ĀRYAVAIDYAN

A PEER REVIEWED QUARTERLY JOURNAL ON AYURVEDA AND ALLIED SCIENCES

Regn. No. 55127/87 ISSN 0970 - 4086 Vol. 36, No. 2 November 2022 to January 2023

लाभानां श्रेय आरोग्यम्

Of all the gains,
the most precious is health

ESTD. 1902

Vaidyaratnam P.S. Varier's
Arya Vaidya Sala, Kottakkal, Kerala

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सततध्ययनं वादः परतन्त्रावलोकनम् ।
तद्विद्याचार्यसेवा च बुद्धिमेधाकरो गणः ।।

Constant study, mutual discussion, learning other disciplines and close association with the preceptor - these factors endow one with intelligence and memory

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Front: *Tinospora cordifolia* (Willd.) Miers
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GUEST EDITORIAL
ĀRYAVAIDYAN, Vol. 36, No. 2, November 2022 - January 2023, Pages 05 - 08

Enhancement of *aayurvedic* knowledge system - the need for a multipronged approach

Muraleedharan T.S.*

It is universally accepted that knowledge keeps evolving incessantly. How does knowledge evolve? Is it a seamless phenomenon? Or is it often facilitated by abrupt and disruptive events, to borrow a term from modern management parlance? What is the kinetics of the paradigm shifts that would, indeed, have accompanied such evolutionary processes? At what stages of such shifts does knowledge get metamorphosed into wisdom? And in comparison, is wisdom an irrevocable finality at every stage of its formation, without being subjected to any kind of further evolution? That is, does every state of wisdom remain static eternally? And will further progression happen in a staccato mode? These are, of course, matters of epistemological concern. They may not necessarily cause any direct and immediate consequence on the way knowledge is acquired and practically applied for the purpose of achieving specific and tangible end-results intended to promote the welfare of humankind. Their consideration, instead, becomes relevant while taking a historical view on the development of knowledge systems. This dichotomy between practical and epistemological aspects of knowledge applies to the domain of *aayurveda* too. In fact, it may prove to be quite complex in the case of *aayurveda*, because it happens to carry with it a heavy legacy of traditional knowledge and ancestral wisdom with an uninterrupted history of a couple of thousand years. And there is yet another complicating factor. The rate of change and the resulting acceleratory pressures have become much faster and vigorous in the recent epochs compared to what they were in the distant past. This applies to every domain of knowledge, and particularly so to *aayurveda*.

In this contextual scenario, it may be pertinent to ponder over the modalities that the protagonists of *aayurvedic* growth will need to adopt in order to render the evolutionary sequence more seamless and natural. The first step in this direction seems to be that all those who are partners in the efforts to take forward *aayurveda* to higher levels of achievement should become ready to shed the erroneous and rather complacent notion that this energetic knowledge system has always been remaining in a safe cocoon of self-contained hermitage. That notion does not seem to represent the reality. A lively and vibrant system like *aayurveda* could, obviously, not have remained insulated from the continuously changing knowledge realms of the world all through the past centuries of its evolution. The relentless process of evolution is quite evident in

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its long history starting from the very earliest days of the *Samhita*s. When the philosophical ethos of those early periods is compared with the pragmatic spirit of the *aayurvedic* renaissance of the early 20th century, the extent of the paradigm shifts that had occurred in the interregnum period would become clear.The five decades long span starting from the last quarter of the 19th century and extending through the first quarter of the next century was the period when the structure and functioning of *aayurvedic* health care system started becoming organised as a result of concerted efforts of some illustrious pioneers. They set up Institutions in different parts of the country in the areas of drug manufacture, clinical service and education. For example, Krishnasashtri Puranik established Shri Dhootapapeshwar at Mumbai in 1872, S.K. Burman set up Dabur at Kolkata in 1884, Sandu Brothers started Sandu Pharmaceuticals at Mumbai in 1899, P.S. Varier founded Arya Vaidya Sala at Kottakkal in 1902, J.C. Ghosh started Sadhana Aushadhalaya at Dhaka in 1914, R.N. Sharma started Baidyanath Ayurveda Bhawan at Kolkata in 1917, and Thaikkat Unni Mooss started SNA Oushadhasala at Trichur in 1920. Gananath Sen published his *Pratyakshaṯaareeram* in 1911 and P.S. Varier brought out his *Ashṯaanggaṯaareeram* in 1925. Professional-cum-academic alliances also came into being during this period. All India Ayurvedic Congress was set up by Shankar Daji Pade Shastri at Nashik in 1907 and Aryavaidya Samajam at Kottakkal by P.S. Varier in 1903. Visionaries such as these contributed eminently to the enrichment of the *aayurvedic* system as a whole and, thus, played a major role in the great *aayurvedic* renaissance that happened at the turn of the last century. They brought about a major paradigm shift in its knowledge system. That was the phase when the modern era of *aayurveda* really set in. The expanding frontiers of *aayurveda* became visible to all by then.

When *aayurvedic* clinical service and drug manufacture started becoming organised, their ability to meet the changing needs of the society became more strengthened. Naturally, these sectors benefited by receiving and assimilating inputs from the advancements occurring in other streams of contemporary knowledge. As time passed, the pace and quantum of such assimilation increased. Similarly, when the education sector became organised, the knowledge acquiring process became universalised and standardised. The whole modality of tutelage became progressive as it got exposed to modern ways of general education.

A cue can be taken from a historical perspective on these developments. Looking from the present times, such forward thrusts of knowledge, which happened subtly in the distant past, may appear to have occurred as natural processes. But, on closer examination, it will become evident that they were, in fact, the outcomes of human endeavour. In the absence of motivated and dedicated efforts of people of high calibre, such knowledge enhancement would not have happened. History marked the venerated *aacaarya*s of yore as codifiers of *Samhita*s,

*Nighantu*s, etc. Actually, they should be recognised as contributors to knowledge generation and upgradation, even though their legacies manifest in the numerous treatises they authored. The essence of what all of them did, both in the ancient times as well as in the recent past, can be summed up as upgradation of existing knowledge and generation of new knowledge. And the methodology employed by them had two arms. Firstly, they thoroughly studied and mastered the prior-art. Secondly, they added further value to it by skilfully applying new information and data with the aid of new tools. This modality of their endeavour may not be quite obvious from a cursory point of view, particularly in the case of ancient *aacaarya*s. But an example can be cited to prove the point. The use of metals and minerals became an integral part of *aayurvedic* therapeutic practice only after the *aacaarya*s of the later periods acquired knowledge and skill from the methods of metallurgy which was a parallel stream of knowledge being practised by alchemists.

It is to be noted that the *aayurveda* masters of both the distant andrecent pasts were esteemed academicians and at the same time, they were versatile implementers of innovative ideas too. Thus, they had no trepidation while interfacing with other streams of knowledge. Their dual expertise emboldened them to traverse unbeaten tracks. Whereas, academy, drug sector and the clinical wing of today are, apparently, compartmentalised, with research and innovation being left in a corner of insignificance. An element of schism seems to exist between these sectors. This is a disconcerting situation which is not conducive to the overall growth of *aayurveda*.

It is worth noting that the *aayurvedic* renaissance of the last century happened because its academia and its functional arms moved forward in sync. That inclusive spirit of *aayurveda* needs to be enlivened once again in order to facilitate closer associations between its academia, researchers, clinicians and drug industry. Appropriate utilisation of modern scientific principles, methods and tools will become an integral component of such associations. Some pioneering efforts in this direction have got launched since the last two decades. The mega scientific programme, a Scientific Initiative in *aayurveda*, which led to the formation of the Task Force on *Aayurvedic* Biology under the Science and Engineering Research Board of GOI, facilitated major collaborative research projects which established the scientific veracity of some vital *aayurveda* fundamentals. It should also be mentioned that new Research Journals have come to stay, in which peer reviewed scientific results are being published. Such studies have contributed significantly to the strengthening of *aayurveda* knowledge base. The increasing academic activities have resulted in an overall rise in enthusiasm among young *aayurvedic* protagonists. The recent results on the efficacy of *aayurveda* in preventing and treating COVID infection can be cited as a typical example. The historical importance of that report is that the

potential of *aayurveda* in NCD domain is now being treated seriously. All these developments signal that the knowledge frontiers of *aayurveda* are expanding. One interesting observation in all these scientific endeavours is that they were all undertaken by modern scientists, including modern medicine experts, in collaboration with *aayurvedic* academia, practitioners, and drug developers. From a historical point of view, it is interesting to note that the original knowledge collation of *aayurveda* happened centuries ago by the yeoman efforts of the venerated *aacaarya*s who were wise preceptors as well as practical masters. And the *aayurvedic* renaissance of the 20th century, as shown above, was spearheaded by dedicated entrepreneurs who were erudite scholars as well. And the current upsurge in all realms of *aayurvedic* knowledge, which can actually be perceived as the second wave of *aayurvedic* renaissance of modern times, is being shaped by collaborative endeavours in which the *aayurvedic* academia, clinical experts and experienced drug developers along with modern scientists are equal partners, in spite of their distinct academic status. And that, indeed, augurs well for the future growth and expansion of *aayurveda* health care system as a whole.

One can only hope that this spirit of partnership based on a multipronged approach will continue to flourish and this will facilitate enhancement of *aayurvedic* knowledge system. In fact, that will bring about the right environment for *aayurveda* to become an active participant in the "One Health" policy of WHO, which envisages multi-sectorial collaboration for achieving better public health outcomes. Such a participation will also equip *aayurveda* to take its due role in the global health care scenario. The significance of the Government's decision to establish in India the WHO Global Centre for Traditional Medicine is to be viewed in this perspective.

RESEARCH ARTICLE

ĀRYAVAIDYAN, Vol. 36, No. 2, November 2022 - January 2023, Pages 09 - 18

Anti-neoplasticpotential of *Tinospora cordifolia* (Willd.) Miers stem extract in Dalton's lymphoma ascites cell lines

Shyju O.,[1*] Bindya Liz Abraham,[2] Suresh Nair N.,[3] Dhanush Krishna B.,[4] Muhasin Asaf V.N.,[5] Aravindakshan T.V.[6]

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ABSTRACT: *Tinospora cordifolia* (Willd) Miers [TC] has been recognized as a traditional medicinal agent in the indigenous medical systems of India and has many therapeutic properties finding use in various ailments since ancient times. The present study was carried out to investigate the *in vitro* apoptotic potential of TC stem extract in Dalton's lymphoma ascites (DLA) cell lines. The Gas Chromatography and Mass Spectroscopy (GC-MS) analysis of the methanolic extract of the TC stems revealed the phytochemistry to be consisting of 18 bioactive compounds such as squalene, 10-octadecenoic acid methyl ester, gamma-gurjunenepoxide-(2) and vinyl deconate. The potential apoptotic effect of aqueous TC stem extract was evaluated through dual acridine orange/ ethidium bromide (AO/EB) staining and compared with the reference drug, cisplatin. The results indicated that at any particular time span, DLA cells treated with the IC_{50} concentrations of the TC test extract and cisplatin showed apoptotic signs. Majority of TC treated cells were found to be in late apoptotic stage anda greater number of cisplatin treated cells were in early apoptotic stage. Therefore, it was revealed that in a given time, the progression towards apoptosis was found to be faster with TC-treated cells when compared to the cisplatin-treated cells. The study suggested that TC stem extract induced considerable degree of apoptosis in the lymphoma cells in a shorter time and hence could be developed as a potent anti-neoplastic chemotherapeutic drug for the treatment of various lymphomas and neoplasms.

Key words: *Tinospora cordifolia,* GC-MS, AO/EB staining, Apoptosis

Introduction

A considerable bulk of the population of the world, nearly 80% (around four billion), makes use of botanical remedies for medical purposes, revealed the WHO recently.[1,2,3] *Tinospora cordifolia*, a member of the *Menispermaceae* family, is one of the most versatile medicinal plants in *aayurvedic* medicine and has been in use for centuries to treat a variety of ailments.[4] This plant has attracted the attention of researchers around the world due to its wide range of medicinal properties such as anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic efficacies.[5,6,7,8] Over the past two decades, the herb has undergone extensive phytochemical, pharmacological and clinical

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investigations.[9,10,11] The anti-cancer activity of various alkaloids present in *T. cordifolia* has been reported in Ehrlich ascites carcinoma in mice, lung adenocarcinoma cell lines, DMBA induced skin carcinoma in mice, C6 glyoma cell lines and Hela cells.[12,13,14,15,16]

Tinospora cordifolia is found in deciduous and dry forests throughout India (Figure 1). It is a glabrous climbing shrub with a succulent stem and papery bark that is creamy white to grey in colour.[7] The shrub shoots out aerial roots, usually on neem and mango trees, which could be as long as 30 meters. It bears heart-shaped leaves. The yellow flowers are axillary and long-stalked racemes. The fruit is a pea-sized, sub-globose drupe and red-coloured on maturity. Flowers can be seen in June, while fruits occur in November. Various parts of the plant including leaves, stems, and bark are reported to be used for the treatment of fevers, chronic diarrhoea, dysentery, jaundice, cancer, periodic fever, bone fracture, general debility, cough, pain in the ear, leucorrhea, asthma, skin disease, bites of toxic insects and venomous snake and eye disorders.[7] *T. cordifolia* is also termed a 'divine nectar' or 'heavenly elixir' in *aayurveda* due to its high medicinal valueand rejuvenating potential. Its drug-likeaction has been categorized according to taste, property (*guna*), digestion and metabolism, potency and mechanism of action.[17] It is used as a *rasaayana* to improve the immune system and body resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India.[18] There are a numberof reports on the use of TC as antiallergic, anti-inflammatory, immuno-suppressive, immunomodulatory, anticancer, hypoglycemic and anthelminthic.[19] *T. cordifolia* has earned a special mention not only in *aayurveda* but also in Chinese and other traditional systems of medicine.[20] Some of the active components present in the plant are alkaloids, glycosides, steroids, diterpenoid lactones, sesquiterpenoids, phenolics and aliphatic and polysaccharides.[17,21] The present study was undertaken with the objectives of screening the *Tinospora cordifolia* stems for the presence of various pharmaceutical compounds and also investigate the *in vitro* apoptotic potential of TC stem extract in Dalton's lymphoma ascites (DLA) cell line through dual acridine orange/ ethidium bromide (AO/EB) staining methods.

Figure 1
Tinospora cordifolia

Materials and methods

Preparation of aqueous and methanolic extracts of T. cordifolia stems

The fresh stems of *Tinospora cordifolia* were collected from Chelannur, Calicut, Kerala.The specimens were authenticated at the Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala (CMPR/AIF/PHG/325/01 dtd.

12/03/2022). The aqueous crude extract (called "*ghana*" in *aayurveda*) was prepared as per *Siddha Yoga Samgraha*[22] from the stems which were cleaned, dried under shade, cut into small pieces, soaked in four times water and heated to get a decoction. The decoction was reheated until it became semisolid and dried in the oven at 55°C. The aqueous extract was then stored under refrigeration (4°C) in an air-tight container.

The methanolic extract of TC stem was prepared for Gas Chromatography and Mass Spectroscopy (GC-MS) analysis using the hot extraction method in a Soxhlet apparatus. A Soxhlet extractor is a lab equipment designed for processing certain kinds of solids. This device allows for continuous treatment of a sample with asolvent over a period of hours or days to extract compounds of interest. Typically, Soxhlet extraction is done only where the desired compound has a limited solubility and the impurity is insoluble in that solvent. Twenty grams of the powdered TC stem sample was heated in 300 ml of methanol at 65°C for 8-10 cycles in 24 hours in a Soxhlet apparatus to get the methanolic extract. The extract was then filtered and concentrated by a rotary evaporator first and then by evaporating the solvent in desiccators.

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The active phytochemical principles of TC were analysed using the GC-MS system (QP-2010-Shimadzu, Japan, Rxi-5Sil MS column of 30m length, 0.25mm diameter and 0.25µm thickness) of the Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala. One µl of the filtered sample was analysed under chromatographic conditions. Helium at the flow rate of 1 mL/min was used as the carrier gas and the injector temperature was 260°C. The oven temperature was maintained at 80°C for 4 min, with an increase of 5°C/min to 280°C in 6 minutes under split-less injection mode. EI mass spectra were measured at 70 eV over a mass range of 50-500 amu and ion source temperature maintained at 200°C. The volatile components of TC were identified by comparing the retention times of chromatographic peaks using the database of the National Institute of Standards and Technology (NIST 11) and WILEY.[23]

Daltons lymphoma ascites (DLA) cell lines and the experimental animals

Daltons lymphoma ascites (DLA) cell line was procured from Amala Cancer Research Centre, Thrissur, Kerala. Ten adult Swiss albino mice, six to eight weeks old, weighing 25-30g each bought from the Small Animal Breeding Station (SABS) of the College of Veterinary and Animal sciences, Mannuthy (KVASU), Kerala, were used to maintain the DLA cells intra-peritoneally. The animals were housed in polypropylene cages under standard management, feeding and optimal environmental conditions of air and illumination and acclimatised for a period of one week before the start of the experiment. Routine clinical examinations of all the animals were performed throughout the period of the experiment. The DLA cells were maintained continuously as ascitic fluid in the Swiss albino mice (Figure 2) by serial transplantation through intra-peritoneal injections @ $5x10^5$ cells/mouse counted in a cell counter as 2.5 million cells/ mouse as per the protocol widely in use.[24] The DLA cells thus maintained *in vivo* in the mice were used for the subsequent *in vitro*

Figure 2
DLA cells maintained intraperitoneally in Swiss albino mice

studies. The animal experimentation procedures were approved by the Institutional Animal Ethics Committee of the College of Veterinary and Animal Sciences, Mannuthy, Kerala, as per proposal No. IAEC/22/01 dt. 06/04/2022.

Acridine orange / Ethidium bromide (AO/EB) staining

Acridine orange / Ethidium bromide (AO/EB) staining procedure[25] was followed to differentiate the live, apoptotic and necrotic cells after the treatments with test extract and cisplatin. The vital dye, acridine orange would stain both live and dead cells whereas ethidium bromide would stain only those cells that have lost their membrane integrity.[26]. The cells with green shades were identified to be in either in viable or early apoptotic stage. However, a uniform green colour indicated viable cells. Bright green dotted cells were identified as early apoptotic. Orange colour indicated late apoptosis whereas the red cells were necrotic. A concentration of 5 x 105 DLA cells was seeded into a six-well cell culture plate and treated with $IC_{50}$of test extract and reference drug for 24 hours. The IC_{50}concentrations of *T. cordifolia* stem extract and cisplatin in DLA cell lines identified as 72.05 µg/mLand 65.44 µg/mL respectively in another study by the same investigating team[27] were utilized for this procedure. Twenty-five microlitres of the cells were stained with five microlitres of acridine orange (10 µg/mL) and ethidium bromide (10 µg/mL) and analysed under trinocular research fluorescence microscope (DM 2000 LED, Leica) with blue excitation (488 nm) and emission (550 nm) filters at 40X magnification.[28]

Results and Discussion

Gas Chromatography-Mass Spectroscopy (GC-MS) analysis

The results of the GC-MS analysis of the methanolic extract of *T. cordifolia* revealed the presence of 18 compounds. Some of these compounds such as squalene, 10-Octadecenoic

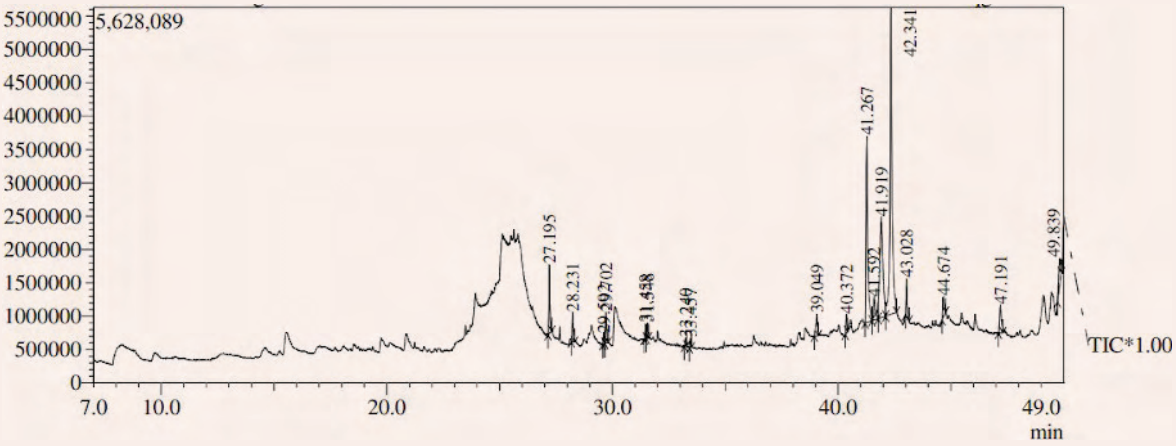
Acid Methyl Ester, Gamma-Gurjunenepoxide-(2) and vinyl deconateare reported to have antioxidant and anti-tumorproperties.[29] Hexadecanoic acid, Methyl ester, 2,6,8-Trimethyl bicyclo[4.2.0]Oct-2-Ene-1,8-Diol and columbinalso are believed to have anti-inflammatory properties.The GC-MS chromatogram of the methanolic extract of *T. cordifolia* are given in Table 1 and Figure 3. The GC -MS chromatogram of the methanolic extract of *Tinospora cordifolia* and the peak report of *T. cordifolia* stem extract depicting the retention time, area and per cent area, height and percent height, the compound names and their m/z values as revealed in GC-MS analysis are depicted in Table 1 and Figure 3.

The phytochemical screening of the aqueous extract of the TC stem in the present study revealed the presence of various bioactive components such as protein, carbohydrate, phenols, alkaloids, flavonoids, saponins, glycosides, quinones and anthraquinones in agreement with the earlier reports.[30,31,32] Terpenoids, coumarin and steroids were present in the methanolic extract only in agreement with earlier workers.[33] Many earlier reports have identified similar polysaccharides with immunomodulatory activity

Table1
GC-MS Chromatogram of the methanolic extract of *Tinospora cordifolia* stems

Peak	R.Time	Area	Area%	Height	Height%	Tentative identification	Base m/z
01.	27.195	3651068	4.46	1038498	7.21	1,2-benzenedicarboxylicacid, bis(2-methylpropyl) ester	149.00
02.	28.231	1582522	1.93	451553	3.14	Hexadecanoic acid, methyl ester	74.05
03.	29.592	465659	0.57	153079	1.06	Octadecane	57.05
04.	29.702	1982478	2.42	467382	3.25	Longipinane, (E)-	109.05
05.	31.458	573102	0.70	229500	1.59	2-methyltetracosane	57.10
06.	31.548	631619	0.77	237244	1.65	10-Octadecenoic acid, methyl ester	55.05
07.	33.240	308932	0.38	126401	0.88	Octadecane	57.10
08.	33.457	413148	0.50	145546	1.01	Heptadecyl acetate	57.05
09.	39.049	1569094	1.91	330690	2.30	1,2-benzenedicarboxylic acid	148.95
10.	40.372	979111	1.19	284649	1.98	Columbin	94.05
11.	41.267	14728035	17.97	2798395	19.44	3-isopropyl-7a-methyl-1,4,5,6,7, 7a-hexahydro-2h-inden-2-one	192.00
12.	41.592	2630918	3.21	342895	2.38	Germacrene a	68.05
13.	41.919	14961227	18.26	1513681	10.51	Vinyl decanoate	71.05
14.	42.341	29016374	35.41	4598604	31.94	2,6,8-Trimethylbicyclo[4.2.0] oct-2-ene-1, 8-diol	124.10
15.	43.028	1910600	2.33	571915	3.97	Squalene	69.05
16.	44.674	1438613	1.76	303924	2.11	1,2-Benzenediol,4-(2-aminopropyl)-	137.00
17.	47.191	2360145	2.88	421477	2.93	Gamma-Gurjunenepoxide-(2)	121.10
18.	49.839	2745278	3.35	381741	2.65	Dodecanoic acid, 1,2,3-propanetriyl ester	71.05
		81947923	100.0	14397174	100.00		

Figure 3
GC-MS peak report of *Tinospora cordifolia* stem extract



in *T. cordifolia* extracts.[34,35] Alkaloids, phytosterols and triterpenoids of phytochemical origin obtained in the study are also reported to exhibit potent cytotoxicity towards MCF-7 cells and proved to prevent cell proliferation by inducing apoptosis.[36,37]

Microscopic examination under Acridine orange /Ethidium bromide (AO/EB) staining

In order to evaluate the cause of growth inhibition of DLA cells by aqueous TC stem extract, the morphological changes in the cell and their characteristic features of apoptosis were studied under a fluorescent microscope. After treating the DLA cells with IC_{50} concentrations of aqueous TC stem extract and cisplatin, Acridine orange / Ethidium bromide staining was performed to detect live, early apoptotic, late apoptotic and necrotic cells. The microscopic pictures of cells under different treatments are presented in Figure 4 (A to C).

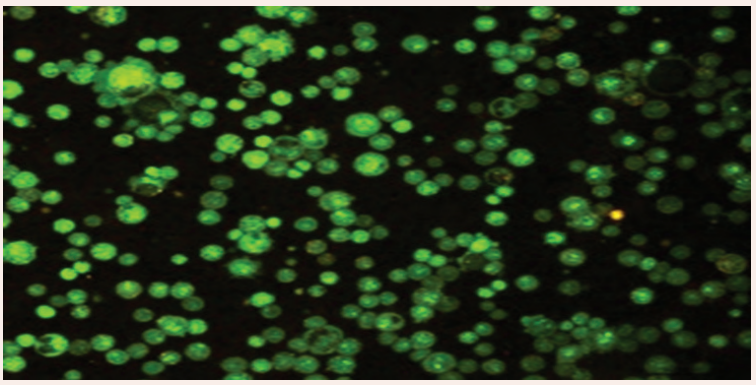
All the treated DLA cells, within a given span of time, showed prominent morphological changes like cell shrinking, rounding of cells, formation of membrane blebs and the appearance of apoptotic bodies which were characteristic features of cytotoxicity and apoptosis in agreement with the earlier reports.[28] The untreated cells of the control group (A) were live and showed green fluorescence with a circular nucleus.

The DLA cells with cisplatin (B) showed apoptotic changes of early stages. Majority of the cells were found to be in early apoptotic stage and they were found in green shades. As a result of chromatin condensation and nuclear fragmentation, the early apoptotic cells stained green and had bright green dots in their nuclei.

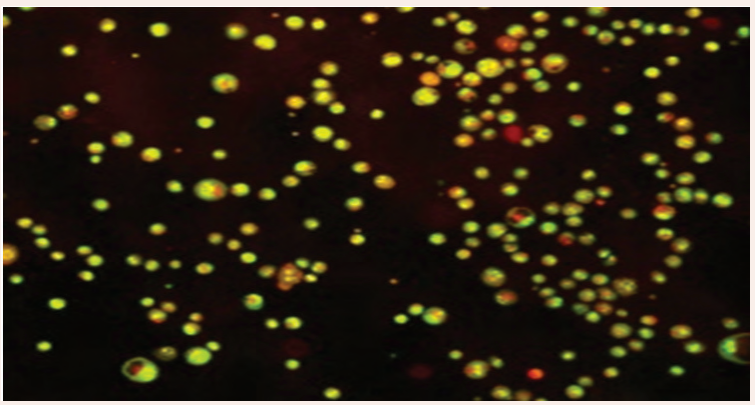
The cells subjected to TC stem extract (C) showed apoptotic changes of late stages. Majority of the cells were found to be in late apoptosis indicated by their orange and red shades. The cells in late apoptotic phase were stained orange. They also had orange-stained condensed, asymmetrical fragmented nuclei characteristic of late apoptosis. The necrotic cells stained red with a nuclear morphology of viable cells, but without a condensed chromatin. The findings of the study

Figure 4
Staining of DLA cells with AO/EB under different treatments (40X magnification)

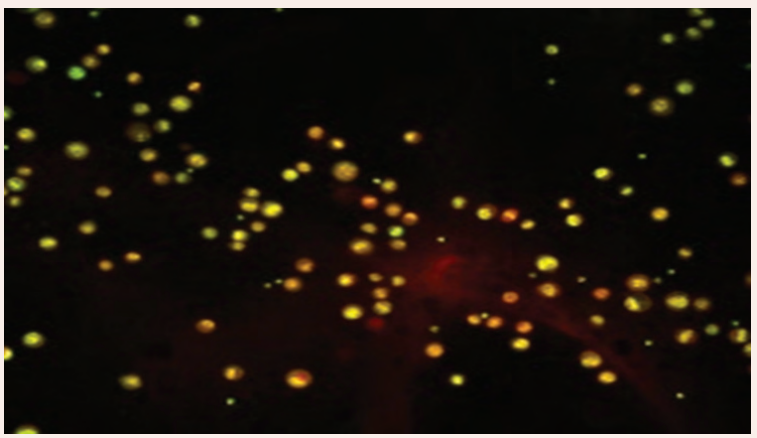
A: Live cells without treatment (control)



B: Cisplatin-treated cells with early apoptotic changes - bright green dots indicating early apoptotic cells



C: TC-treated cells with late apoptotic changes (orange and red indicating late apoptotic cells)



were similar to the earlier reports[38,39] wherein DLA cells treated with *Anthocephalus cadamba* extract signified the presence of green nuclei with uniform chromatin for live cells, orange nuclei and a condensed chromatin for apoptotic cells and red nuclei for necrotised cells.

The results of the staining procedures indicated that the TC treated cells moved faster towards

apoptosis than cisplatin-treated cells in a given time period pointing out the possibility of IC_{50} concentration of TC to exert a faster apoptosis in cells when compared to cisplatin. The study indicated that *Tinospora cordifolia* stem extract induced considerable degree of apoptosis in lymphoma cells and its cytotoxic and cell inhibition activity were indicative of the anti-neoplastic action. The findings suggest that *T. cordifolia* stem extract has the potential to emerge as a promising therapeutic agent for the treatment of various neoplasms like lymphomas.

Conclusion

The study confirms that *Tinospora cordifolia* stem extract prevented cell proliferation by inducing apoptosis in Dalton's lymphoma ascites cells, making it a potential candidate for anti-neoplastic action against lymphomas. The apoptotic potential of the herb may be attributed to the different phytochemical compounds identified in the study with known anti-inflammatory, antioxidant and anti-tumor properties. The findings could also be used to leverage the therapeutic properties of the plant to provide an effective means for the prevention and cure of various neoplastic conditions. Further research is needed to identify the active anticancer components of *Tinospora cordifolia* and understand the relevant molecular mechanisms underlying it's anti-neoplastic action in order to develop effective natural therapeutic variants of the herb.

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CASE STUDY

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Clinical approach in the management of Hirschsprung disease based on *aayurvedic* principles - a case study

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ABSTRACT: Hirschsprung disease (HSCR) or congenital intestinal aganglionosis, is a birth defect characterised by complete absence of neuronal ganglion cells from a portion of the intestinal tract most commonly the large intestine. This can extend proximally to variable distance according to the failure of descent of the neuroblasts. The aganglionic segment does not relax and causes functional obstruction, the normal proximal bowel hypertrophies and eventually dilates. Overall incidence of HSCR is 1 in 5000 live births. The most common presenting symptoms are chronic constipation, abdominal distension, and failure to pass meconium in the first 24 hours of life. Here is a case of Hirschsprung disease presented with the major complaint of chronic constipation which is well managed through *aayurvedic* treatment. In *aayurveda* this may be correlated to *vaatika gulma*. The therapeutic interventions were done based on the general *cikitsaasootra* of *vaatika gulma.* Medicated *sneha* in the form of *paana* (intake), *anna* (mixed with food), *abhyangga* (oil massage) and *vasti* (medicated enema) were used. Among which *vasti*was taken as the major *pancakarma* procedure. Assessment was done by before after X-ray barium meal study, Wexner constipation score and subjective analysis; final results showed no evidence of HSCR in the X-ray barium meal study and out of 8 parameters of constipation scoring scale, 7 showed remarkable change with a satisfactory improvement in subjective analysis. Experience of this case with pure classical *aayurvedic* protocol has shown a positive result in the management of HSCR, further trials of the same on the basis of case series may generate a satisfactory management protocol of HSCR in the field of *aayurveda*.

Key words: Hirschsprung disease, *Vaatika gulma*, *Aayurvedic* management, *Vasti*, *Sahaja pakvaasayagata vyaadhi*

Introduction

Hirschsprung disease, or congenital aganglionic megacolon, is a developmental disorder of the enteric nervous system, characterised by the absence of ganglion cells in the submucosal and myenteric plexus, extending proximally for a variable distance.[1] This causes inadequate relaxation of the bowel wall and bowel wall hypertrophy, which can lead to intestinal obstruction. In the majority of cases (80%), the aganglionic tract involves the rectum and the sigmoid colon only (short segment HSCR), while in 20% of cases it extends toward the proximal end of the colon (long segment HSCR).[2] Overall incidence of HSCR is 1 in 5000 live births with a female to male ratio of 1:4.[3,4] There is an increased familial incidence in long segment disease, HSCR may also be associated with other

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congenital abnormalities like trisomy 21, urogenital or cardiovascular abnormalities etc.[1]

The symptoms of HSCR varies widely in severity, but almost always appears shortly after birth. The most common presenting symptoms are constipation, abdominal distension, and failure to pass meconium in the first 24 hours of life.[5] Failure to thrive with weight less than fifth percentile is evident in infants who are diagnosed after they reach two months of age.[5] Enterocolitis is the most dangerous complication of HSCR and which has high mortality rate.[5]

In *aayurveda*, this disease may be correlated to *vaatika gulma.* Major symptoms of *vaatika gulma* such as *vit̲sanga*[6] (constipation), *vyadhasoocivat*[6](abdominal pain), *kaars̲ya*[6] (emaciation), *vishamavahnita*[6] (irregular appetite), *aantrakoojana*[6] (abdominal sounds) are similar to that seen in HSCR and when complicated with infections the symptoms of *jvara*, *kr̲cchrocchvaasa* (breathing difficulty) may be seen.

Surgery to bring the ganglionic bowel down to the anus is the only treatment that could be done in modern science. Here is a case report of a child diagnosed with HSCR, well responded with *aayurvedic* treatment protocol, mentioned in the classics under the management of *gulma*- including both *aayurvedic* internal medications and *pancakarma* procedures.

Patient information

A 7-year-old male child, born as the second child of non-consanguineous parents, with a birth weight of 2.75Kg, had a history of delayed passage of meconium during the neonatal period, all other developmental milestones were attained normally with timely attained bladder habits; presented with the complaints of severe constipation since birth with irregular bowel habits of once or twice in a week. The stool resembled pellet-like with a profuse foul smell, often seen as traces of liquid or pasty stool in child's underwear. Parents ignored this until the child was 3 years old, and gradually they noticed intense fear in the child towards toileting activities. Different home remedies were used by the parents to treat constipation but none of them were satisfactory. Parents noticed exacerbation of the condition on intake of food like meat, chappati and rice without vegetables, and mild relief was noted on intake of fruits like oranges, watermelon, etc. As the condition got severe with further increase in constipation (marked by bowel evacuation only on intake of food articles having laxative properties) associated with irregular appetite, occasional painful abdomen, and painful stool evacuation, modern consultation was taken and was advised with laxatives and anal suppositories to treat the same. As there was no remarkable relief and due to lack of curability by these methods, the patient was referred to higher centre for further investigations, where he was diagnosed with short-segment Hirschsprung disease and was advised for surgical correction. As the parents were reluctant for surgical management they approached Govt. Ayurveda College, Thiruvananthapuram, Kerala, to know the possibility of *aayurvedic* management in this disease and got admitted in the IPD. Before admission, scope and limitations regarding the disease and management were well communicated with the parents.

Clinical findings

The boy seemed to be anxious, but well cooperative, alert and oriented. Gait was normal

with all vital signs and anthropometric measures within normal range. On examination of oral cavity tongue was non coated, dentition normal with dental caries. Abdominal examination revealed mildly distended abdomen with no visible mass on inspection, tenderness over left iliac fossa with loaded abdomen on palpation, dull notes on left hypochondriac region and tympanic notes over upper abdomen on percussion and rectal examination revealed an empty rectum. Haematological values including CBC and thyroid profile were within normal limits. No relevant findings were observed on stool examination. Clinical examination in *aayurveda* is tabulated under *dasavidhapareeksha* in Table 1.

Case timeline

Timeline of the case is given in Table 2.

Table 1
Dasavidhapareeksha

Dooshya *Dosha* : *Vaata pradhaana tridosha* (Involvement of *apaana vaata, samaana vaata, paacaka pitta* and *kledaka kapha*; *lakshana's* include *malasangga, aadhmaana, aatopa, bhaya, agnimaandya*) *Dhaatu* : *Rasa, rakta, maamsa, medha* (*Baalya* is the *dhaatu poshana kaala*, diseases chronically affecting *annavahasrotas* and *pureeshavahasrotas* can hamper *dhaatu poshana* and affect *bala* even if the *lakshanas* are not seen in the early stages) *Mala* : *Pureesha*
Desa : *Bhoomi desa - Aanoopa saadhaarana* *Deha desa* : *Pakvaasaya*
Bala : *Rogeebala - Madhyama* *Rogabala - Madhyama* *Kaala* : *Kshanaadi - Greeshma* *Vyaadhyavastha* : *Puraana* *Anala* : *Vishama*
Prakrti : *Vaata kapha* *Vaya* : *Baala*
Satva : *Madhyama* *Saatmya* : *Madhyama*
Aahaara : *Abhyavaharanasakti - Avara* *Jaranasakti - Avara* *Vyaayaamasakti - Madhyama*

Table 2
Case timeline

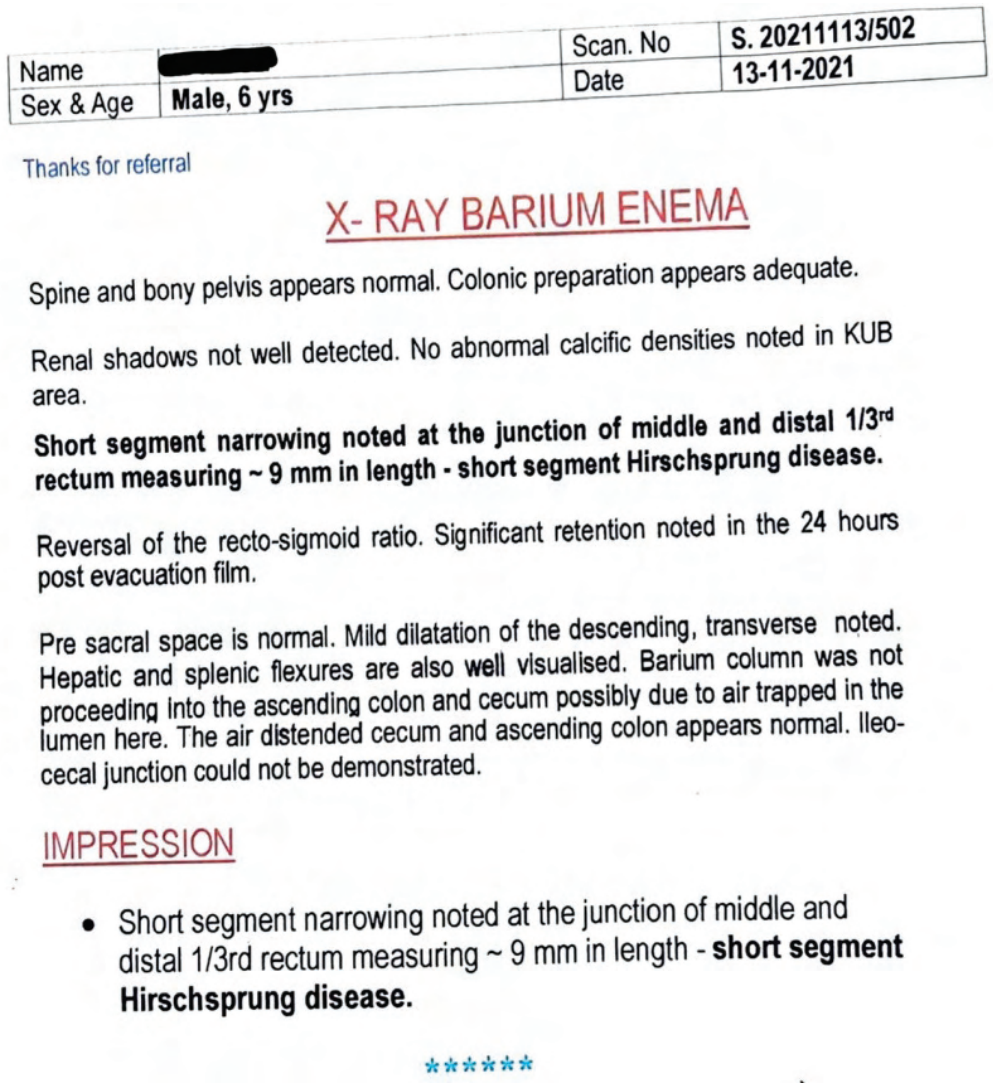
Year	Clinical events
2015	Patient had delayed meconium evacuation during birth and chronic difficulty in passing stool since then.
2018 - 2021	Took modern consultation from nearby hospital, advised with diet modifications and laxatives.
2021	Consulted with paediatric gastroenterology in SAT Hospital, Thiruvananthapuram, Kerala. Clinical workouts, investigations and X-ray barium enema were done, which suggested short segment Hirschsprung disease.
2022	Anal manometry was done from Amrita institute of medical science, which showed that Recto anal inhibitory reflex could not be elicited consistently and was suggested for biopsy confirmation of Hirschsprung disease.
2022	Consulted Govt. Ayurveda medical College, Thiruvananthapuram, Kerala, took 45 days IP management and discharged with clinically significant improvement of unaided formed stool evacuation in every 1 or 2 days.
2022	Visited OPD after one month of IP management, patient was able to pass normal volume of stool in every 1 to 3 days without any assistance.

Diagnostic testing and assessment

The history of delayed passage of meconium with chronic constipation is suggestive of HSCR,[7] which was later confirmed by Anal manometry and X-RAY barium enema, which revealed mild dilation of the descending and transverse colon with short segment narrowing noted at the junction of middle and distal 1/3rd rectum measuring ~ 9mm in length. Figure 1.

Haematological evaluations were done to exclude the other differential diagnosis including celiac disease, intestinal motility disorders, acquired mega colon, etc. In this particular case, the probable diagnosis can be *vaatika gulma* considering *grahani*, *pakvaasayagatavaata*, *udaavarta,* and *arsas* as the other differential diagnosis. In *grahaneeroga*, the *agnyadhishthaana* (*pakvaamaasaya madhyastha*)[8] and

Figure 1
X-ray Barium enema taken before treatment



Name	[redacted]	Scan. No	S. 20211113/502
Sex & Age	Male, 6 yrs	Date	13-11-2021

Thanks for referral

X- RAY BARIUM ENEMA

Spine and bony pelvis appears normal. Colonic preparation appears adequate.

Renal shadows not well detected. No abnormal calcific densities noted in KUB area.

Short segment narrowing noted at the junction of middle and distal 1/3rd rectum measuring ~ 9 mm in length - short segment Hirschsprung disease.

Reversal of the recto-sigmoid ratio. Significant retention noted in the 24 hours post evacuation film.

Pre sacral space is normal. Mild dilatation of the descending, transverse noted. Hepatic and splenic flexures are also well visualised. Barium column was not proceeding into the ascending colon and cecum possibly due to air trapped in the lumen here. The air distended cecum and ascending colon appears normal. Ileo-cecal junction could not be demonstrated.

IMPRESSION

- Short segment narrowing noted at the junction of middle and distal 1/3rd rectum measuring ~ 9 mm in length - **short segment Hirschsprung disease.**

******

annagrahana is affected; whereas in this case, the affected location is *pakvaasaya* which is not the *agnyadhishthaana* and *annagrahana* is not affected here. As a visible *maamsakeela* is absent in this particular case, *arsas* can be ruled out. Symptoms of *pakvaasayagata vaata* shows more of a syndromic approach and *udaavarta* may be considered as the later stage symptoms of this disease. *Vitsangga* (constipation), *vishamavahnita* (irregular appetite), *vyadha-soocivat* (abdominal pain), *aantrakoojana* (abdominal sounds) were the symptoms of *vaatika gulma* presented in this patient.

Therapeutic intervention

The therapeutic interventions were done based on the general *cikitsaasootra* of *vaatika gulma.* Use of medicated *sneha* for *paana* (intake), *anna* (mixed with food), *abhyangga* (oil massage) and *vasti* (medicated enema) were done as per *cikitsaasootra* along with *avagaahasveda.*[6a] Among which *vasti* was taken as the major *pancakarma* procedure, as it is predominantly indicated in *pakvaasayagata vaata gulma.*[6b]

Considering the age of child, started *maatrvasti* with a lower dose of 50ml for the initial two days, *ksheera vasti* for the next 15 days - dose of *vasti* increased to 300ml successively (calculated as maximum dose for 7 year old child).[9] On reaching the dose of 300ml, started *yogavasti* for the next 8 days, with alternate *pippalyaadi anuvaasana taila*[6c] *vasti* and *erandamoola- nirooha vasti*[6d]. After *yogavasti, Mustaadi-raajayaapana vasti*[9a] - 3 in number for regular 3 days, next 3 in alternate days and last 3 in a gap of 2 days were administered. Table 3.

Externally, *abhyangga* with *Laakshaadi taila*[6e], *naabheepicu* with *Pippalyaadi anuvaasana taila*[6c] and *avagaaha* in water boiled with

Table 3
Internal medications used

Internal medication	Mode of administration	Rationale
Ashtacoorna[6f]	3gm powder with 3g *Daadimaadi ghrta* with the initial bolus of food thrice daily	Indicated in *vaatika gulma agnideepana, vaataanulomana*
Daadimaadi ghrta[6g]	3gm powder with 3g *Daadimaadi ghrta* with the initial bolus of food thrice daily	*Moodhavaataanulomana, deepana, gulmahara*
Pooteekaasava[6h]	5ml thrice daily after food	*Agnideepana, paacana, anulomana, gulmahara*
Gandharvahastaadi ksheera kashaaya[10]	25ml twice daily half hour before food Preparation - *kashaaya sookshma coorna* 5g + 25ml milk + 200ml milk reduced to 25ml	*Agnideepana, vaatahara,* helps in *malasodhana*. Given as *ksheera kashaaya* as it act as *snigdha* to *koshtha*
Sukumaara ksheera kashaaya[6k]	25ml twice daily half hour before food Preparation - *kashaaya coorna* 5g + 25ml milk + 200ml milk. Reduced to 25ml	*Vitvibandhahara, gulmahara*. Used for *anulomana* in *sukumaaras* for prolonged use. Site of action is *pakvaasaya*.
Sukumaara ghrta[6k]	3g before *peya* in the evening	*Vitvibandhahara*. *Ghrta* preparation helps to improve *agni* and provide *brmhana* effect.

vaatahara patras were done for those 45 days. Table 4.

Along with the external procedures and *vasti*, oral medications used were - *Ashtacoorna*[6f] 3g with 3g *Daadimaadi ghrta*[6g]thrice daily with initial bolus of food, *Pooteekaasava*[6h] 5ml thrice daily after food- for the initial 1 week, after which continued *Daadimaadighrta* 3g at 6pm and *Gandharvahastaadi ksheera kashaaya*[10] 25ml twice daily half hour before food. Table 5.

Table 4
External therapy done

External treatment	Medicine used	Rationale
Abhyangga	*Laakshaadi taila*[6e]	*Balya, brmhana, vaatahara*
Naabheepicu	*Pippalyaadi anuvaasana taila*[6c]	*Naabhi* is the *moolasthaana* of *dhamani* which is helping in proper functioning of *pakvaasaya*. It is also one of the practise in traditional *keraleeya aayurveda*.
Avagaaha[6a]	Water boiled with *vaataharapatra*	Correction of *Apaanavaayu*

Table 5
Vasti's administered

Vasti	Mode of preparation	Rationale
Maatraa-vasti	*Pippalyaadi anuvaasana taila*[6c] -50ml and *saindhava* - 3g	To bring *vaataanulomana* *Pippalyaadi anuvaasana taila- vaatavarca-vinigraha, moodhavaataanulomana*
Ksheera-vasti	*Erandamoola ksheera kashaaya* - 75ml *Pippalyaadi anuvaasana taila* - 25ml *Sukumaara ghrta* - 25ml, *madhu* - 25ml, *saindhava* - 3g Initial starting dose - 150ml. Dose increased to reach a dose of 300ml within 15days	*Mrdu sodhana vasti*, used in *sukumaara's* for *sodhana* and *snehana* in *pakvaasaya*
Yoga-vasti	Alternative days (total 8 days) *Snehavasti*: *Pippalyaadi anuvaasana taila* - 50ml + *saindhava* - 3g *Kashaaya vasti*: *Erandamoola nirooha vasti*[6d] *Erandamoola kashaaya* - 160ml, *Pippalyaadi-anuvaasana taila* - 40ml, *Satapushpa kalka* - 10gm honey - 40ml, *dhaanyaamla* - 50ml, *saindhava* - 5gm *madanaphala* - 2 in no.	*Erandamoola kashaaya vasti* given in *yogavasti* pattern prevent *vaata* vitiation due to *rookshana* of *kashaaya vasti* alone *Erandamoolaadi vasti* - special indication in *koshtha* and *guhya soola, gurutaam vibandha*, *gulma, grahani* and *gudottha roga*
Mustaadi-raajayaapana vasti[9a]	*Ksheera kashaaya* - 160ml, *kalka* - 10ml *Sukumaara ghrta* - 80g, honey - 40ml *saindhava* - 5g, *aja aantra siddha maamsarasa* - 50ml used	*Sadyobalajanana*, *rasaayana*. Indicated in *gulma, udaavarta, kukshi soola,* etc. *Maamsarasa* prepared out of *aja aantra* is considering *saamaanya visesha cikitsaatatva*.

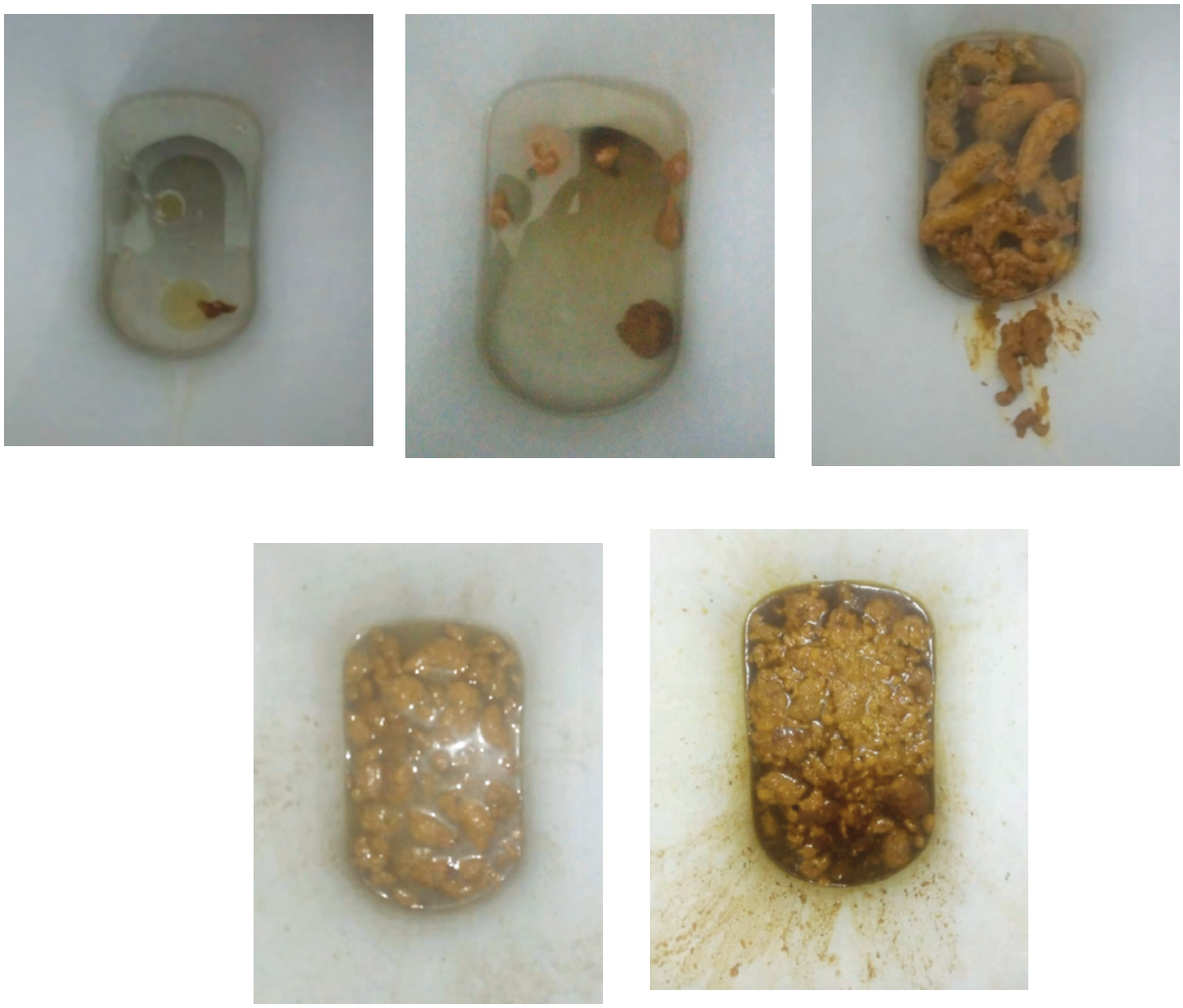
Peya[6i]prepared with *yava*, *aamalaka*, and *pippali* was used in the evening as a dietetic preparation for those 45 days.

Patient was discharged on 45th day with the advice of a dietetic preparation prepared with *aja aantra siddha maamsarasa, daadimarasa, dhaanyaka, naagara* and *ghrta*[6j] for the first 7days during evening. And there after to continue the use of *ksheera kashaaya* prepared with *Sukumaara kashaaya yoga*[6j] - 25ml twice daily before food, *sukumaara ghrta*[6k]- 3g before *peya* in evening. Two preparations of *peya* were advised to use daily in the evening 1) with *yava* and *ksheera*[6l], 2) with *ajamaamsa*, *tila*, *maasha* and vegetables added with rice[9b].

Follow-up and outcomes

Patient's condition before and after the study were assessed by using X-ray barium enema, Wexner constipation score[11] and subjective analysis. Bowel passed each day was assessed by capturing photographs in order to ascertain the consistency and the amount evacuated per day. Figure 2.

Figure 2
Bowel evacuated during different stages of treatment



Patient passed good volume of stool with formed consistency without any kind of assistance or pain for the first time on 7th day of treatment, which was then continued for the next days with sufficient volume of bowel evacuation everyday either along with the evacuation of *vastidravya* or before *vasti* in the morning hours.

Wexner constipation score - 19/30 (before treatment), 4/30 (after treatment).

Other subjective improvements observed were - child became more active, playful and socially interactive, no more fear for toileting activities, improvement in appetite with timely food intake, no episodes of foul smelling stool / flatulence, no episodes of soiling undergarments, improvement in body weight of about 1.5kg within 45 days of IP treatment.

Patient was on follow up for the next month by continuing all internal medications and diet regulations. During the period, patient was able to pass good volume of stool in every one to three days without any assistance or pain, no episodes of abdominal pain or soiling undergarments. No concurrent modern medications were used during that period of time.

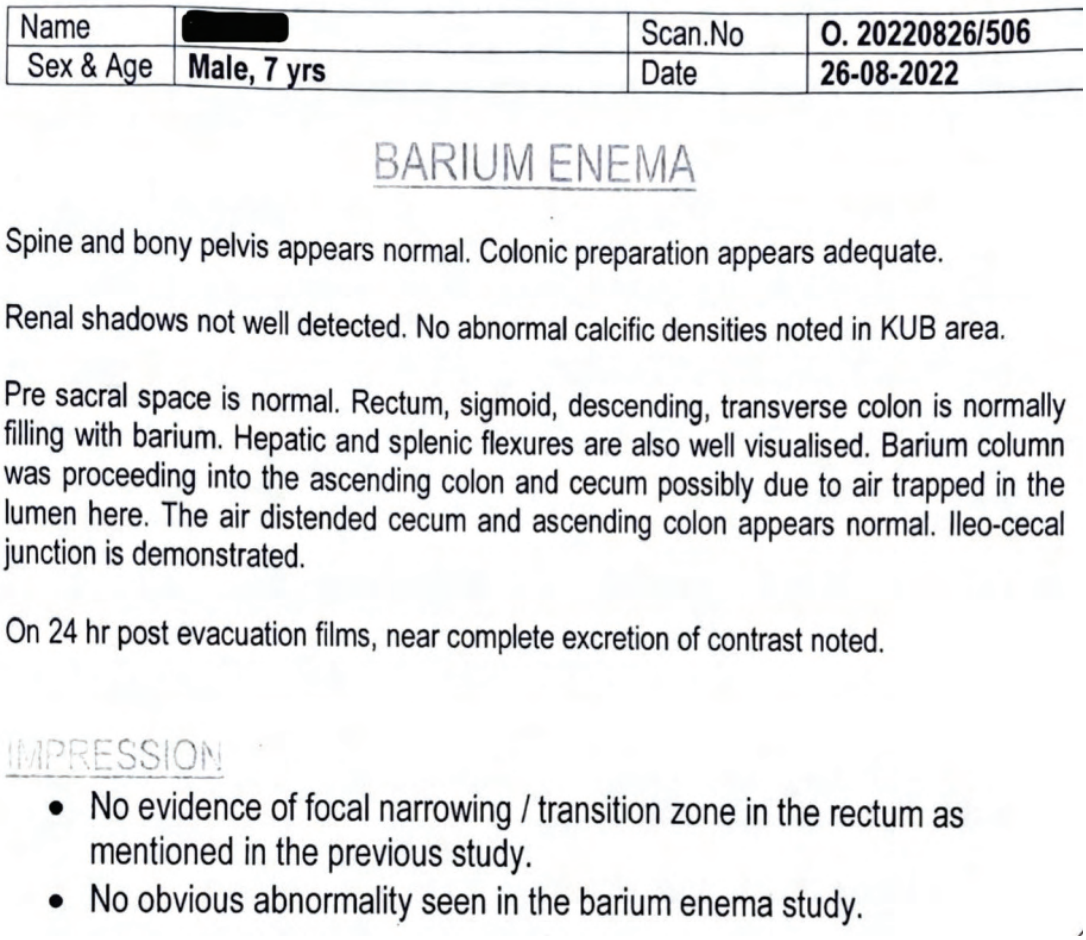
X-ray barium enema after one month of discharge showed no evidence of focal narrowing/transition zone as mentioned in previous study. Figure 3.

Discussion

Hirschsprung disease is a congenital disorder characterised by the absence of ganglionic cells in the terminal rectum that extends in a variable distance proximally. Lack of peristalsis in the aganglionic segment and failure of the internal anal sphincter produce varying degrees of intestinal obstruction, and the bowel proximal to the aganglionic segment become gradually dilated and hypertrophied for a variable length, as the peristaltic waves try to propel stool through the obstructing aganglionic segment. Oral medication in HSCR does not work in most of the cases. Other temporary management options include use of suppositories and enemas. Currently, the only treatment for HSCR is surgery. Failure to surgically treat HSCR can be fatal due to malnutrition or sepsis following bowel perforation. Although surgery is the routine therapy for HSCR patients, surgical outcomes can vary widely, with a range of long term consequences, such as constipation, faecal incontinence and enterocolitis.[12]

In *aayurveda* - diseases such as *vaatika gulma, udaavarta and pakvaa<u>s</u>ayagata vaata* show similar kind of presentation due to vitiation of *vaata* in *pakvaa<u>s</u>aya.* As HSCR is congenital in origin, it can be understood as a *sahaja vyaadhi* affecting *pakvaa<u>s</u>aya;* which might have arised due to *maatura aahaara-vihaara-apacaara* during *garbhaavastha*. *Sahaja vaata prakopa* during *garbhaavastha* in *<u>si</u>s<u>u</u>* may affect the main site of *vaata* i.e *pakvaa<u>s</u>aya*, causing a decrease in normal *snigdha, ush<u>n</u>a, cala gu<u>n</u>a* of *pakvaa<u>s</u>aya* and increases the *rooksha*, *<u>s</u>ushka*,

Figure 3
X-ray Barium Enema taken after one month of discharge



Name	[redacted]	Scan.No	O. 20220826/506
Sex & Age	Male, 7 yrs	Date	26-08-2022

BARIUM ENEMA

Spine and bony pelvis appears normal. Colonic preparation appears adequate.

Renal shadows not well detected. No abnormal calcific densities noted in KUB area.

Pre sacral space is normal. Rectum, sigmoid, descending, transverse colon is normally filling with barium. Hepatic and splenic flexures are also well visualised. Barium column was proceeding into the ascending colon and cecum possibly due to air trapped in the lumen here. The air distended cecum and ascending colon appears normal. Ileo-cecal junction is demonstrated.

On 24 hr post evacuation films, near complete excretion of contrast noted.

IMPRESSION

- No evidence of focal narrowing / transition zone in the rectum as mentioned in the previous study.
- No obvious abnormality seen in the barium enema study.

kharaguṇa of *vaata* in *pakvaaśaya;* thereby obstructing the normal *paaka* and *gati* of *anna* through the *pakvaaśaya,* causing obstruction and accumulation of *mala* and further vitiation of *vaata* in *pakvaaśaya*. Eventually, this accumulation can increase the size of *pakvaaśaya* proximal to the site of obstruction and may be felt on palpation as dilated loops of colon, which may be correlated with *granthi-roopita sparśopalabhya gulma.*

Treatment was aimed at pacifying the vitiated *vaata* and to increase the *snigdha ushṇa cala guṇa* in *pakvaaśaya*. Every treatment principle that pacify *vaata* can be adopted, *vasti* has been given the major importance, as it has the highest potency to pacify vitiated *vaata* in *pakvaaśaya* i.e *"pakvaaśaya gate vasti";*[6b] *"vastikarma param vidhyaat gulmaghnam"*[6m]. *Vasti* can act in *pakvaaśaya* through different ways- by administering bulk amount of medicine through

anal route, it provides dilatory mechanism to the colon and by the property of medicinal drugs used in *vasti*, it pacifies *vaata* from its root. Altogether, *vasti* sensitises the chemo and stretch receptor nerve endings in the colon near to the aganglionic segment and has the ability to cleanse the entire *mahaasrotas*, which might have helped in bringing the outcome.

Initial *maatraavasti* helped in softening the hard impacted stool; *ksheera vasti* provided *mrdu<u>s</u>odhana*; *yogavasti* helped in further *<u>s</u>odhana* of *pakvaa<u>s</u>aya* without vitiating *vaata.* Finally *Mustaadi raajayaapana vasti* provided *rasaayana* and *balya* effect to the *pakvaa<u>s</u>aya*. All the internal medications were aimed at improving *agnideepana* and providing proper *vaata* and *mala anulomana*. Externally *abhyangga*, *avagaaha* and *naabheepicu* were done as a general measure to pacify vitiated *vaata* in the whole body.

With the completion of 45 days of IP management, the case had shown the effectiveness of classical treatment protocol in HSCR. There was good improvement in overall well being of the child which was evaluated by increased body weight, loss of fear to toileting activities and active involvement of child in every activities like running, playing, etc. There was no adverse effect during any stage of treatment and the need for using modern medication in between the management. For a complete cure, further management might be needed in following years up-to an age of adulthood. Also *patya aahaaraas* having *anulomana* property can be incorporated in the regular diet in order to avoid constipation. Episodes of chronic constipation can be avoided by the use of mild medicaments having laxative property whenever in need, if bowel does not evacuate in more than three days. With the advancement of age of the child there may occur new neuronal development over the aganglionic region. Result in this case has widened the area for future trials that could be initiated in a more early period of age. A collaborative research in this area by adhering with all the research guidelines may provide an effective management protocol for the management of HSCR from the field of *aayurveda*.

Conclusion

Hirschsprung disease or congenital intestinal aganglionosis is an uncommon condition presented with chronic constipation and much more complications, which can be considered as a *sahaja pakvaa<u>s</u>ayagata vyaadhi* and may be correlated with *vaatika gulma*. Treatment mainly focused on correction of *vaata* in *pakvaa<u>s</u>aya*; the management which goes in hand with treatment principles of *vaatika gulma, udaavarta and pakvaa<u>s</u>ayagata vaata* had shown imminent results in the present case and can be adopted in further cases with similar presentation.

Patient perspective

Parents of the patient and patient himself was

happy and satisfied with the treatment provided.

Informed consents

Written permission for publication of this case study was obtained from the parents of the patient.

Source of funding

None

Conflict of interest

None

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6h. Ibidem., Cikitsasthana 8/144

6i. Ibidem., Cikitsasthana 1/30-31

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CLINICAL RESEARCH
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Exploration of the relationship between metabolic markers and the functional aspects of *pitta* and *rakta* through the assessment of treatment response in Psoriasis

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ABSTRACT: In the present study, the relationship between metabolic markers and the functional aspects of *pitta and rakta* was being investigated through the assessment of treatment response to *pitta samana-rakta suddhikara dravya* in psoriasis. Therefore, 15 psoriatic patients between the age group of 20-50 years were selected as per the inclusion and exclusion criteria. Data including basic details, *dasavidhapareeksha* along with a detailed case history were collected using the proforma. Diagnosis was confirmed using internationally accepted diagnostic criteria for psoriasis. Their Serum LDH and CRP were analysed and subjects with either elevated LDH or elevated CRP were selected for the study. They were instructed to consume *Saaribaadi gana kvaatha* for a period of 30 days and *sramsana* with *Avipatti coorna* was done a day before and after the medication. Re-evaluation of *pitta-rakta dushti* and Serum LDH and CRP tests were done immediately at the end of the intervention and whatever results obtained were analyzed statistically. The analysis showed a significant regulatory effect of intervention on *pitta-rakta dushti*, elevated levels of serum LDH, CRP and ESR. It was also observed that there was a significant relationship between elevated LDH and *pitta vrddhi*. Hence, it has been proven that there exists a relationship between such metabolic markers and the functional aspects of *pitta and rakta*.

Key words: Pitta, Rakta, Metabolic markers, LDH, CRP, Psoriasis

Introduction

Aayurveda has evolved its own biochemistry, where the theories like *panjcamahaabhoota, shad- padaartha and tridosha* are applied brilliantly. Further fundamental and unique perspectives in *aayurveda* like *agni, ojas, manas, bala, rogamaarga* can be helpful in understanding and treating the fatal maladies of modern era, such as metabolic disorders, autoimmune disorders, cancer, etc. The objective of this study was to explore the association between metabolic markers and the functional aspects of *pitta and rakta* in autoimmune diseases w.s.r. to psoriasis.

The etiology and patho-physiology of autoimmune diseases[1] is not completely known till the day,

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whereas incorporating *aayurvedic* concepts on autoimmune diseases can postulate better patho-physiology as well as line of treatment for it. Psoriasis[2] is a major autoimmune disease, where certain metabolic markers[3] were seen to be elevated. Currently, intensive works are taking place to develop more effective metabolic markers, which are also seen as the key to personalized medicine.

Whereas the notion of auto-immunity according to *aayurveda* revolves around four factors:

Agni vaishamya: One among the normal functions of *agni* is to opsonize exogenous substances. *Agni* or its components carries out the whole metabolic process in human body. *Mandaagni* is the cause for all diseases. It will later leads to *agnivaishamya* in *dhaatu* level and manifests *dhaatuvaishamya* and *dhaatupaaka*. In other words, *agnivaishamya* in *dhaatu* level causes metabolic errors which will bring about further morbidity.

Aama: living body is a platform for complex chain of transformations, either anabolic or catabolic, which are ultimately sustaining the life ahead. If these transformations are getting hindered due to any of the *trividhahetu*[4], then that state is called *aaama*. Any substance that has not attained finality in the metabolism can be considered as *aama*. When the *parin̲aaama* is not completed, the ultimate function of that *srotas* or tissue gets impaired resulting in a disease.

Doshavaishamya: The involvement of *dosha* gets complicated as per the chronicity and the severity of the disease. In many of the auto immune conditions, all three *dosha* are getting vitiated in an altered manner. Mutation in *vaata-dosha* is responsible for defects in coding and signal transduction. There can be qualitative defects in *pitta* which will bring about various metabolic changes in the body later may cause increased or defective immune reactions. A qualitative depletion of *kapha* can be observed along with decreased immune capacity.

Ojodushṯi: The *taijasabhaava* of *ojas* is responsible for the immune reactions. In autoimmune disorders this *bhaava* is getting mutated by *aama*. As stated early, the *agni-vaishamya* and *aama* leads to *dosha-dhaatu vaishamya* which indeed manifest *ojodushṯi* as *ojas* is the *saara* of all other *dhaatus*. *Ojodushṯi* is of 3 types[5] and almost all autoimmune disorders show *visramsa* and *vyaapat lakshan̲aas*.

In *aayurvedic* parlance of psoriasis, *sampraapti* of *kushṯha* composes of the following sequence,

- *Nidaana* (aetilogical factors)
- *Agnimaandya* (loss of power of digestion)
- Formation of *aama* due to *agnimaandya* (metabolic error)
- *Srotodushṯi* (vitiation of the channels/pathways of metabolism)
- *Dosha-dooshya sammoorcchana* (morbid conglomeration of *dosha-dooshya*)
- *Roga vyakti* (complete manifestation of the disease)

It is observed that, the levels of various metabolic markers in autoimmune diseases are suggestive of metabolic errors which can be interpreted as a deranged *agni or pitta* status in human body. Recently, an eminent *aayurvedic* physician observed the incidence of elevated metabolic markers like LDH and CRP in *pitta-rakta* predominant autoimmune diseases, especially in psoriasis. This study aims to explore the inter-connection between some of the metabolic markers and *pitta-rakta* through the assessment of treatment response to *pittaṣamana-rakta-ṣuddhikara* medicine provided in such cases. This will immensely assist in diagnostic, preventive and curative aspects of psoriasis as well as other auto immune ailments.

Methodology

This study was carried out to discover the relationship between *pitta-rakta dushṭi* and metabolic markers[6] (LDH and CRP) by assessing their response to the administration of *Ṣaaribaadi gaṇa dravya* on psoriasis patients with elevated LDH or CRP levels in their blood. The study type was interventional, designed as a before and after comparison study without control design considering the sample size availability and affordability. The study design was selected under guidance of experts in the field of research and statistics. The study setting was Kriyashareera Research OPD, Govt. Ayurveda College Hospital, Kannur, and the study population was 15 psoriasis patients between the age group 20-50 years selected as per inclusion and exclusion criteria. Total duration of the study was 18months from June 2013. A Proforma with diagnostic criteria[7] for psoriasis and *pitta-rakta dushṭi* was used to confirm the diagnosis along with a detailed case sheet. Severity of psoriasis was measured using PASI score. Basic information such as biodata, *satva, prakṛti* and *saatmya* was collected using the proforma. Inclusion criteria screened in diagnosed psoriasis patients with elevated CRP or LDH level in blood, aged between 20-50 years and the exclusion criteria ruled out patients having mental illness and other systemic illnesses and any patients taking allopathic medicine for psoriasis.

Intervention

The subjects were advised to consume *Ṣaaribaadi gaṇa*[4a]*kvaatha* for a period of 30 days. *Sramsana* with *Avipatti coorṇa*[4b] was done before and after the medication. Necessary lab investigations like blood routine, serum LDH[8] and serum CRP[9] were done before and after the intervention. The results were analyzed statistically, tabulated and represented graphically and the conclusions were made based on the analysis. Paired 't' test was used to analyze the effects of intervention and Pearson correlation test was used to analyze the correlations. Table 1 and 2.

Table 1
Effect of intervention on LDH level
(*Normal LDH value 225-450 U/L)

	Mean	Std. Deviation	t value	P value
LDH before	511.47	86.860	4.408	0.001
LDH after	424.93	63.201		

Table 2 Effect of intervention on CRP level (*Normal CRP value: Upto 6 mg/L)				
	Mean	Std. Deviation	t value	P value
CRP before	10.20	18.952	2.122	0.05
CRP after	5.20	13.040		

Observations and Results (Figures 1 and 2)

• In this study, out of 15 subjects, all 15 had positive Auspitz sign, 12 of them had shown koebner phenomenon negative and 3 of them had shown it positive.

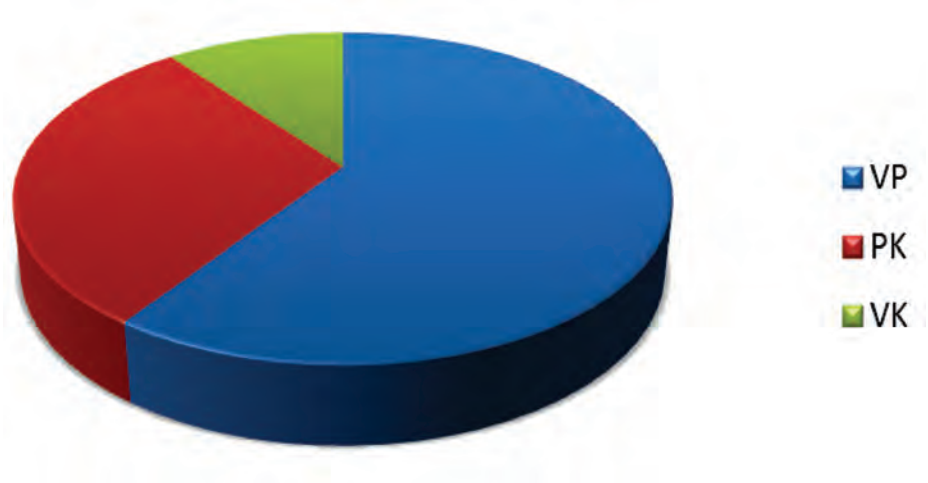
• In this study, out of 15 subjects, 13(86.7%) were of type A (stressed) and 2 (13.3%) were of type B (calm) personality.

• On analysis (Pearson correlation test) of the relation between *pitta-rakta* and LDH, it is seen that, there is a significant relation between *pitta vr̲ddhi* percentage and elevated LDH levels before and after the intervention. Table 3.

• The relations between *pittakshaya* and LDH level, *raktavr̲ddhi* and LDH level, *raktakshaya* and LDH level before and after the intervention were not significant on Pearson correlation test.

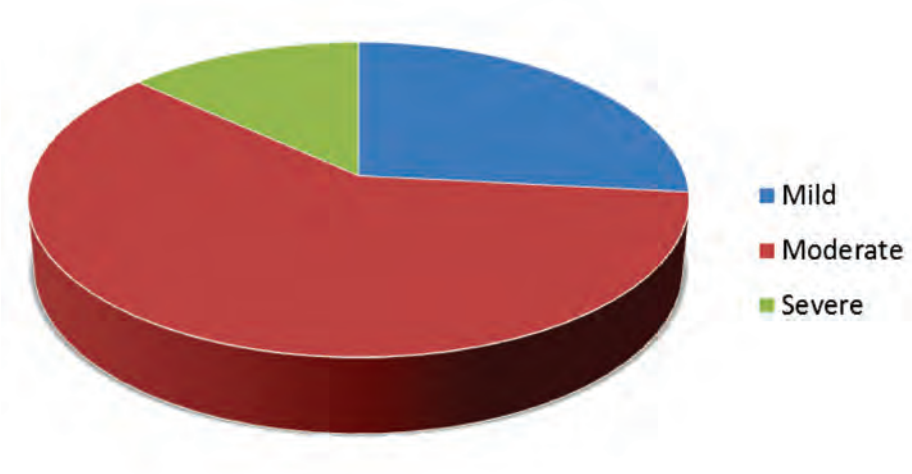
Table 3 Relation between *Pitta-Rakta* and LDH			
Correlations between *pittavr̲ddhi* and LDH after intervention			
		Pittavr̲ddhi % after	LDH after
Pittavr̲ddhi % after	Pearson Correlation	1	.542*
	Sig. (1-tailed)		.018
	N	15	15
LDH			
after	Pearson Correlation	.542*	1
	Sig. (1-tailed)	.018	
	N	15	15
*. Correlation is significant at the 0.05 level (1-tailed).			

Figure 1
Distribution of *Prakr̲ti*



Out of 15 subjects, 8 (53.3%) were of *vaatapitta*,
4 (26.7%) were of *pittakapha* and 3 (20%) were of *vaatakapha prakr̲ti*

Figure 2
Distribution according to severity



Out of 15 subjects, 9 (60%) had moderate, 4 (26.7%) had mild and 2 (13.3%) had severe degrees of psoriasis

• On analysis (Pearson correlation test) of the relation between *pitta-rakta* and CRP, it is observed that, there are no significant relations between *pittavr̲ddhi* and CRP, *pittakshaya* and CRP, *raktavr̲ddhi* and CRP and *raktakshaya* and CRP before and after the intervention.

• The results have shown that there is a relationship between the metabolic markers and the functional aspects of *pitta* and *rakta.*

Discussion

Lactate dehydrogenase (LDH or LD)[10] is an enzyme found in nearly all living cells. LDH catalyzes the conversion of lactate to pyruvate and back, as it converts NAD^+ to NADH and back. This shall be considered as a *dhaatu* level *agni* which flares up only when there is a great amount of pyruvate (fuel) available. This shows the *paaka* of same *dhaatu* and *aamatva* in successive *dhaatu* level (ie. the inability of Krebs cycle[11] to be carried out partially or completely). *Aacaarya Vaagbhaṭa*[12] describes that a kind of *vr̲ddhi* and *kshaya* of *dhaatus* take place due to the respective debility and intensity of the *ams̲as* of *dhaatvagni* present within the *dhaatus.* Just like forest fire, intermittent flaring up and dimming down depending on the nature of the fuel available, the *pari̲naama* of *dhaatus* may increase or decrease depending upon the nature of fuel. So elevated LDH level can be an indicator of abnormal *dhaatu* level *agni* in its respective locations.

C-reactive protein (CRP)[13] is an annular (ring-shaped) pentameric protein found in blood plasma, whose circulating concentrations rise in response to inflammation. CRP is a strong indicator of *aama* condition. It can be taken as a response of *agni* to an *aama* factor. CRP is getting elevated as a response to inflammatory cytokines. Here inflammation can be taken as the action of *agni*

over the harmful substances. So the elevated levels of CRP suggest the *aamatva* in body. As CRP is the first response for inflammation to enhance the *taijasa bhaava* to execute the *aama*, it can be taken as an *amsa* of *pitta* only. Susrutaacaarya states that all *aagneyaamsa* present in the body are none other than *pitta*.[5a]

Pitta-rakta: *Pitta* includes all those factors responsible for digestion and metabolism due to the properties of *agni* present in *pitta*, attending to burning, digesting and such other functions, *pitta* itself is considered as *agni*. *Aacaarya Caraka* has quoted *ooshma* as a synonym for *agni* while explaining *bhootaagni*. *Agni* by virtue of *pitta* performs different functions in *saareera*[14]. *Agni* is explained as *hetu* for *ojas* and *teja*. *Dalhana* comments that, *ooshma* is also called as *ojas*. *Ojas* is defined as *parateja* of *rasaadi dhaatus* by *aacaarya Vaagbhata* and *Susruta*. This *ojas* is the originator of every physical and mental faculty. This states that *pitta* has a clear role in digestion-metabolism in *bhootaagni* and *dhaatvagni* level. The *taijasa bhaava* of *ojas*, bestowed by *pitta*, is responsible for the immune reactions. Whenever there is a fault in *pitta*, it affects *rakta* within a short period or vice versa. This is due to the *aasraya-aasrayee* relationship between *pitta* and *rakta* which is a result of *samaana gunas* between these two components. *Rakta* is the only *dhaatu* linked with *pitta* and they share many common *gunas*. Both are *aagneya* and *aapya*. Whenever there is a defect in *pitta*, there definitely will be a qualitative defect in *rakta*. Above mentioned markers can also be taken as the indicators of such derangements and are detectable in the blood analysis.

Pitta dushti in psoriasis may possess either *vrddhi* or *kshaya* or both at different levels. It depends on the causative factors, severity and chronicity of the disease. It is not mandatory that either *vrddhi* or *kshaya* of a *dosha* only exist at a time. Usually *vrddhi* is the dominant factor that leads to a disease condition. All three *doshas* are involved in *kushtha*, where *pitta* and *rakta vrddhi* can be seen initially due to *nidaana visesha* and later, due to the chronicity and *vrddhi* of other *doshas,* signs of *pitta* and *rakta kshaya* can also be seen.

The intervention, *pitta samana-rakta sodhaka* drugs, is aiming to correct the *pitta-rakta dushti*. The administration of *Saaribaadi gana-kashaaya* along with *sramsana* can bring down the percentage of *pitta vrddhi* significantly. The derangements in *rakta* are easier to compensate as it is much more *moorta* than the *pitta*. *Saaribaadi gana* has an exceptional capacity to handle the *rakta dushti*. *Saariba* is therapeutically *rakta sodhaka* in action, and almost all other drugs in this *gana* are of *seeta guna* and *pitta saamaka* in action, in turn subsides *rakta dushti* efficiently.

Elevated LDH levels in the serum can grossly be interpreted as the derangement of *agni* or dysfunction of *pitta*. LDH can be taken as an *amsa* of *pitta* or *agni* which under normal conditions is not much operative in metabolism. Whenever there is an inflammatory reaction, there is excessive consumption of oxygen which eventually leads to local hypoxia. This is followed by pyruvate accumulation (due to lack of oxygen to carry out Krebs cycle), and LDH converts this pyruvate to lactate for energy production. But this is an altered *agni vyaapaara* which leads to the *paaka* of same *dhaatu.* This alteration also affects successive *dhaatvagni vyaapaara* resulting in *aamatva* at various levels. Administration of *Saaribaadi gana kashaaya* along with *sramsana* corrects the *agni* at respective levels to remove the *aamatva* and to bring back the normal pathway of energy production, thereby the LDH levels are brought down.

CRP can be taken as a response of *agni* due to the *aamatva* to bring about an increase in *taijasa bhaava* for a natural *deepana-paacana*. Any incapability of *agni* to perform its duties will lead to the formation of *aama* at respective levels. Here the distortions are predominantly at the level of *rakta*. The administration of *Saaribaadi gana kashaaya* along with *sramsana* corrects the *agni* and removes the *aamatva* of *rakta* which in turn reduces the CRP.

Conclusion

Increased LDH level shall be taken as an indicator of 'deranged *dhaatu* level *agni* and increased CRP level is a strong indicator of *aama* condition (a response of *agni* to an *aama* factor). Significant regulatory effects on *pitta-rakta dushti* as well as on elevated LDH and CRP levels are obtained as the result of the intervention. As per the analytical data, there is a significant relationship between the levels of LDH and the functional status of *pitta dosha*.

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REVIEW STUDY

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A conceptual comparative study of Multiple sclerosis and Systemic sclerosis in the perspective of *aayurveda*: a review

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ABSTRACT: Multiple sclerosis (MS) is an autoimmune disease mainly affecting the central nervous system. Description of MS is mainly available from contemporary medicine. Contemporary medicine lacks a complete overview of the events that happen in MS due to its complexity. The uncertainties that exist in the field of MS create difficulty in its management. Some research reports show that MS patient is affected by other autoimmune disorders such as Systemic sclerosis (SSc). The objectives of the study are to understand the etiopathogenesis and co-existing conditions of MS and SSc with *aayurveda* perspectives. It can enhance the insight of *aayurveda* practitioners to provide better treatment. The description of MS and SSc were collected from different sources such as contemporary medicine books, research articles, etc. The information is analyzed to understand the etiopathogenesis of MS and SSc in *aayurveda*. The etiopathogenesis is compared to find out any relation between MS with SSc. MS and SScdiffer in some factors of etiopathogenesis such as the site of manifestation. So, they should be considered as two different diseases. The presence of *aama*, the relevance of *vaata,* and the similarities of the *adhishṯhaana* have been found in MS and SSc. The coexistence of MS and SSc is due to the characteristics of *aama*, the relevance of *vaata,* and the nearness of the *adhishṯaana* of MS with SSc. The similarities in the *aayurvedic* etiopathogenesis of MS and SSc will be helpful to explore the characteristics of autoimmunity from the *aayurveda* perspective.

Key words: *Aama*, Multiple Sclerosis (MS), Systemic Sclerosis (SSc), Autoimmune disease, Etiopathogenesis

Introduction

Multiple sclerosis (MS) is a multifocal inflammatory autoimmune disease that grossly affects the CNS white matter resulting in progressive neurodegeneration in genetically susceptible hosts.[1] Possibly the earliest documentation of multiple sclerosis is the case of Lidwina the Virgin, who lived in Schiedam, Holland. In 1935, at age 16 years, Lidwina developed an acute illness and subsequently fell while skating on a frozen canal. Some commentators have considered there to be sufficient evidence for a diagnosis of MS.[2] MS affects about 2.5 million people worldwide and is the most common cause of neurologic disability in young and middle-aged adults. MS is twice as common in women than in men. The onset of the disease usually occurs between 20 and 50 years of age with a peak at about 30 years of age. It has been recognized across all ethnicities.[3]

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Systemic sclerosis(SSc) is a connective tissue disorder; manifested by hardening and thickening of the skin, abnormalities involving the microvasculature and larger vessels, and fibrotic degenerative changes in various body organs including the heart, lungs, kidneys, and gastrointestinal tract.[4] The peak age of onset is in the fourth and fifth decades, and it has a 4: 1 female: male ratio.[5] It is subdivided into Diffuse cutaneous systemic sclerosis (DCSSc) and Limited cutaneous systemic sclerosis (LCSSc).

It is striking to note that some contemporary treatments in MS can trigger another autoimmune disease like SSc.[6] The report also states the difficulty of treating patients with MS associated with SSc. Some other report shows that the MS can associate with SSc.[7] Most of the *aayurveda* studies try to co-relate MS to *aayurveda* disease terminologies like *vaatavyaadhi, majjagata vaata*, etc., and explain their treatments.[8,9,10,11,12,13] There are studies in SSc try to correlate SSc with *uttaana vaatarakta, tvaggatavaata*, etc.[14,15,16,17] Most of the *aayurveda* studies separately mentions MS and SSc and there may be no mention of co-existing conditions of MS and SSc. Hence, this study tries to solve the research gap of the co-existence of MS and SSc through *aayurveda* perspectives. Certain traditional medicine research from Chinese and Iranian medicines explores the diet habits, regimens, and malnutrition risk in SSc.[18,19]

Aayurveda shows promising results in MS and SSc. Most of the patients of MS come to treatment in *aayurveda* after the contemporary treatment, with a greater chance for SSc. In this scenario, it is relevant to understand and compare the etiopathogenesis of MS and SSc with *aayurveda* perspectives to provide better treatment in MS-associated SSc. Hence, the study tries to understand the etiopathogenesis and co-existing conditions of MS and SSc with *aayurveda* perspectives.

Materials and methods

The data needed for the analysis of MS and SSc have been taken from contemporary science treatises, articles, internet sources, and *aayurveda* treatises. The data have been analyzed to understand the etiopathogenesis of MS and SSc with *aayurveda* perspectives such as *anuktavyaadhi* protocol and *rogapareeksha*. The etiopathogenesis is compared to find out any similarities.

Analysis and Result

The innate nature of the disease can be understood with the *rogapareeksha- nidaana*, *poorvaroopa*, *lakshaṇa*, *sampraapti,* and *upaśaya-anupaśaya*. The *nidaana*, *lakshaṇa,* and *upaśaya-anupaśaya* of MS and SSc are explained below.

Nidaana

The etiology of MS is unknown in contemporary medicine. Contemporary medicine points out that genetic factors, infections, environmental factors, and vitamin D deficiency have the main role in the etiology of MS. In MS, multifactors can trigger the disease process. Meat of beef, veal, lamb, chicken, fish, seafood, canned tuna and hamburgers, sausages, and pizza, as well as fruits like watermelon, apricots, cherries, peaches, nectarine (sour, sweet, salty), greengage (European plum)-sweet and sour taste, grapefruit,

oranges, tangerines, pomegranates, plums, and strawberries, as well as seasonings such as chips, cheese balls, salt, sugar, can increase the risk of MS.[17] A high-fat diet that includes butter, olive oil, animal fat, margarine, and hydrogenated vegetable oil makes MS more likely. Most of these foods are *madhura*(sweet), *amla*(sour), *lavana*(salt) in taste, and *guru*, *snigdha*, *seeta,* and *ushna* in properties. Recent Chinese research has suggested that low antioxidant or vitamin B 6 levels may be a factor in some foods' cold essence.[18] Some other contemporary research on diet and nutrition suggests plant-based diets with moderate intake of some foods such as fish, poultry, and low-fat dairy leads to MS.[20] Some *aayurveda* case reports point out the presence of *aama* as the etiological factor which initiates an *aavarana* pathology.[21,8] Some other studies report the unnecessary gut microbiota and presence of toxins in MS.[22,23] All these factors ensure the presence of *aama* or *aamavisha* in MS.

The etiology of SSc is unknown in the contemporary medicine. Genetic factors, environmental factors, and infections have a greater role in SSc. Consumption of salt, and alcohol, and sleep less than 7 hours increase the risk for SSc. Intake of oily foods, fish, cereals, meat, non-alcoholic beverages, eggs, and dairy products have an association with malnutrition risk in SSc. The intake of carbohydrates, protein, and fat also in excess causes *aama* which increases malnutrition. It is noted that the malnutrition risk is low in those who consumes a less-calorie diet. The subjects reported with the presence of dysphagia and early satiety had a higher malnutrition risk.[9] The incompatible foods, irregular food habits, and improperly digested foods can lead to *aama* formation which can trigger the unwanted immune response.

Poorvaroopa

Aayurveda medicine case reports the prodromal symptoms of MS such as numbness, loss of movement, and tingling sensation in the initial stage of MS.[8,24,25] These symptoms continue as the disease progress. Fatigue, pain, fibromyalgia, migraine, bowel issues, bladder issues, sleep disturbances, anaemia, cognitive impairment, and dermatological issues are reported as prodromes in contemporary medicine.[26,27]

Hence, it can be taken as *avyaktalakshana* explained in *aayurveda*. *Avyaktalakshana* is an incomplete part of the main symptoms according to *Cakrapaani*. The prodromal symptoms of SSc can be taken as the *poorvaroopa* of *kushtha* and *vaatarakta* such as stretching, thickening, tightening of skin, itching, and *tvak vaivarnyata*-(blackening).[14,16] Contemporary case reports point out the fatigue, arthralgia, tightening, stretching of the skin, persistent fatigue, xerostomia, dysphagia, bilateral lower extremity weakness, Raynaud's phenomenon, joint pain in both hands that improved with activity and worsened with rest, morning stiffness lasting longer than one hour, dyspnea with exertion, diarrhea, painless oral ulcers, unintentional weight loss.[28]

Lakshana

The symptoms of MS and SSc are taken from previous studies and contemporary textbooks of medicine, summarized in Table 1 and 2. MS and SSc can involve multiple systems of the body.

Table 1
Symptoms of MS and Etiopathogenesis

Symptoms of MS[7,8,9,10,12]	Etiopathogenesis	References
Loss of sensation/diminished sensation (*acetana*)	*Sarvaanggakupita vaata*	Ca.Ci. 28/25
Numbness(*suptata*)	*Vaatakopa, kaphakopa, raktagatavaata tvaggatavaata, sarvaanggagatavaatakopa, raktaavṛtavaatakopa*	Ca.Soo. 20/12,Ca.Soo. 20/18, Ca.Soo. 20/18, Ca.Ci. 28/30, Su.Soo. 1/31, Su.Soo. 1/33
Weakness / loss of power (*daurbalya*)	*Majjaagata vaata, praaṇaavṛta vyaana, udaanavṛta praaṇa, samaanaavṛta vyaana, kaphaavṛta vaata, kaphaavṛta udaana* or *praaṇa*	Ca.Ci. 28/33, Ca.Ci. 28/202, AH.Ni. 16/52, AH.Ni. 16/52, Su.Ni. 1/35, Ma.Ni. 22/22, Ca.Ci. 28/35
Difficulty in walking (*gatisangga*)	*Kaphaavṛta vaata*	Ca.Ci. 28/229
Lack of coordination (*skhalatgati*)	*Kaphaavṛta vyaana*	AH.Ni. 16/50
Tingling sensation (*cimicimaayana*)	*Vaatakopa*	AH.Ni. 15/55
Burning sensation (*daaha*)	*Vaata* is obstructed by *pitta*	Ca.Ci. 28/61
Pain(*ruja*)	*Majjaagata vaata, pittaavṛta vaata*	Ca.Ci. 28/33, Ca.Ci. 28/61
Stiffness(*stabdhata*)	*Vaatakopa, majjaagata vaatakopa, kaphaavṛtavyaanavaata*	AH.Soo.12/51, AH.Ni.15/15, Ma.Ni. 22/26
Heaviness in the body (*gaurava*)	*Kaphaavṛtavaatakopa, saamavaata*	Ca.Ci. 28/ 62, AS.Soo. 16/30
Incontinence of urine/ bowel	*Apaanaaavṛta vyaana*	Ca.Ci. 28/212
Burning micturition (*mootra daaha*)	*Saamapitta*	Ma.Ni. 1/5
Pin needle sensation (*toda*)	*Vaatakopa, sarvaanggagata vaata, tvaggata vaatakopa, raktaavṛta vaatakopa*	Ca.Soo. 20/12, AH.Ni.15/15, Ma.Ni. 22/15, Su.Ni. 1/33
Slurred speech (*avyaktavaak*)	*Majjaavahasrotas* affected by *vaata* like in *arddita*	Ca.Ci. 28/41
Involuntary movements (*kampa*)	*Vaatakopa*	Ca.Soo. 20/12, Ma.Ni. 1/5
Fine and gross motor movements are affected (*ceshṭa haani*)	*Kaphaavṛta vyaana, pittaavṛta vyaana*	Su.Ni. 1/39, AH.Ni. 16/44
Back pain (*pṛshṭharuja*)	*Sarvadhaatvaavṛta vaata kopa*	AH.Ni. 16/43
An electric shock-like sensation that occurs on flexion of the neck	*Vaatakopa* leads to *majjavahasroto dushṭi* like in *ardita*	Su.Ni. 1/70
Memory problems (*smṛtikshaya*)	*Praaṇaavṛta vyaana*	Ca.Ci. 28/202

Table 2
Symptoms of SSc and Etiopathogenesis

Symptoms of SSc[13,14,15,16]	Etiopathogenesis	References*
Tightening of skin on the face etc. (*aayata*)	Can seen in *uttaana vaatarakta*, *vaataadhika kushtha*	Ca.Ci. 29/20
Roughness, hardness, hyper pigmentation, hypo pigmentation like white patches (*parusha, kathina, tvakvaivarnyata*)	*Vaatakopa, vaataadhika kushta, tvaksthita kushtha*	Ca.Soo. 12/8, 49/23 Ma.Ni. 25/11
Toes and fingers turn bluish/blackish (*syaavaneelavarna*)	*Vaatakopa* in the skin - blackish, *pittakopa* in leads into bluish discolouration, *vaataraktasampraapti vaataadhikakushtha*	Ma.Ni. 1/5 Ma.Ni. 23/ 5,6,7 Ma.Ni. 49/23
Multiple joint pain (*sandhi soola*)	*Tvaksthita vaata kopa*, *asthi, majja, sandhi, snaayugata vaata kopa*, *kaphaavrta vyaana*, *aamavaata sampraapti*, *vaatarakta sampraapti*	Ca.Ci. 28/30, AH.Ni. 15/12, Ca.Ci. 28/ 33, Ca.Ci. 28/37, AS.Soo. 19/23, Ca.Ci. 28/30, Ma.Ni. 25/7, Ma.Ni. 23/5,6,7
Stiffness(*stabdhata*)	*Saamavaata, vaatakopa, raktagata vaata, maamsagata, medogata, majjagata and snaayugata vaata, aamavaata sampraapti*	Ca.Ci. 12/51, Ca.Ci. 28/31, AH.Ni. 15/31, AH.Ni. 15/11, AH.Ni. 15/15, Su.Ni. 1/27, Ma.Ni. 25/11
Swelling on joints, forearm, face, etc. (*svayathu*)	*Saamavaata, vaatarakta*	Ma.Ni. 1/5, Ma.Ni. 25/6, Ma.Ni. 23/8,10
Burning sensation (*daaha*)	*Saamapitta*	Ma.Ni. 1/5, Ma.Ni. 25/11
The fullness of the stomach (*atipoorna koshthata*)	*Vaatakopa*	Ma.Ni. 1/5
Regurgitation of gastric content (*hrllaasa*)	*Saamapitta*	Ma.Ni. 1/5
Belching (*amlodgaara*)	*Saamapitta*	Ma.Ni. 1/5
Vomiting (*charddi*)	*Aama*	Ma.Ni. 25/10
Weight loss (*kaarsya*)	*Rasapradoshaja, vaatakopa*	Ca.Soo. 28/10, AH.Soo. 11/5
Hair fall (*kesasaata*)	*Rasapradoshaja, asthikshaya*	Ca.Soo. 17/67
Generalweakness/ fatigue (*daurbalya*)	*Aama, dhaatupaaka*	AH.Soo. 13/12, Ma.Ni. 38/16
Fever (*jvara*)	*Aama*	Ma.Ni. 25/6
Breathlessness (*svaasa*)	*Kapha* blocks *vaata* and upward *gati* of *vaata*	Ma.Ni. 12/15
Cough (*kaasa*)	*Aama*	Ma.Ni. 11/1
Constipation (*mala vibandha*)	*Aama*	AH.Soo. 13/12
Difficulty in swallowing (*krcchraabhyavhrti*)	Due to *hanugraha*	Su.Ni. 1/53
Difficult to open mouth (*mukham krchrenavivrtati*)	*Vaatakopa* in *hanu sandhi*	Ma.Ni. 22/21
Decreased appetite (*alpakshut*)	*Aama*	Ma.Ni. 1/5, Ma.Ni. 25/6
Itching (*kandu*)	*Kaphakopa, vaata, rakta, kaphaadhikakushtha-sampraapti, raktaadhikakushtha sampraapti*	Ma.Ni. 1/5, Ma.Ni. 23/10,12 Ma.Ni. 49/24, Ma.Ni. 49/26
Sleeplessness (*alpanidrata*)	*Dhaatupaaka*	Ma.Ni. 25/9-10
Excessiveurination (*atimootrapravrtti*)	*Aama*	Ma.Ni. 25/9
Varicose veins (*siraayaama*)	*Vaata* in *siraa*	Ma.Ni. 38/16
Blisters on distal phalanges (*sphota*)	*Pittakopa*	Ma.Ni. 1/5
Digital ulcers (*vrana*)	*Pittakopa, vaatarakta*	Ma.Ni. 23/ 11

*Ca.Ci. - *Carakasamhita Cikitsaasthaana*, Ca.Soo.- *Carakasamhita Sootrasthaana,* Ma.Ni.- *Maadhavanidaana*, AH.Soo. - *Ashtaangaahrdaya Sootrasthaana,* AH.Ni. - *Ashtaanggahrdaya Nidaana,* AH.Ci. - *Ashtaanggahrdaya, Cikitsaasthaana,* Su.Ni. - *Susrutasamhita Nidaanasthaana*

Here, common primary symptoms are taken into consideration. The corresponding symptoms are converted into *aayurveda* terms with the help of the NAMASTE portal and *aayurveda* treatises. The etiopathogenesis behind each symptom and the corresponding references are also mentioned.

The analysis of symptoms gives an idea of about different factors like *aama, dosha dushṭi, aavaraṇa, dooshya, dhaatupaaka,* and *dhaatukshaya*. From this, the general *sampraapti* of disease can be inferred.

Sampraapti

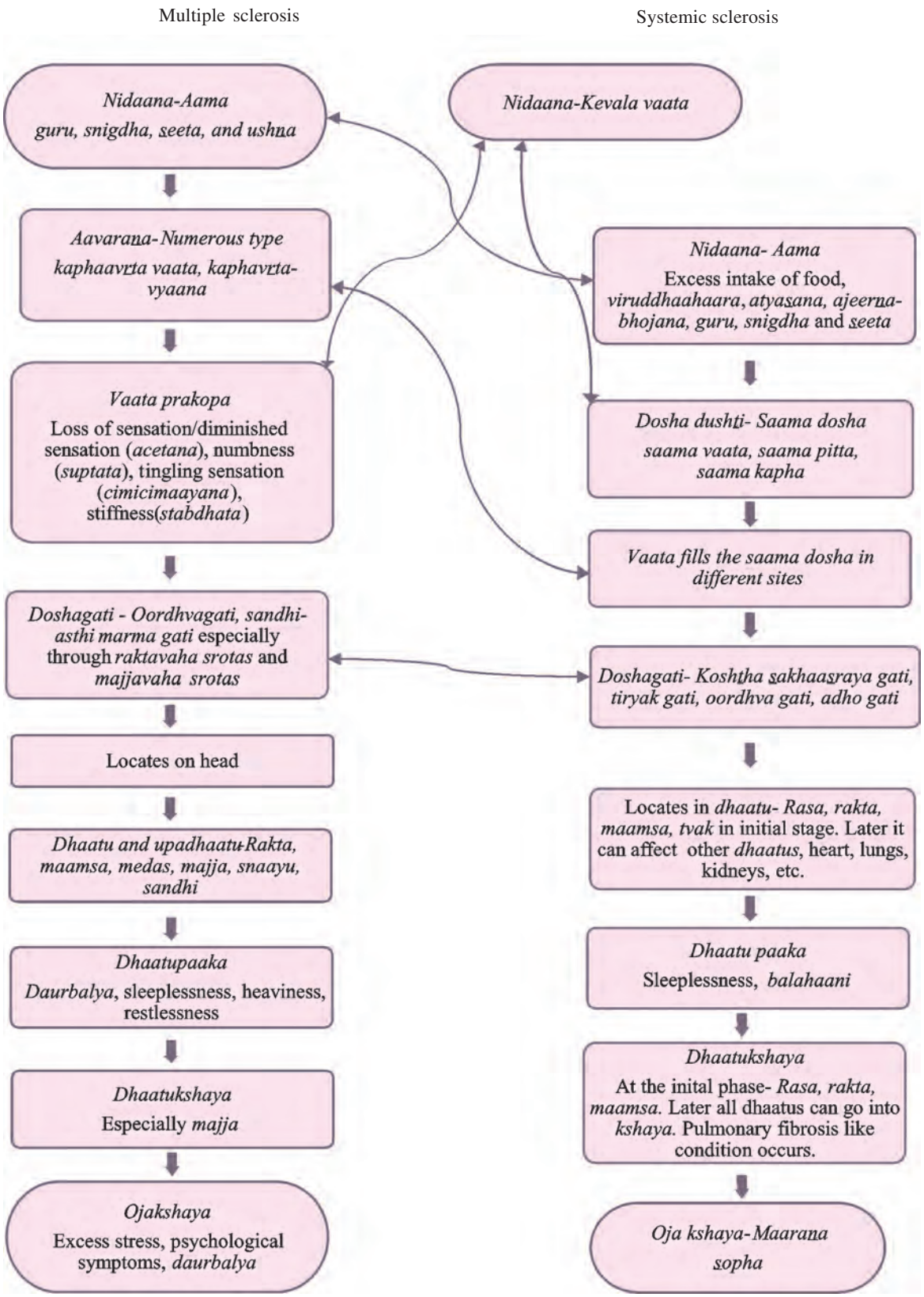
The arrow marks connect the *sampraapti*, provide the possibility of one *sampraapti* can enter the other. The MS has a complex *sampraapti*. The *nidaana* is usually started with the formation of *aama* or *aamavisha*. The *aama* can make *srotorodha* and can act as *aavaraṇa*. According to the type of *aama*, the nature of *aavaraṇa* also changes. In case of *kaphaavṛta vaata, aavaraka* is *kapha*. Due to the complexity of MS, the characters of *aavaraṇa* changes in each patient of MS. Due to the *aavaraṇa*, the *gati* of *vaata* is obstructed and *vaata* becomes in *prakupita* state. The *vaata* goes into *oordhvagati* or *sandhi-asthi-marma gati* to locate in *majja* of *śiras* and in *rakta, maamsa, medas, snaayu* and *sandhi*. The *vaata* distorts the *agni* in these places and starts the *dhaatupaaka*. The *dhaatupaaka lakshaṇa* are the symptoms of *daurbalya*, sleeplessness, heaviness, and restlessness. After the period of *dhaatupaaka*, the *dhaatu* starts to detiorate. The *ojakshaya* features appear. In certain cases, the *vaatakopa* occurs due to the etiologic factors of *vaata*. Then that *vaatakopa* can cause distorted *dosha gati* same as that of *aavaraṇa*.

The general *sampraapti* of SSc starts with the *aama* formation. The *viruddhaahaara, atyaśana, ajeernabhojana, guru, snigdha*, and *śeetaguṇa* cause the *aama* formation. The *aama* along with vitiated *dosha* goes to the *rasa, rakta, maamsa, tvak in the* initial stage through *koshṭhaśaakhaaśraya gati* and to *sandhi, asthi*, and *marma* through *sandhi-asthi-marma gati*. The lodged *dosha* causes the *dhaatupaaka* and *dhaatukshaya*. In the extreme *dhaatukshaya*, the pulmonary fibrosis like symptoms can be seen in SSc. At the final stage, *śopha* as part of *ojakshaya* can be seen in SSc. Another possible etiology of SSc is *kevalavaatakopa* which can initiate the *sampraapti* of SSc. The general *sampraapti* of MS and SSc are shown in figure 1.

Discussion

The typical features of MS and SSc can vary from patient to patient. MS and SSc are multifactorial autoimmune diseases. So, the physician must differentiate each patient to understand the specific *sampraapti* from the general *sampraapti*. The complex general *sampraapti* of MS and SSc gives the idea about the different diagnoses such as *vaatavyaadhi, aavaraṇajanya roga, vaataadhika koshṭha, tvaksthita koshṭha, vaatarakta* and *aamavaata*. The general *sampraapti* of MS and SSc is taken into consideration in this study. The etiopathogenesis of MS and SSc are comparable in *aayurveda* and can be associated with each other.

Figure 1
Sampraapti of Multiple sclerosis and Systemic sclerosis



The *aama* formation, *vaatakopa* and *adhishṭhaana* are common in MS and SSc. The *aavaraṇa sampraapti* is also noticed in both diseases. The same *aama* in the patients of MS can initiate SSc as a *svatantra vyaadhi*. If the *doshagati* is distorted in MS due to some factors, the *dosha* can enter into SSc *sampraapti*. The wrong treatment modalities adopted in MS deviate the *gati* of vitiated *dosha* and cause more *aama* formation. The relapsing and remitting nature of MS also favours the movement of *dosha* in MS. The initial *adhishṭhaana* of SSc is *koshṭha* and *śaakha* however it enters *madhyama roga-maarga* in later phase. The similarities in the *adhishṭhaana* also make MS susceptible to SSc.

Conclusion

MS and SSc can simultaneously find in some patients due to the similarity of *aama*, the relevance of *vaata,* and the similarity of the *adhishṭhaana*. This emphasizes the importance of *aayurveda* treatment modalities such as *nidaana parivarjana, aamapaacana,* and *vasti* like *vaata* pacifying treatment in MS. The *rasaayana* treatment has a main role in *dhaatu* debilitating diseases like MS and SSc. Eventhough some similarities are found in the basic *sampraapti* of MS and SSc, they differ in some factors of etiopathogenesis and the site of manifestation. So, they should be considered two different diseases. The similarities in the *aayurvedic* etiopathogenesis of MS and SSc will be helpful to explore the characteristics of autoimmunity from the *aayurveda* perspective. More *aayurveda* research in autoimmune diseases is needed to explore hidden *sampraapti* of autoimmune diseases and their interrelation.

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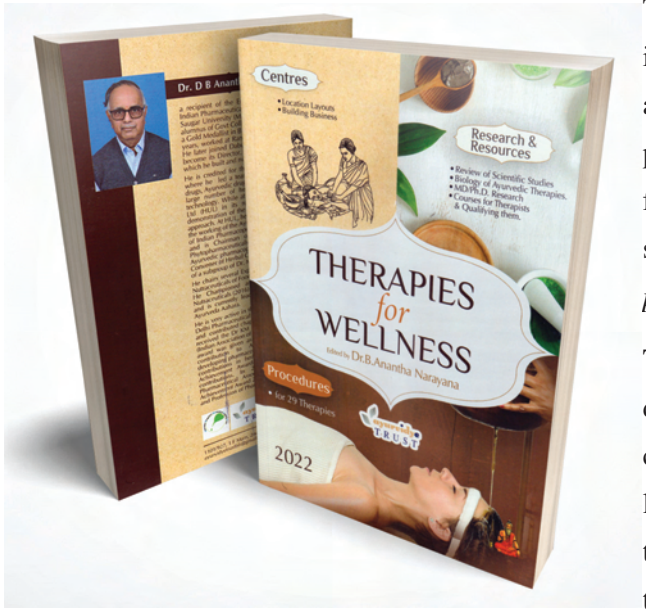
BOOK REVIEW

ĀRYAVAIDYAN, Vol. 36, No. 2, November 2022 - January 2023, Pages 49 - 50

'Therapies for Wellness' - book review

Anandaraman Sharma P.V.*



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Published: 2022
Pages: 376 pages
(8 printed art paper for color print)

A growing interest and popularity has been observed in the field of *aayurveda* especially *pancakarma* therapies among the patients for their chronic problems as well as by healthy to maintain their well-being.

This unique book contains 37 chapters like infrastructure and marketing, available evidences and research, human resources, common working procedures of therapies, hygienic practices to be followed in *aayurvedic* therapy centres and the standard operative procedures of various *panjjakarma* therapies.

The first part about infrastructure and marketing describes about the various considerations for constructing a stand-alone *aayurvedic* centre, a layout has also been suggested for a single, double treatment room as well as an 8-room multiple treatment centre with in-patient services and the considerations to be kept in mind for setting up an *aayurveda* treatment centre in a rented building. It explains very detailed information about importance of marketing strategies, branding and layout, location, pricing of services, generation of footballs, understanding the financials, how to maintain customer relationship, how to do follow-up after therapies, data documentation and how to manage the customer's misconception about Spa.

The second part of the book deals with the published works on *pancakarma* therapies, mode of action of *poorvakarma*, all *sodhana* therapies, *sirodhaara* and massage therapy along with the

* *Medical Superintendent, Professor and Head, Department of Panchakarma, All India Institute of Ayurveda, Sarita Vihar, New Delhi, India. Email: dr.ananthramsharma@gmail.com*

scientific data supporting its benefits. A list of post graduate and PhD theses published in different universities conducting *aayurveda* courses in India has been included in this part.

The third part contains the role of *paricaaraka* in the therapies and it has been correlated to the need of Human Resource Development, the syllabus, modules and skill in Certificate course for Panchakarma Assistants, guidelines for the qualification of therapists.

The fourth part contains the common working procedures of 29 Therapies, details of how a therapy room should be, the instructions to be followed post therapy at the centre, and the hygienic practices to be followed. It also contains detailed SOP of 29 therapies.

The purpose, need, definition, references, room preparations, pre-examinations, duty of therapists, detailed SOP, post therapy, general information like benefits, indications, contraindications, appearance, attitude, stance, position and factors to be considered by therapists, preferred medications according to conditions of each of the therapies has been mentioned. 29 therapies like *sarvaangga abhyangga* by both single and two therapists, *paadaabhyangga, sirobhyangga*, all types of *sthaanika basti*, different types of *pinda sveda*, *drava sveda* and *ooshmasveda, udvartana,* all *moordhni taila, maatra basti, nasya, lepa, talam, talapodiccil.* The annexures contain a comprehensive list of *taila,* herbs, *coorna, ghrta* and glossary of Sanskrit terms.

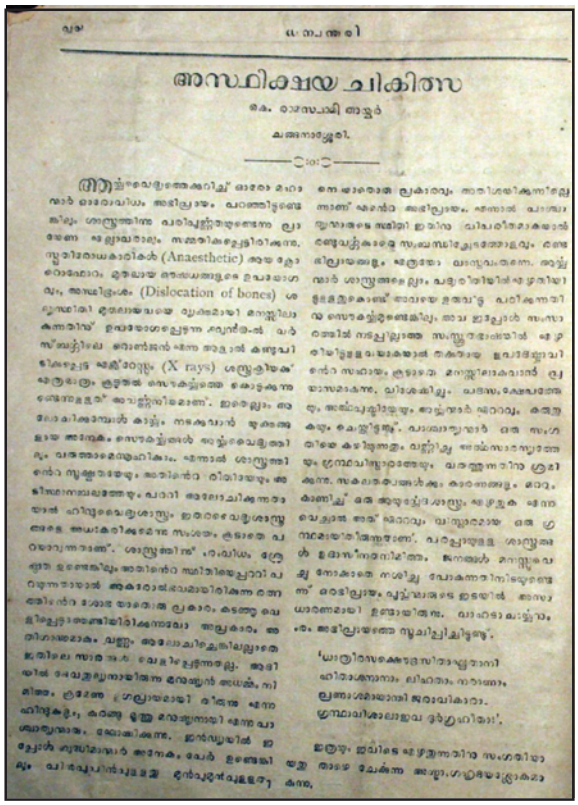
This book can be referred byall doctors as well as entrepreneurs who wish to start an *aayurveda* therapy centre or even a full-fledged multilevel in-patient hospital. The book is structured into various sections, with each section on relevant topics, and has a table of content, index, citations, references, and figures. The book follow an evidence-based approach with extensive citations and reference lists which can be valuable for obtaining more information on a specific topic of interest. Thus, the book has comprehensive documentation on the therapies meant for wellness.

ĀRYAVAIDYAN, Vol. 36, No. 2, November 2022 - January 2023, Pages 51 - 52

Asthikshayacikitsa

Ramaswami Iyyer K., Changanasseri

Dhanvantari is the first medical journal in Malayalam published every month by Vaidyaratnam P. S. Varier from Arya Vaidya Sala uninterruptedly for 23 years from 1903 to 1926. This clinical note was published in its column on Book No. 6, 1084 (Malayalam Era) *Vr̥scikam* (Malayalam Month) 1909 (CE) Issue, Article No. 9, Page 84.



അസ്ഥിക്ഷയ ചികിത്സ

ക. രാമസ്വാമി അയ്യർ

ചങ്ങനാശ്ശേരി.

The common factor cutting across the numerous commentaries on the various texts of *aayurveda* is the fact that the science is effective. The advent of anaesthetics and x-rays in 1895 by Wilhelm Conrad Röntgen in Wüzburg along with other technologies in the field of allopathic medicine have made diagnosis much more accurate and simple. The incorporation of the advanced technologies will and should be applied in *aayurveda*. Yet, when we reflect on the nuances, treatment protocols and the fundamentals of *aayurveda*, its superiority over the Western medical sciences is evident. Despite the said cult status, the fact remains that we have witnessed only the glimmer of the shiny diamond. Only the wise and blessed can wipe off the soot of carbon and witness the true form in all its glory for countless are the hidden meanings that lie between the lines. The Indian scriptures depict the tale of the fall of man from divinity to the animaldom because of the deviation we took from the path of *dharma* whereas the Westerners put forth the theory of evolution of the man from the apes. The deeds of the population in these respective parts of the land stand witness to both the claims. The narrative being in verse makes it easier to learn but since the medium is the ancient language of Sanskrit, the need of a master is indispensable. The brevity and the choice of the deep meaningful words is something that authors of the texts were

Translated by: Rati Vijayan, Centre for Textual Studies and Publications, Arya Vaidya Sala, Kottakkal, Kerala.

particular about because had they tread the western path of detailed descriptions, the texts would have been twice the size and people would abstain from going through it in full. *Vaagbhata* shares the same apprehension in the following verse,

Dhaatreerasakshaudrasitaaghrtaani
hitaasanaanaam lihataam naraannaam ।
Pranaasamaayaanti jaraavikaara-
grandhaavisaalaa iva durgrheetaah ॥

The inspiration for the aforementioned account happens to be the following verse from *Ashtaanggahrdayam*

..... asthi samkshayaat ।
Jaataan ksheeraghrtaistikta
samyuktairvastibheestathaa ॥

According to the quote, *"Tatraasthini sthito-vaayuh"* bones are the seat of *vaayu* and hence, responsible for the degradation of the same. Yet, how did *tiktarasa*, the arch rival of *vaayu*, the trigger for *dhaatukshaya* and vitiation of *vaata* (the verse to corroborate the same is given below), became the significant *rasa* of majority of the medicines for *Asthikshaya*?

Dhaatukshayam caalavyaadhee
naatiyogaat karoti sah ।
Tiktam katu ca bhooyishtha-
mavrshyam vaatakopanam ॥

The drugs that are lubricant and absorptive in nature often provide solidity and that might be the rationale behind it. But the same can be applied to the *rasa* of *katu* and *kashaaya* and yet that is not the case. So why the specificity of *tikta*? I welcome the response from the erudite readers of *Dhanvantari.* I would also like to point out that even though there are numerous ingredients in *Guggulutikta ghrta*, the primary *rasa* is that of *tikta.* It is deemed best for vitiated *vaata* and *pitta*. Let me conclude with the verse where the afore mentioned medicine is prescribed for the treatment of *visarpa*. Though *sneha* is judged contraindicatory to the ailment, exceptions according to the condition and diagnosis seem prudent.

Niraame sleshmani ksheene
vaatapittottare hitam ।
Ghrtam tiktam mahaatiktam...

INSTRUCTIONS TO AUTHORS

Author: Those who have substantially contributed to the reported work is to be considered as author. Corresponding author is responsible for all the communication with the journal. Non author contributors may be acknowledged in the relevant portion of the paper.

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Submission can be in form of original research articles, review articles, short communications, case studies and book reviews. All submissions should be made through email: publications@aryavaidyasala.com.

The language of the journal is English. For Devanagari script please follow the transliteration key given and make them in Italics.

Transliteration Index

अ	आ	इ	ई	उ	ऊ	ऋ	ए	ऐ	ओ	औ	अं	अः
a	aa	i	ee	u	oo	r̲	e	ai	o	au	am	ah̲

क्	ख्	ग्	घ्	ङ्	च्	छ्	ज्	झ्	ञ्
k	kh	g	gh	ng	c	ch	j	jh	ṇj

ट्	ठ्	ड्	ढ्	ण्	त्	थ्	द्	ध्	न्
t̲	t̲h	d̲	d̲h	n̲	t	th	d	dh	n

प्	फ्	ब्	भ्	म्	य्	र्	ल्	व्	श्	ष्	स्	ह्	ळ्	क्ष्
p	ph	b	bh	m	y	r	l	v	s̲	sh	s	h	l̲	ksh

क	का	कि	की	कु	कू	कृ	के	कै	को	कौ	कं	कः
ka	kaa	ki	kee	ku	koo	kr̲	ke	kai	ko	kau	kam	kah̲

Manuscripts submitted will undergo internal editorial review and external peer reviewing.

Kindly go through the details below before submitting the article.

Manuscript presentation

Article must be clear in delivering the idea. It should be devoid of any grammatical mistakes. Ayurvedic and Sanskrit terms must be in italics. Manuscripts must be typed double spaced with margins of one inch (2.5cm) at the top, bottom and the sides and all pages numbered starting from the title page. 12 pt Times New Roman font must be used and remain uniform throughout the text.

There is no need of translating the fundamental words of Ayurveda in English. Eg. *Dosha*- Humors, *Agni*- Bio fire, etc. Use the transliteration key given, for writing Sanskrit words.

Research articles, review articles and short communications must be limited to 5000, 4000 and 2000 words in length respectively.

Structure of the manuscript is presented below.

(i) Title page: This page should contain title of the article with affiliation and addresses of all the authors, including corresponding author with an asterisk. E-mail ID of the corresponding author should be provided as a foot note on the title page.

(ii) Abstract: Second page should contain a well structured abstract of 6-7 sentences or maximum 300 words for full papers and reviews (200 words for short communications). It should contain the key points of the article. Introduction, materials and methods, results and conclusion are to be well reflected in the abstract. There is no need of references here. Expansion of the abbreviations used in the abstract is to be given in brackets.

(iii) Key words: A list of up to six relevant keywords should be given. Need of adding general terms. Do not use plural terms. Keywords are important for indexing and searching the article.

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(v) Introduction: Objectives of the investigation with enough background of the subject must be stated in the introduction. The significance of the work in relation to the earlier ones has to be explained with relevant references. Introduction can be concluded with the aims and objectives of the study.

(vi) Materials and methods: All the materials that have been used to conduct a study along with the procedures adapted has to be included in detail. Adequate details of the methodology (study degisn) of the work should be provided so that others can reproduce it. Previously reported methods can also be cited with proper references. Modifications done to it has to be described. It is in this section, that ethical approval, study period, sample size, grouping, evaluation criteria, exclusion criteria and statistical methods should also be described in sequential manner.

Study Designs: Selection and Description of Participants: Describe the method of selection of the observational or experimental participants (it may be patients or laboratory animals, including controls) clearly. Their eligibility and exclusion criteria are to be explained. A clear description of the source population is also necessary. In case if specific apparatus is used, give the manufacturer's name and address in parentheses. Procedures involved in the study are to be given in sufficient detail so that other workers are able to reproduce the results. References to established methods, including statistical methods are to be provided.

Reporting of randomized clinical trials needs present information on all major study elements, including the protocol, methods of randomization, concealment of allocation to treatment groups, and the method of masking (blinding), based on the CONSORT Statement.

(vii) Observations and Results: It should be very clear and precise. This section should include the findings of your study. Presentations of the findings include: tables, charts, graphs, and other figures. But these should be kept to the minimum.

Statistics: As far as possible, quantify the research findings. Try to present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Losses to observation (such as, dropouts from a clinical trial) should also be reported. Do not forget to specify the statistical methods used in analyzing the results. Define the statistical terms, abbreviations, and most symbols. Specify the computer software used.

(viii) Discussion: This section includes the interpretation of the results. It is a contextual analysis of the data explaining its meaning in sentence form. It should be in an organised manner from general to specific. Your findings are to be linked to the literature. It should also be converted to theory, then to practice if appropriate. Results from other studies can be compared. If it is not consistent possible reasons can be explained. Limitations of your study has to be revealed. So that reviewers and readers understand that you have considered your experiment's weaknesses. If there are inconclusive results that also can be explained. Additional experiments needed, can also be suggested.

In core, discussion is nothing but what your results may mean for other researchers in the same area, other areas and also the general public. Can your findings have an application? How do you relate the findings with previous studies? These are also a thought to be added in the discussion.

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After restating the research topic its importance can be summerisedin one sentence. The thesis of the research can be put up next.

Even though you write same matter that was mentioned in the introduction, the wording should be different. Main points of your paper have to be summed up, next. Main points of your arguments with their significance can be stated. The conclusion should offer a new insight and creative approaches for framing another research problem based on the results of your study.

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Due acknowledgement has to be given to all those who helped the author intellectually, academically or professionally. In certain occasions credits for images are also to be given.

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b. Conflict of interest declaration

c. Author agreement

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AIMS AND OBJECTIVES

Aim of ĀRYAVAIDYAN is to promote research in aayurveda and allied subjects by disseminating the outcome into the scientific fraternity. The journal wishes to inculcate scientific writing especially among *aayurveda* scholars. It will promote incorporation of current knowledge with *aayurveda* to make it in tune with times. Each issue of ĀRYAVAIDYAN is expected to reflect the new developments in the fields of *aayurveda* and related knowledge systems. Documentation, validation and dissemination of principles and practices of Kerala school of Ayurveda is also an area of special interest to ĀRYAVAIDYAN. Journal will promote originality and innovation in scientific work. Documentation of evidence based *aayurveda* is the principle aim of the journal. ĀRYAVAIDYAN will not support any kind of malpractices in publication.

Objectives: The journal is devoted to publish the issues in the months of January, April, July and October with peer reviewed conceptual and clinical research articles on *aayurveda* with its different branches ie. *Kaayacikitsa, Kaumaarabhṛtya, Maanasarogacikitsa, Śaalaakyatantra, Śalyatantra, Agadatantra, Rasaayana* and *Vaajeekaraṇa*. The foundational systems of *aayurveda* like *dosha-siddhaanta, Dravyaguṇavijnaana, Śareeravijnaana, Bhaishajyakalapana, Rasaśaastra, Pancakarma, Svasthavṛtta, Padaarthavijnaana* are also considered.

Works from allied Indian knowledge systems like *Nyaaya, Vaiśeshika, Saangkhya, Yoga, Vedaanta* and *Poorvameemaamsa* in relation to aayurveda is included in the journal. Information from other ancient branches of Sanskrit knowledge systems like *Hastyaayurveda, Aśvaayurveda, Vṛkshaayurveda, Vaastutantra, Aagama,* etc. if scientifically interpreted may be considered for publication. Outcome of literary researches related to *aayurveda* texts so as to enrich the study of evolution of *aayurveda* will also be accepted for the journal.

Research articles related to drug production like processing, quality assurance etc. and plant related studies in botany, biochemistry, pharmacognosy, phytochemistry, pharmacology, ethnobotany survey of medicinal plants, nutraceuticals, drug standardization, biotechnology, agriculture, horticulture, pharmacovigilance, etc. are important to the journal.

ĀRYAVAIDYAN will provide an inter-disciplinary platform for linking traditional knowledge with the current one. Types of articles include original research articles, review articles, short communications and case studies and book reviews. Finally the articles should be illuminating to*aayurveda*. It also should be educative. Clinical interest is given more stress.

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