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Original article

Nano-formulation of herbo-mineral alternative medicine from *linga chenduram* and evaluation of antiviral efficacy

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ABSTRACT

Traditional medicine is becoming a primary source of health care in many countries in recent years. The current study proposes a new dimension of understanding a traditional origin treatment, using herbo-mineral preparations in nanoform. The herbo-mineral preparation, *Linga chenduram* [HMLC], was prepared according to the ancient palm script protocol dates back to 1000 years. In search of alternative therapy for the coronavirus, an attempt was made to determine this ethnic medicine formulation's therapeutic potential for viral hepatitis infection. The Hepatitis C virus [HCV] has several genomic similarities with SARS-CoV-2 viruses. The herbo-mineral formulation (HMLC) were analyzed using UV–vis, EDAX, FTIR, XRD, SEM, and TEM studies. SEM images confirmed the ' presence of nanoparticles with agglomerated conditions having an average grain size of 18 to 25 nm. EDAX studies showed the presence of metallic components in oxide or sulfide form in HMLC. The HCV inhibitory effects of HMLC indicated a good response. The cytotoxicity of this preparation against the Huh-7 human hepatoma cell line was significant. The HMLC showed a strong inhibitory effect on HCV replication in a dose-dependent manner. The genomic component of HCV is similar to COVID −19 virus. The Hepatitis C virus (HCV) NS3/4A protease has a striking three-dimensional structural similarity to the SARS-CoV2 M^{pro} protease, particularly in the arrangement of key active site residues. So HMLC can be tried to treat coronavirus infection. At higher concentrations, HMLC exhibited over 100-fold inhibition. In the MTT assay, HMLC did not show any apparent cytotoxic effect on cell viability at the concentrations 1–100 μg. Histological studies indicated that the liver and kidney did not experience any toxicity by 7 and 15 consecutive days of administration of HMLC on experimental Wistar rats. Hence, the HMLC can be tried as a therapy for COVID −19 infections using the preparations strictly according to ethnopharmacological protocol and optimum doses.

1. Introduction

The traditional healers in South India are taking the technology of herbo- mineral preparation from ancient citations of saint Agasthiar, Bohar, and others as a therapy for the present-day illness. Even some thousands of years back, the saints learned the art of preparing metals based medicine in nanoformulations using herbal treatment. In the traditional Siddha system of medicine, several herbo-mineral formulations are used as Parpam (oxide form of mineral/or metals), Chenduram (sulfide form of mineral/or metals), Chunnam (a caustic oxide preparation) and Pathangam (a product of sublimation). Parpam and Chenduram types of drugs are highly effective, and the shelf life of parpam and chenduram is more than 100 years and 75 years, respectively (Rama Devi *et al,*. 2019).In Siddha medicine, metal and minerals are predominantly used. Metals possess longer shelf life, greater efficacy with a little dose, and the potential therapeutic efficacy. The purification of these metals in the Siddha system of medicine cuts toxicity and enhances effectiveness. But due to the myth of metal toxicity among the public, their clinical usage is minimal. And also, minimal scientific validation has been carried out on these formulations: Parpam (mineral/metallic oxides), Chenduram (mineral/metallic sulfides), Chunnam (caustic or major oxides) and Pathangam (sublimation). Among them, Parpam and Chenduram types of

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medicines are widely used, having potential therapeutic values2. Parpam retains their potency (shelf life) for 100 years and Chendhooram for 75 years (Rama Devi et al., 2019). In Siddha medicine, metal and minerals are predominantly used. Metals possess longer shelf life, greater efficacy with a little dose, and the potential therapeutic efficacy. The purification of these metals in the Siddha system of medicine cuts toxicity and enhances efficacy. However, due to the myth of metal toxicity among the public, their clinical usage is minimal. Moreover, minimal scientific validation has been carried out on these formulations. A traditional Siddha medicine formulation, "Visha sura kudineer" was reported to possess an excellent pharmacological property, including antiviral properties (Shailaja et al. 2017).

A metal is toxic at the macro level, but when it is reduced into nano-sized particles and the appropriate dose is taken, it does not harm the consumer (Devanesan et al., 2020). It does not get accumulated in their body (AlSalhi et al., 2016). The art of transforming raw metals into nano-size particles is exciting. With modern scientific advancement, researchers are trying to determine the particle size of the metals present in different herbo-mineral preparation. Studies on metal-based herbal remedy used as an ethnic medicine for treating infectious and other chronic diseases have given adequate attention in recent years (Alfuraydi, et al., 2019; Devanesan et al., 2020; AlSalhi et al., 2019). With the help of modern analytical tools X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Infrared Spectroscopy (I.R.) and Transmission Electron Microscopy (TEM) and other analysis, the bioactive components in the drugs are easily traced.

The Siddha herbo-mineral preparations exhibit a good antibacterial activity (Razak et al., 2020). One of Shilajit's traditional herbs-mineral preparations is a good antiviral agent. The plant materials used in traditional medicine are available in hilly areas freely so the treatment cost is less than modern medicine (Mann et al., 2008). Herbal formulation made from five plants called Deva-5 effectively inhibited influenza A virus H3N8 at a dose level of 0.5–1% in *in-vitro* testing (Oyuntsetseg et al., 2014). The herbal plants synthesize many secondary metabolites that act as potent antimicrobials, pesticides, and many pharmaceutical drugs. Different types of viral infections are pandemic, and there is an urgent need to contain the viral infection. Because of frequent genetic changes, it isn't elementary to develop a vaccine or antiviral drug. So adequate antiviral chemotherapeutic agent preparation is challenging. Currently, the corona viral infection is one such problem to eradicate or treat using much modern medicine. Although significant progress has been achieved in developing antiviral drugs in recent years, lacunae still exist to treat many recent viral epidemics. Many of the newly developed drugs are analogs of nucleoside-like thymidine kinase-dependent viral enzyme inhibitors. As an alternative medicine for bioactive compounds in natural products, it can be researched to find potential antiviral drugs based on different in vitro and in vivo approaches (Velmurugan et al., 2013). A herbo-mineral formulation, Sarva Noi *Linga Chenduram* was beneficial for urolithiasis (Punitha et al.,2017). Several plants used in the traditional Siddha system of medicines are good antiviral agents because of their phytochemicals, such as the flavonoids, terpenoids, lignans, proteins, and peptides, etc. Many phytochemicals inhibit viral replications (Sathya et al., 2014). A herbal formulation, Nilavembu kudineer, effectively treats CHIKV and DENV-2 viral infection and research is going on in this aspect. The traditional medicine drug's action will be effective if the cellular availability of drugs is high (Jain et al., 2020). Ben-Shabat et al., (2020) identified many herbs to contain good antiviral agents. They stressed the need to find out smart pharmaceutical nanotechnologies to take antiviral phytochemical drugs to target intracellular sites and follow specific molecular signals to release them. As Najjari et al. (2015) found, the extracts of the fruits of fig and olive and selenium nanoparticles effectively inhibited the avian influenza virus subtype. The herb Panax's root extract is not a ginseng enhanced interferon- and natural killer cell-mediated immune response to inhibit the influenza viral infection. It is reported that the herbal juices are capable of reducing the metals in to nanoparticles and the nanosized metal particles promote health effects. (Arun Sudha et al., 2009). The nanonature of metals and plant bioactive compounds enhance the usefulness of herbo-mineral drugs to treat cancer (Sheikh et al., 2012). The toxic macro or micro-particles of heavy metals are converted to nontoxic nanoparticles by herbal extract treatment (Dasgupta and Hammett-Stabler, 2010).

Although the herbo-mineral preparations are effective antiviral drugs, it needs a thorough standardization as modern drugs (Meenadevi et al., 2010). In order to overcome these difficulties, a novel attempt has been made to standardize the Siddha drug by using modern techniques in the present study.

A herbo-mineral formulation 'Linga Mathirai' prepared using Poondu (Allium sativum), Venkaram (Borax), and Lingam (Red sulfide of mercury/Cinnabar) were reported to exhibit an excellent antiviral and antibacterial activity. Cinnabar or quicksilver used in the preparation of HMLC contains mercury and sulfur and it is a good antibacterial and antiviral agent (Savarimuthu Michael et al., 2011; Jiji Mol et al., 2014; .Elansekaran et al., 2016].. There is a general perception in the western world that the use of metal-containing remedies is unacceptable. But these preparations are used since ancient times with safety (Mao and Desai, 2019). The question of safe practices has to be addressed before commercialization. Despite its richness in medicinal value, many people are still afraid of using this herbo-mineral preparation due to metal toxicity. However, the herbo-mineral practice *Linga Chenduram* (HMLC) is used in Indian Siddha medicine for treatment over several thousand years with no side effects. In the current study, experiments are to be conducted to determine its efficacy against an RNA-HCV virus. The study's outcome can be used to evaluate its inhibitory effect on another RNA virus, SARS-CoV-2 virus, with similarities with HCV.

2. Material and methods

2.1. Purification and detoxification of ingredients before drug preparation.

2.1.1. Lingam (Natural cinnabar (or) Lingam (or) Virmilion)

Alangium bark (*Alangium savlifolium*) −1400 g powder was added with vinegar 5.2 l and placed in dews in the night. After one day, it was crushed further. Thirty-five grams of cinnabar was taken in a small cloth bag and put in the above liquid taken in a mud pot. This pot was left inside another pot, and this part was a cloth loaded with mud. After a day, the content in the pool was dried and exposed to do for one day. It was then heated with low-intensity wood fire, and the liquid was fully dehydrated for 24 h. After this processing, the cinnabar was taken out of the pot and cleaned well. This procedure was repeated using the vinegar and powder of the whole plant *Vitis lanata* (Puli karunai) and Indian Sarsaparilla root to get purified cinnabar (Thiagarajan and Anandhan, 2008).

2.1.2. Preparation of Linga Chenduram

The following ingredient's used for the Preparation of *Linga Chenduram*

Cow's ghee 7 g; Saerangottai (*Semecarpus anacardium*) −7 g; Valuluvai arisi (*Celastrus paniculatus)* −7 g; Lingam (*cinnabar*)- 21 g; White arsenic- 7 g; Kasthoori (Secretions from musk deer)

– 3.5 g; Saffron (*Crocus sativus Linnaeus*) 3.5 g; Ginger juice (*Zingiber officinale* Rosc)- as required and Lemon juice (*Citrus Limon* – *Rutaceae*) as required

At first, the Saerangottai and Valuluvai arisi and were ground together and mixed well with the Cow's ghee. Then the cinnabar was placed inside the mixture and burnt into the furnace for 12 h. Then 14 g of the above mixture was mixed with the white Arsenic, Kasthoori, Saffron, and ground well in a Kalvam by adding the required amount of ginger juice. The mixture was then dried in the hot sun for 42 h, and again, it was ground into a fine powder. This powder was then grounded well with the required amount of Lime juice and dried in the shade for 36 h (Swamy, 1975). Continuous grinding (trituration) increases the power of the drug. Hence, the herbo -mineral medicines are ground and 'atomized' because they operate powerfully and act rapidly when administered small doses. This means the grinding of minerals and metals into various herbal extracts, which also means the same thing as combining solutions of metals and minerals with tinctures and extracts (De Chane, 2011). The Physico-chemical characterization of HMLC was analyzed using U V spectrophotometer, XRD, SEM, EDX, I.R, and TEM. The results showed that the metals and minerals were in the oxide or sulfide form. The physicochemical characterization of these herbo -mineral contributes to providing useful hints on its therapeutic properties. Proper standardization techniques are to be ensured for checking the quality before use.

2.2. U.V. Spectral analysis

The elements present in HMLC were monitored by measuring the U.V. - Vis Spectrum (Hitachi UV-1700 Spectrophotometer, Japan) after 24 h incubation by diluting a small aliquot of the sample with distilled water. U V –VIS spectral measurements were made in the wavelength range from 250 to 800 nm with a bandwidth of 1.0 nm and 120 nm.

2.3. XRD (X-Ray Diffraction) analysis

The characterization of HMLC was carried out using X-ray diffractometer. Powder XRD studies of the solid samples were recorded from Philips 1710 x-ray diffractometer using CuKα radiation (ƛ-1.5405 A^0) filtered by a nickel foil over the range of diffraction angle 10 – 70^0 operating at 30 kv and 20 mA. The pattern was recorded for the angle (2 θ) ranging from 5 –80 degrees at a scanning rate of 3 degrees/second and the crystal size was calculated using Scherrer equation. $t = \frac{\lambda 0.9}{\beta} \times \cos\theta$

2.4. SEM with EDAX analysis

The prepared HMLC drug was characterized for its size using a Scanning Electron Microscope (JEOL-JSM 6390, Japan). EDX (Energy Dispersive X-ray) analysis of these medicines was carried out using the same instrument to confirm the sample's elemental composition.

2.5. FTIR (Fourier transform Infrared) analysis

Infrared spectroscopic analysis of HMLC was done. From the spectrum, the functional groups were identified and correlated with the drug's curative nature.

2.6. TEM (Transmission Electron Microscope) analysis

The TEM images of HMLC were taken by Philips Technai-10 equipment. TEM was used to confirm the presence of nanoparticles.

HCV inhibition assay

For effective antiviral treatment in HCV infection, Interferon-a (PEG IFN- α) combined with ribavirin (RBV) is used. However, it has specific side effects, and the response was slow in patients infected with HCV genotypes 1a and 1b (Feld and Hoofnagle, 2005; Zeuzem et al., 2000). Several antiviral inhibitors are showing a promising approach against HCV.JFH-1, a genotype 2a HCV strain based on intra-genotype 2a chimeric HCV was used to study viral replication (Arumugaswami et al., 2008). A human hepatoma (Huh) based cell line was used for propagating HCV. The Huh cells were pretreated with various drugs for 1 h and were infected with *Renilla* luciferase, reports HCV. The infected cells were incubated with drugs for an additional 48 h. The cells were lysed to measure *Renilla* luciferase activity. Clean DMSO (drug vehicle) was included as a control. The percent of infectivity was obtained by calculating the HCV replication ratio between drug-treated and DMSO. The mean and standard error of percent inhibition in three observations of HCV replication ratio was calculated for the different doses of HCV

2.7. MTT cell proliferation assay for drug cytotoxicity

The MTTassay (3,-(4,5-dimethyl thiazolyl-2)- 2,5-diphenyl tetrazolium bromide) was performed to determine the toxicity of the drug. The human hepatoma cells (Huh-7) were plated at $3x10^3$ densities in each well of 96 well plates (Gonzalez et al. 2009). Twenty-four hours post-plating various concentrations of selected Siddha drugs were added to each well. The experiment was done in duplicate. The cell viability and cell proliferation were measured using MTT assay (ATCC), used according to the manufacturer's recommendation. After Forty-eight hours post-treatment, ten µl of MTT was added to each well, and the plate was incubated at 37 degreesC with 5% CO_2 for four h. Then 100 µl of detergent was added to each well, and the plate was kept in the dark at room temperature for two h. The absorbance of each well was read at 570 nm wavelength using a plate reader. The values were normalized against DMSO (drug vehicle alone) and presented in a bar graph as percent viability. As HCV replication in cell culture is limited to human hepatocytes and their derivatives, several reports showed that HCV could replicate in Huh-7 cells through detection of viral genes and viral copy number by real-time PCR in both cells and supernatants (Molina et al., 2008).

2.8. Histological changes in liver and kidney tissue due to the usage ofHMLC

Generally, herbo-mineral preparations are rejected by several people thinking that it contains toxic metals. If the Indian medicines are appropriately prepared, the metals' toxicity is nullified, and the phyto-detoxified metal in nano form does not give any histopathological changes in the organs. Hence an attempt has been made to study the histology of vital organs like liver and kidney in rats, which were treated with selected HMLC chronically.

2.9. Animals:

For animal studies, male albino rats of Wistar strain (180 – 200 g) were used. According to the guidelines of CPCSEA, the animals were maintained under the Animal Ethics Committee's supervision [1949/P.O./Re/S/17/CPCSEA, Prathyusha engineering college Thiruvallur]. The experimental animals were acclimatized to laboratory conditions before use and fed with pelleted chow (supplied by Poultry Research Station, Chennai) and water *ad libitum*.

2.10. Histopathological studies

The animals were divided into three groups, each with 6 rats. Group, I was treated as control. For the other groups, the drug HMLC suspension in 1% carboxymethyl cellulose was orally administered in doses of 10 and 20 mg/kg/bodyweight for 60 days. The studies' doses were chosen based on the preliminary toxicity report of (Ilango et al., 2009). After 31st and 61st days, during the control of drug-treated animals, liver and kidney were removed, washed with 1% ice-cold saline, and fixed in neutral buffers with 10% formalin.

2.11. Tissue fixation

The tissues were processed following the procedure (Pearse, 1985). The liver and kidney tissues were fixed in Zenker's fixative. The tissues were fixed in the fixative for 12 h and washed in the running tap water overnight, and dehydrated in isopropyl alcohol's ascending grades. Two changes in absolute alcohol were followed by clearing in methyl benzoate. The tissues were then left in 1% celloidin dissolved in methyl benzoate. The celloidin in filtered tissues was left in toluene till they became translucent. After moving toluene, the tissues were in filtered with paraffin wax on melting point 56-58°C. Three wax changes, totaling 90 min, were sufficient for infiltration. The tissues were then embedded in wax. Thus double embedding in celloidin wax was employed.

Using a rotary microtome sections were taken from the tissues embedded in wax. The sections were floated on water poured over albumenized slides. The sections were allowed to expand by gently warming the slide on a hot plate maintained around 50°C. When the sections became flat and embedded, the water was drained. The slides were left overnight on the hot plate maintained at a constant temperature of 45°C. The sections were stained by monochrome, bichrome, and Heidentain's iron hemotoxylin to get cellular and sub-cellular structural details. In the dichromate method, Weighert's iron hemotoxylin and Biebrich scarlet stains

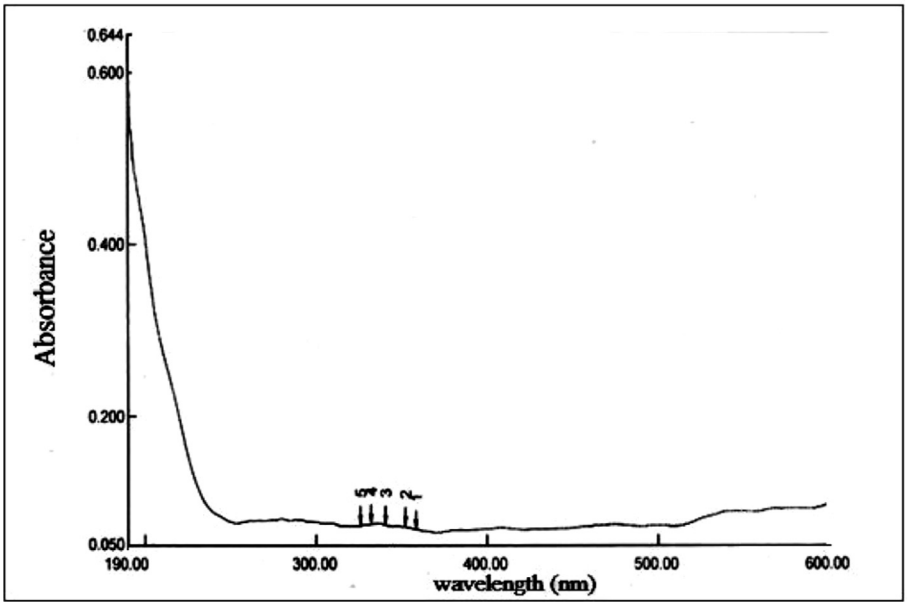

Fig. 1. U.V. Spectrum of HM LC.

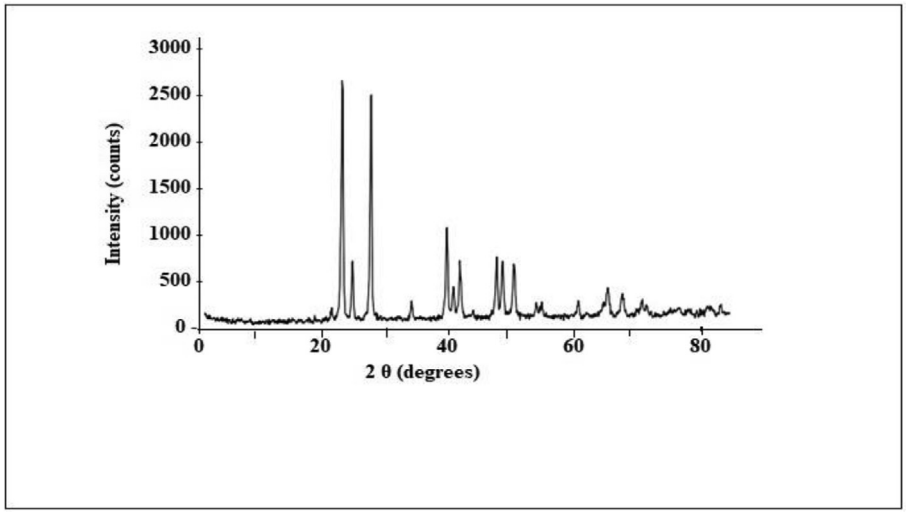

Fig. 2. XRD pattern of HMLC.

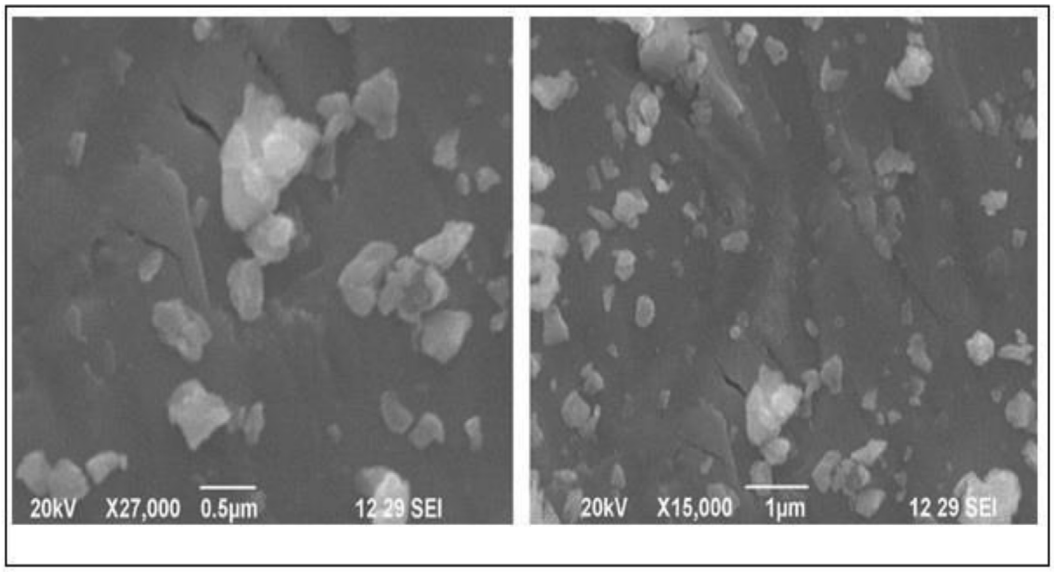

Fig. 3. Confirming the elemental composition of HMLC by SEM.

Table 1
EDAX analysis of HMLC.

Element Present	HMLC	
	wt (%)	At (%)
C	8.2	45.32
N	–	–
O	0.34	1.40
Mg	–	–
Al	0.15	0.37
Si	–	–
P	–	–
S	11.92	24.50
Cl	0.16	0.29
K	–	–
Ca	0.12	0.20
Fe	0.12	0.14
Cu	0. 88	0.91
As	0.10	0.09
Mo	2.77	1.90
Ag	0.48	0.29
Ba	0.25	0.12
Hg	74.44	24.45

were used. The section was deparaffinized in xylene and rehydrated with descending grades of isopropyl alcohol. As mercuric chloride was used in Zenker's fixative tissues, it was passed through 70% alcohol with 0.5% iodine to remove mercury precipitates from the tissues. The tissues were further treated for 5 min and left in distilled water until they were stained. The stained sections were rapidly dehydrated in ascending grades of isopropyl alcohol or acetone. The latter proved to be a better dehydrating agent, as it was gentler to most of the stains. After two absolute alcohol or acetone changes, the sections were passed through 1:1 acetone – xylene/alcohol-xylene and cleared into xylene. The sections were mounted in DPX mountant and microphotographs were taken.

3. Results

U V-vis spectrum of the HM LC exhibits a peak absorption band at 462 nm. The UV–vis spectrum showed that there is no absorption band between 360 and 600 nm. The present study illustrates that HMLC has good transparency in the visible and near-IR regions. From the U V –vis absorption studies, it can be inferred that HMLC has good transparency in the visible region. The U V spectrum of HMLC indicates five peaks at 358.40, 352.10, 340.20, 331.90, and 325.80 nm confirming the nanosized (Fig. 1).

The average grain size of the HMLC nanocrystal was calculated and it was 18 nm to 25 nm, respectively. XRD pattern (Fig. 2) of HMLC showed peaks due to mercury oxide and sulfide.

SEM images of HM LC (Fig. 3) showed a difference in the particles' size and agglomeration as reported. The SEM images of *Linga chenduram* confirm the size difference and aggregation of the particles. The elemental composition of sample HMLC is presented in Table 1. Carbon, mercury, and sulfur were found common in HMLC. Further, it is interesting to note the presence of molybdenum (Mo), copper (Cu), iron (Fe), calcium (Ca), chlorine (Cl), oxygen (O), carbon (C), nitrogen (N), and silver (Al) in the sample. From this analysis, it is confirmed that 74.44% Hg present in HMLC is equivalent to 92.63% mercury sulfide (HgS), potassium (K), arsenic (As), iron (Fe), and mercury (Hg). Other major elements present were carbon, nitrogen, and stoichiometric excess oxygen. The presence of large amount of carbon was due to repeated cycles of calcination in the presence of plant juices. The presence of carbon would be responsible for the positive nature of the oxide. It may decrease its toxicity and, on the contrary, impart the therapeutic value to the mercury and sulfur oxide, thus prepared. However, detailed systematic investigations are warranted to ascertain the role of carbon and nitrogen present in HMLC.

The IR spectrum of HM LC (Fig. 4), showed 13 peaks of absorption in different wavenumber (cm^{-1}) (3313.48, 3197.76, 1674.10, 1456.16, 1402.15, 1336.58, 1191.93, 1122.49, 810.05, 752.19, 655.75, 601.75 and 462.88 cm^{-1}). The absorbed frequency (cm^{-1}) and the respective probability functional group are represented in Table 2. The main functional groups present in the HMLC are alcohols, phenols, amines, amides, carboxylic acids, alkenes, alkanes, aromatic, nitro compounds, alkyl halides, aliphatic amines and alkynes.

The TEM study of HMLC showed that the particles in HMLC are in the nano range (Fig. 5) and the particles are not of uniform size. However, spherical shaped particles are more in number, and other particles are trigonal, cubic, and hexagonal. Particles are in the range of 9.71 to 18.40 nm. The results contribute to the standardization of the traditional drug specifications and provide useful hints on its therapeutic properties.

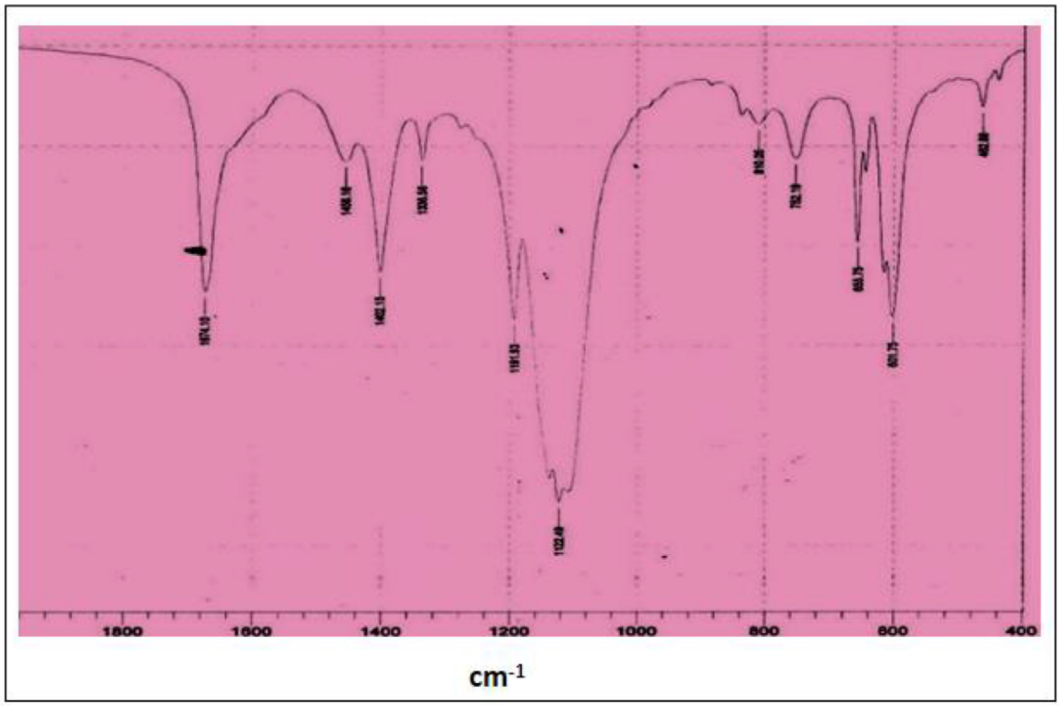

Fig. 4. Functional groups analysis of HMLC using FTIR.

Table 2
Absorption, Probable Bond and Probable Functional group of HMLC.

S.No	Absorption cm^{-1} (LC)	Probable Bond	Probable Functional group
1.	3313.48	O—H stretch H— bonded N—H stretch	Alcohols, Phenol 1°, 2° amines Amides
2.	3197.76	O—H stretch	carboxylic acids
3.	1674.10	—C═C— stretch N—H bend	Alkenes 1 degrees amines
4.	1456.16	C—H bend	Alkanes
5.	1402.15	C—C stretch	Aromatics
6.	1336.58	N—O symmetric stretch C—N stretch	Nitro compounds Aromatic amines
7.	1191.93	C—H Wag (—CH_2 X) C—N stretch	Alkyl halides Aliphatic amines
8.	1122.49	C—N stretch	Aliphatic amines
9.	810.05	N—H Wag	$_1$oo , 2 amines
10.	752.19	C—H —OOP‖ C—Cl stretch	Aromatics Alkyl halides
11.	655.75	—C≡C—H: C—H bend	alkynes
12.	601.75	C—Br Stretch	Alkyl halides

. The present research on the anti-HCV effect of HMLC and its cytotoxicity can help to design drugs with improved specificity and activity. HMLC strongly also inhibits HCV growth in a dose-dependent manner. HMLC at higher concentration exhibited over 100 fold inhibition on HCV (Fig. 6), and the cytotoxicity analyses (Fig. 7.) exhibited the cell viability after drug treatment. The traditional medicine HMLC to Huh-7 cell culture resulted in dose-dependent cytotoxicity after 24 h exposure, and no cytotoxic effect on cell viability was observed in the tested concentrations (1 and 100 μg).

Microphotograph of control Wistar rat liver Fig. 8 (Plate1) is showing normal architecture. Rich Secretary (RS) activity is seen. Hepatocytes are normal. A nucleus (N) is prominent in hepatocytes. Sections of the liver of Wistar rat treated with LC-1 for 30 days showing no marked changes in liver cells' histology (L.C.) and secretions (s) are standard. The liver of Wistar rat treated with LC-1 for 60 Days shows marked changes in the liver. Hepatocytes (H), Secretions, and Nucleus (N) are normal. Rich hepatic circulation was seen. The liver of Wistar rat treated with LC-2 for 30 and 60 Days shows that secretary activity, Kupffer cells (K), and sinusoids (S.S.) are standard. Portal areas (P.A.) and central venus system (CV) are also normal.

Section through Kidney of Wistar rat treated with LC-1 for 30 and 60 Days in Fig. 9 (Plate 2) shows a histological structure similar to the rat's kidney's normal histology. Capillary lumen (CL) and basement membrane (B.M.) is standard. A rich supply of blood is seen in the capillary epithelium. The glomerular filtration is functioning well, and it is proved by the filtered particles and the elimination of HM LC nano particles. This indicates that the kidney can filtrate the HMLC. The nucleus of blood cells and urinary spaces are normal. This indicates that HMLC does not damage the kidney even after 30 days of treatment. In the 60 days of treatment, evidence showed that had led to mild cystic problems and renal displacia were developed. Disorganized architectures like dilated tubule (D.T.) and immature cartilages (I.C.) are seen. The immigration of white cells into the lumen is seen. Haemorrhage is seen in the kidney. However, the phagocytosis (P C) of L.C. nanoparticles is also seen. In some of the cells, the nuclei are hypertrophied and fragmented (F). But the circulation of blood is not much affected. In Bowman's capsule (B C), a crescent-like structure is seen. Haemorrhage is seen inside the glomerular cells in a certain region. This result indicates that prolonged exposure of HMLC may affect the histology of the kidney. The microphotograph of the Wistar rat kidney treated with LC-2 for 30 and 60 days shows no marked changes in the 30 days of treatment. But the 60 days of treatment shows glomerular sclerosis and lipoid necrosis. The basement membrane is loosened. Messengial hyperplacia is also seen. Histopathological studies on the impact of the HMLC-mineral drugs treated Wistar rat showed that the treatment of 30 days does not damage the kidney function. However, chronic exposures lead to some changes.

The present observations also reinforced the importance of strict adherence to traditional medical practitioners' prescriptions in dosage and duration while using these metallic preparations. Any over-usage or self-medication may invariably lead to adverse reactions, as mentioned in the old literature. The study indicates that the liver and kidney did not produce any toxicity by 7 and 15 consecutive days of administration of HMLC. Nanomedicines on experimental Wistar rats. These herbo-mineral nanomedicines can be made functional if the doses are shallow and not chronically administered.

4. Discussion

The powder X-Ray Diffraction (PXRD) commonly used in the pharmaceutical industry for checking drugs was used to study the structure of HMLC (Sudhaparimala et al., 2011). The crystallite size was calculated using the Scherrer equation.

$$t = \frac{\lambda 0.9}{\beta} \times \cos\theta$$

Where t is the crystalline size for (h k l), plane, λ is the wavelength of the incident X radiation [CuK_α (0.1542 nm)], β is the full width at half maximum (FWHM) in radians, and θ is the diffraction angle for (h k l) plane (Anoop Austin, 2012).

Due to repeated cycles of calculations, the particles' agglomeration occurs (Arun Sudha et al., (2009). EDAX analysis to study the chemical form of the elements (Arvelakis and Frandsen, 2005) indicated the presence of mercury (Hg) and sulfur (S) in nanoform. So far, there appears to be no reference for using HgS in nanoform in traditional medicines.

FTIR is an important tool to identify the nanoparticle's different types of chemical bonds (Ravindra, 2009). FTIR spectra of the HMLC samples showed that they do not contain organic compounds. The absence of organic matter confirms proper incineration. This is in agreement with the earlier infrared study of

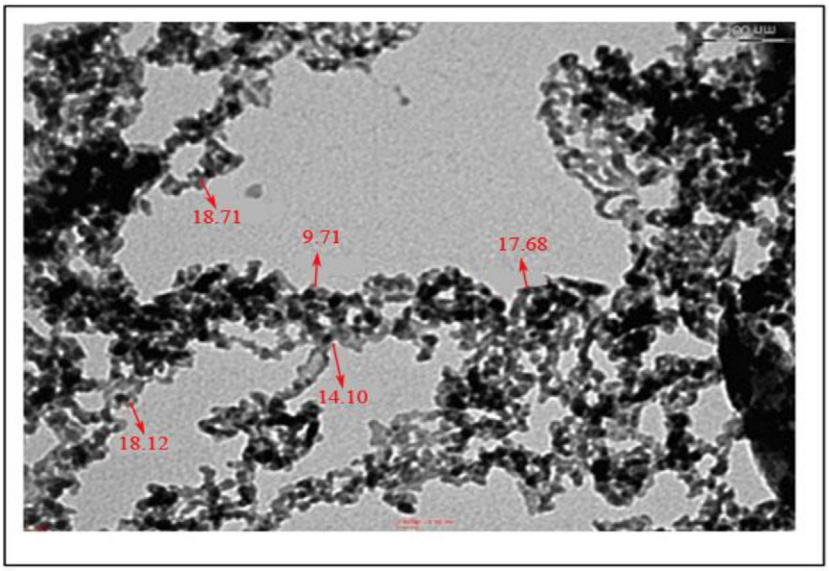

Fig. 5. Confirming the presence of nanoparticlesof HMLC by TEM.

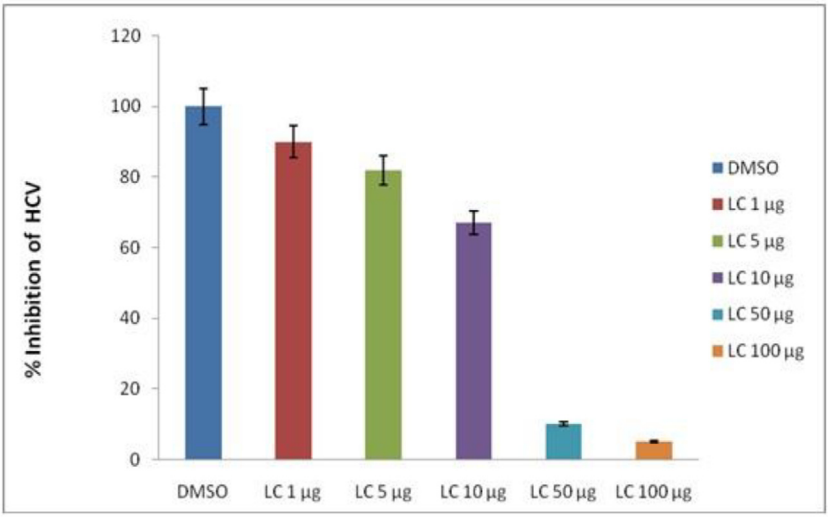

Fig. 6. Anti-HCV activity of HMLC showing the dose-dependent inhibition of HCV replication.

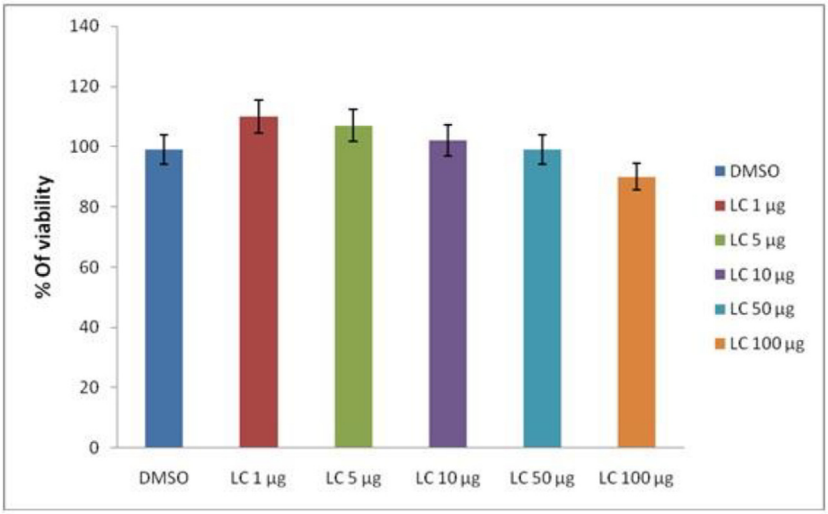

Fig. 7. The cytotoxic effects of HM LC on human hematoma cells (Huh-7) determined by the MTT assay. Huh-7 cells were exposed to L.C. (1–100 µg) for 24 h.

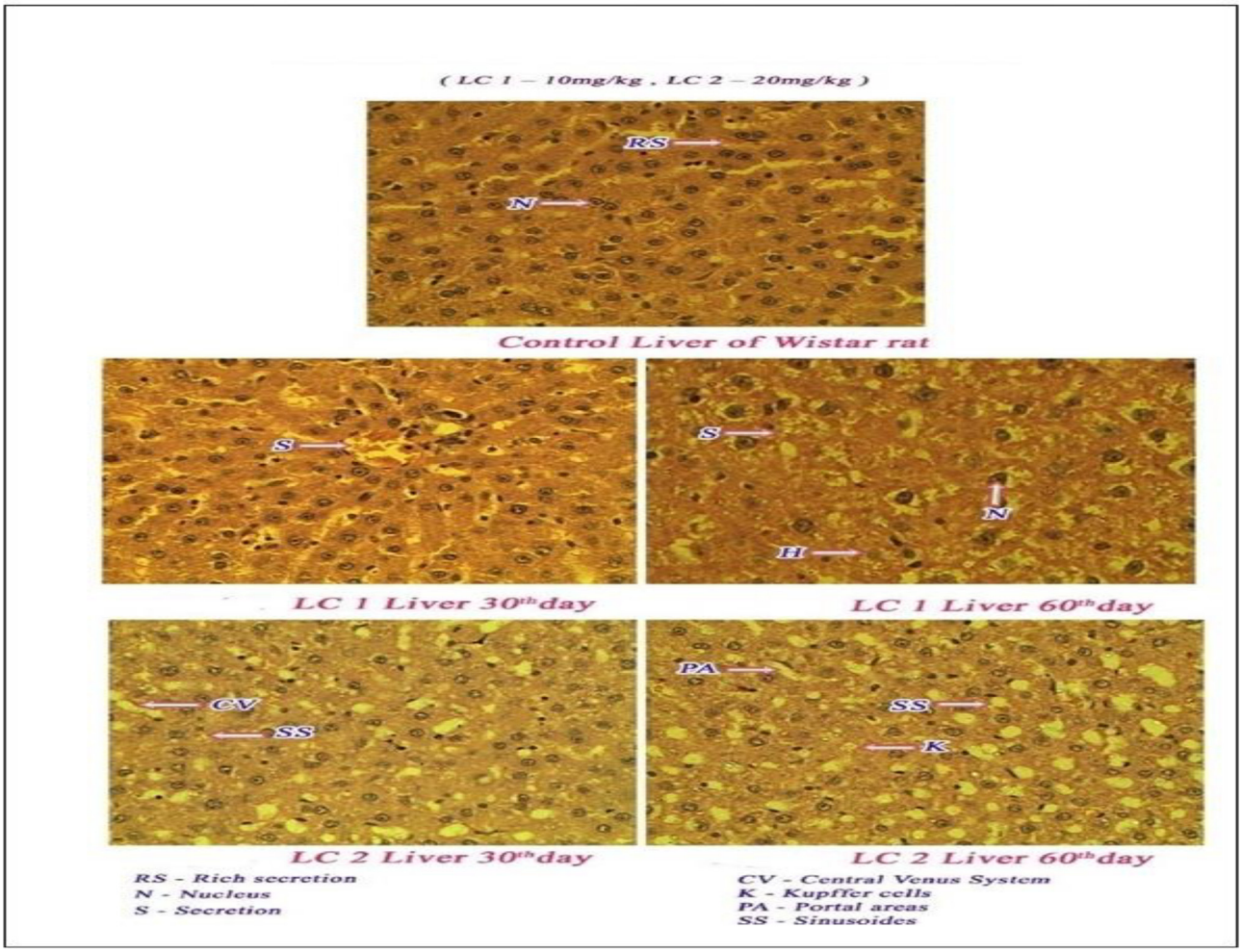

Fig. 8. Photo micrograph showing the histology of control Wister rat liver and treated with LC-1 and LC-2.

Bhasmas (Brown et al., 2007). I.R. Spectrum of PCC (Poorna Chandrodaya Chenduram) is similar to *Linga Chenduram* (Arun Sudha et al., 2009). The peak at 592 cm^{-1} in Ks Kc (Kshaya Kulanthaga Chenduram) is due to Fe_3O_4 (Ma et al., 2007). The broad peaks at 3400 and 1600 cm^{-1} in all the samples are due to the characteristic O.H. stretching, H- O.H. bending vibrational bands due to adsorbed

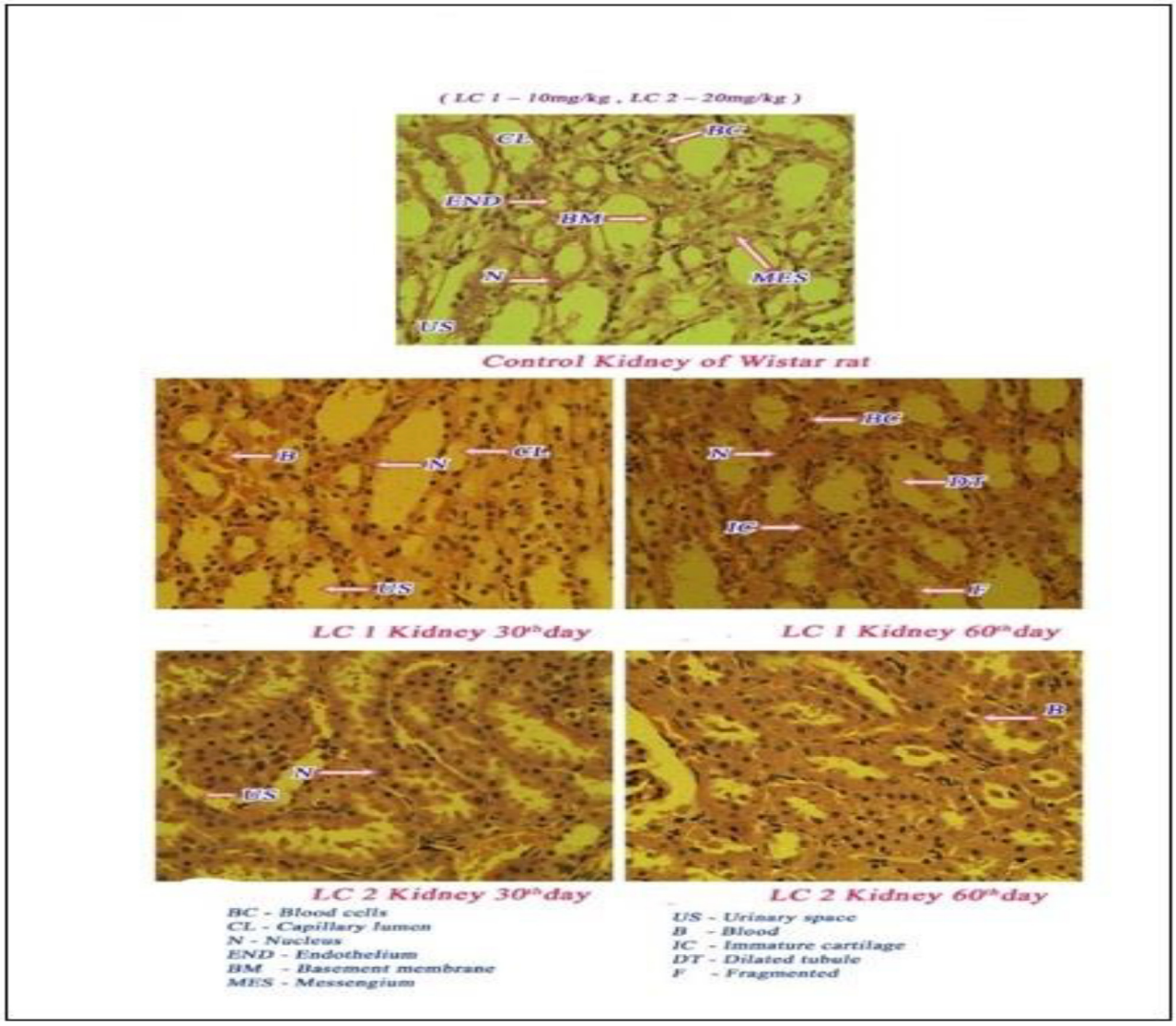

Fig. 9. Photo micrograph showing the histology of control Wister rat kidney and treated with LC-1 and LC-2.

water in the sample (Predoi, 2007). In Indian traditional medicine, Swarnabhasma, globular particles of gold with a size of 56–57 nm promotes its rich therapeutic value (Brown et al., 2007).

Hepatitis C virus (HCV) is a single-stranded RNA virus that belongs to the Flaviviridae family. HCV virus causes acute hepatitis. Chronic HCV infection damages the liver causing cirrhosis and hepatocellular carcinoma (Kim and Chang, 2013). It is reported that Hepatitis C virus (HCV) NS3/4A protease has a striking three-dimensional structural similarity to the SARS-CoV2 M^{pro} protease, especially in the key active site residue arrangement (Bafna et al., 2020). Because of the structural similarity of the chymotrypsin-like protease of SARS-CoV-2 with HCV and HIV proteases, the drug, grazoprevir, a protease inhibitor used to treat HCV infection, has been made to treat COVID-19 patients (Alsofayan et al., 2020; Somily and BaHammam, 2020). The CoVs are positive-sense, single-stranded RNA viruses with nonstructural proteins, structural proteins, and accessory proteins similar to other single-stranded RNA viruses like the hepatitis C virus (Xu et al., 2020). So the HMLC drug that is effective in inhibiting HCV can be given to treat COVID-19 infection.

HCV shares many risk factors for viral transmission. As the drug HMLC was found satisfactory to eliminate HCV, there is a scope to test the possibility of HMLC to prevent corona viral infection. But it needs further study. Current therapy for HCV consists of interferon and ribavirin, which results in 70–80% sustained virologic response (SVR) in patients infected with genotypes 2 and 3, and SVR is significantly lower for genotype 1, at 29–56% (Moore et al., 2004). In India, the available HCV treatment is expensive to afford for many infected individuals. The use of drugs targeting both cellular and viral targets minimizes the evolution of drug-resistant HCV (Arumugaswami *et al .,* 2008). Despite good antiviral drugs, the emergence of resistant viruses is also increasing, and there is an alternative solution for therapeutic failure (Emery, 2008).

Furthermore, many antiviral drugs are toxic and expensive. So there is a need for the development of low cost-effective new drugs. Many traditional herbo-mineral medicines are also evaluated to find potential antiviral drugs (Li et al., 2004; Abad et al., 2000).

Pandey and Sudheesh, 2019, reported that metals are present in the ayurvedic preparations in Sodhit form and leave any objectionable behavior. However, before concluding, there is a need for pharmacovigilance to adopt a standard protocol to ensure the safety and efficacy of ayurvedic preparations.

5. Conclusion

The study proposes a new dimension of understanding of herbo-mineral medicine as ethno-nanomedicine in the surgical era of nanomedicine. The Siddha system of medicine advocates the use of herbs and herbal preparations to treat various ailments. The system frequently employs unique metallic preparations with extracts of herbal juices for curing diseases. The herbo-mineral drug preparation is unique to the Siddha, Ayurveda, and Unani. The physicochemical characterization of HMLC was found to have more number of particles in the nano range. The average grain sizes of nanocrystals are 18 to 25 nm. The inhibitory and cytotoxicity effects of HMLC were examined on the Huh-7 human hepatoma cell line. The HMLC possess a strong inhibitory effect on HCV replication in a dose-dependent manner.

At higher concentrations, all these medicines exhibited over 100-fold inhibition. Cytotoxicity study of HMLC using MTT assay, did not show any apparent cytotoxic effect on cell viability at all concentrations (1–100 μg). A study on the impact of HMLC on the histology of liver and kidney of Wistar rats showed that in the treatment regime for 30 days no damage was seen in the architecture of liver and kidney. However, chronic exposure leads to some changes. So HMLC is safe if the doses were low and not chronically administered to eliminate RNA viral infection. Further study is planned for pharmacovigilance because it is an **advanced** drug monitoring study to determine adverse drug reactions when using herbal, traditional, and complementary medicines.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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