial acetic acid and 2 volumes of chloroform, added 0.5ml of potassium iodide, allowed it to stand for 1 minute, add 30ml of water titrate gradually with vigorous shaking with 0.1M sodium thiosulphate until the yellow color disappears. Add 0.5ml of starch indicator continued the titration until blue color disappears.
Peroxide value= 10(a-b)/W
Where W= weight in g of the substance

Viscosity
The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula
$\eta 1= \rho 1t1 \text{ X } \eta 2$
$\rho 2t2$
$\eta 1$ – Viscosity of sample
$\eta 2$ - Viscosity of water
t1 and t 2- time taken for the sample and water to pass the meniscus
$\rho 1$ and $\rho 2$ – Density of sample and water
X= Specific gravity of sample x 0.9961/specific gravity of water
Π= X x Time for sample x1.004/specific gravity of water x70sec

Sample preparation for HPTLC
Sample obtained in the procedure for the determination of unsaponifiable matter is dissolved in 10 ml of chloroform this was followed for all the sample of *Ghee*, and chloroform soluble portion was used for HPTLC.

HPTLC
3, 6 and 9μl of the above sample was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm

using Linomat 5 TLC applicator. The plate was developed in Toluene – Ethyl acetate (9:1) and the developed plates were visualized under short UV, long UV and after derivatisation in vanillin-sulphuric acid spray reagent observed under white light. Scanned under UV 254nm, 366nm.R_f, colour of the spots and densitometric scan were recorded.

3. Results

Table 1: Results of standardization parameters

Parameter	Results n = 3 %w/w
	LT
Refractive index	1.4767
Specific gravity	0.9409
Acid value	10.02
Saponification value	240.27
Iodine value	135.52
Unsaponifiable matter (%)	2.78
Peroxide value	0.20
Viscosity	52.57
Rancidity	Fat is not oxidised

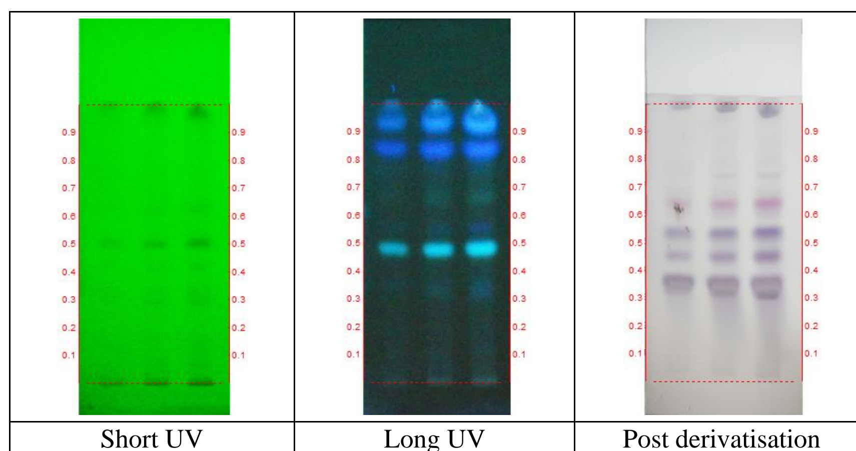
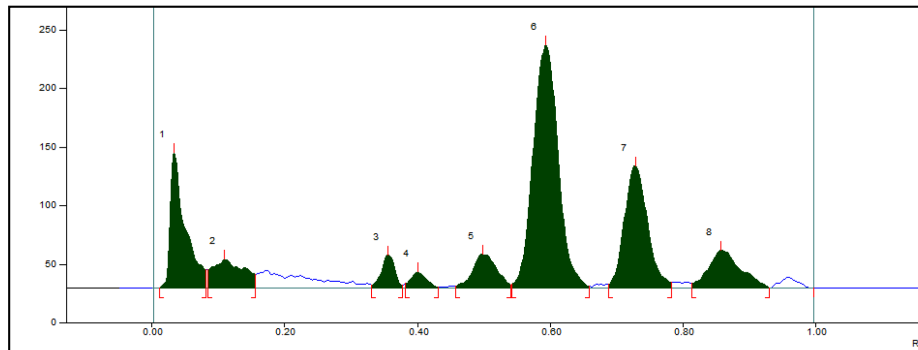


Fig 4: TLC photo documentation of chloroform extract of *LT*
Track 1- *LT* - 3µl
Track 2 - *LT* - 6µl
Track 3- *LT* - 9µl
Solvent system: Toluene: Ethyl acetate (9:1)

Table 2: Rf values of sample of *LT*

254nm	366nm	Post derivatiation
0.07 (L. green)	-	-
0.29 (L. green)	-	-
0.34 (L. green)	0.34 (FD. blue)	0.34 (D. purple)
0.42 (L. green)	-	-
-	-	0.45 (D. purple)
-	0.48 (FD. blue)	-
0.51 (D. green)	-	-
-	-	0.53 (D. purple)
-	0.56 (FD. blue)	-
-	-	0.60 (L. purple)
0.64 (L. green)	-	0.64 (D. pink)
-	0.66 (FD. green)	-
-	-	0.75 (L. purple)
-	-	0.79 (L. purple)
-	0.84 (FD. blue)	-
-	0.92 (FD. blue)	-

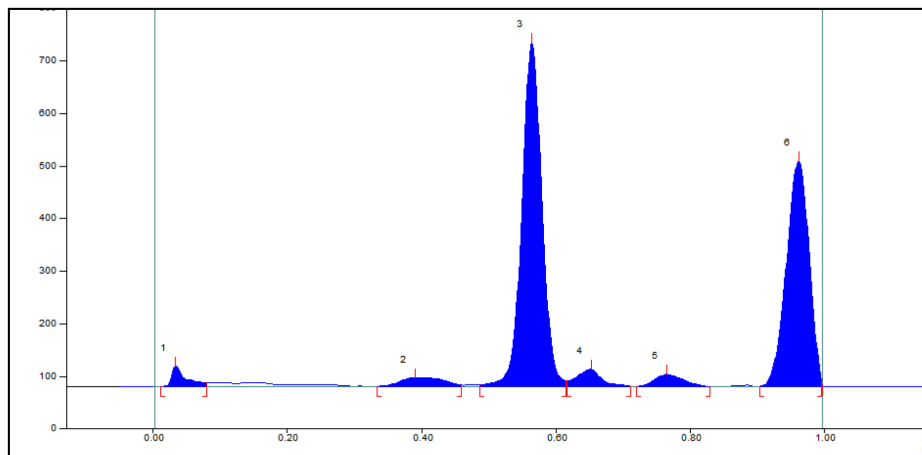
*D – dark; L – light; F - fluorescent



Track 3, ID: Lashuna taila

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.2 AU	0.03 Rf	115.9 AU	20.91 %	0.08 Rf	15.3 AU	1886.6 AU	14.02 %
2	0.08 Rf	15.3 AU	0.11 Rf	24.4 AU	4.41 %	0.16 Rf	11.9 AU	825.8 AU	6.14 %
3	0.33 Rf	2.7 AU	0.36 Rf	28.1 AU	5.07 %	0.38 Rf	2.6 AU	411.8 AU	3.06 %
4	0.38 Rf	3.9 AU	0.40 Rf	13.8 AU	2.48 %	0.43 Rf	0.7 AU	232.1 AU	1.73 %
5	0.46 Rf	1.8 AU	0.50 Rf	28.7 AU	5.18 %	0.54 Rf	2.8 AU	761.1 AU	5.66 %
6	0.54 Rf	3.1 AU	0.59 Rf	207.3 AU	37.39 %	0.66 Rf	1.7 AU	5607.7 AU	41.69 %
7	0.69 Rf	3.6 AU	0.73 Rf	104.1 AU	18.78 %	0.78 Rf	4.8 AU	2609.9 AU	19.40 %
8	0.81 Rf	3.9 AU	0.86 Rf	32.0 AU	5.78 %	0.93 Rf	0.1 AU	1116.6 AU	8.30 %

Fig 5a: At 254nm



Track 3, ID: Lashuna taila

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.1 AU	0.03 Rf	39.2 AU	3.28 %	0.08 Rf	6.8 AU	613.0 AU	2.23 %
2	0.33 Rf	0.6 AU	0.39 Rf	17.0 AU	1.43 %	0.46 Rf	3.2 AU	818.3 AU	2.98 %
3	0.49 Rf	3.2 AU	0.56 Rf	654.1 AU	54.72 %	0.62 Rf	11.3 AU	14037.4 AU	51.08 %
4	0.62 Rf	11.3 AU	0.65 Rf	32.9 AU	2.75 %	0.71 Rf	0.8 AU	881.0 AU	3.21 %
5	0.72 Rf	0.1 AU	0.77 Rf	23.4 AU	1.96 %	0.83 Rf	0.1 AU	716.8 AU	2.61 %
6	0.90 Rf	0.7 AU	0.96 Rf	428.8 AU	35.87 %	1.00 Rf	9.9 AU	10412.1 AU	37.89 %

Fig 5b: At 366nm

Fig 5: Densitometric scan of the sample of LT

4. Discussion

Importance of constituents used in preparation of LT

The processed, edible form of sesame oil which was used in the formulation contains maximum number of unsaturated fatty acids (80%). [15]It has a variety of antioxidants like Sesamin, Sesamolin, Sesamolin glucosides, Lignans, Linoleate, Alpha tocopherol. A systematic review article [16] concludes that, of all the sesame derivation, sesame oil is more frequently effective than any other derivations and decreases the lipid peroxidation and thus decreases the endothelial dysfunction originating from reactive oxygen species (ROS) formation. Along with the above mentioned antioxidants, it also contains Vitamin E which is a supportive for antioxidation. Sesame oil increases the non enzymatic

antioxidants like vitamin C, vitamin K, beta carotene and enzymatic antioxidants like Superoxide distumase (SOD), Glutathione (GSH), Glutathione peroxidase (GPx), Catalase (CAT) and decreases the oxidative stress markers like Thiobarbiuturic acid reactive substance (TBARS), Malondialdehyde (MDA). Vitamin K and Sesamol which is an excellent antioxidant, is important in antioxidant defence system preventing the free radical damage.

Another study [17] claims that garlic has potent bio active compounds largely allylsulfidederivates. Allicin is the major ingredient produced when garlic is crushed. Apoptosis is stimulated by garlic. Some of the antioxidants present in garlic are Alliin, Allicine, S- allyl cysteine, Diallyl disulphide, Ajoene, Saponins, Fructans, Salenium, Allyl methyl trisulfinate. Proper heat treatment and aged processing could be used to enhance the amount of bio active compounds and antioxidant capacity of garlic. Garlic extracts exhibit significant protective effects against DNA damage induced by hydrogen peroxide (HO) and hydroxynonenal(HNE) which might be related to antioxidant activity. Garlic exhibits cytoprotective effects by inducing Glutamate cysteine ligase (GCL), which protects the cells from oxidative injury by increasing Glutathione (GSH) content. Ajoene activates the Protein kinase C delta (PKC delta) dependant Nuclear factor erythroid 2-related factor 2 (NrF2) and thus transcriptionally regulates the expression of gene encoding for GCL and other cysteine metabolizing enzymes. Thus the whole study concludes the action of garlic on genes and DNA through antioxidant property.

The Lecithin [18] which is abundantly found in cow's milk is a food additive, an amphiphilic which acts as stabilizing agent as well as smoothes food texture. It also acts as emulsifier, homogenizing liquid mixture. The authors confirms that from this study, the main aim of adding cow's milk into the formulation of LT might have been fulfilled. Another study [19] continues as, Lecithin has phosphatidylcholines [20], a source of choline, an essential nutrient to humans. A study [21] on Casein supports that it is a major supplier of amino acids, carbohydrates, calcium, phosphorous.

5. Conclusion

Despite the advent of modern technology in standardisation of Ayurveda formulations, only very few are available in the standardised form. With the current standardised parameter which was derived through standard procedure, a proper identification of LT can be done. The purpose of the study was obviously to ensure the effectiveness of LT. As LT contains wide range of fatty acids and antioxidants, it is essential to validate its content and the therapeutic utility through preclinical and clinical studies should be considered as recommendation for further studies.

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