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In Vitro Hepatoprotective Activity and Qualitative Phytochemical Analysis of "Kamalaiku Chooranam" - A Siddha Formulation

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Abstract: ***Liver is the major and important organ which undergoes damages frequently by many causes. Liver has the ability to repair and regenerate by itself. Once it crosses the limit damage can't be reversed so the liver health has to be preserve for that purpose many herbals and herbomineral drug, are used to treat the disease and to protect from diseases before it exists. Jaundice is a very common symptom especially in the developing countries. It is associated with several hepatic diseases which are still major cause of death. There are many different approaches to jaundice treatment; many medicinal plants are used for the treatment of jaundice. The liver performs a vital role in metabolism, secretion, storage and detoxification of endogenous and exogenous substances, oxidative stress and free radicals enhances the severity of hepatic damage which can be overcome by the oxidative mechanism. Plant extracts can be the best source of such antioxidents and mediate hepatoprotective activity. These include "KAMALAIKU CHOORANAM" is the effective remedy for jaundice. Objective: The main objective of the study is to evaluate the hepatoprotective and biochemical activity and the biochemical analysis of kamalaiku chooranam. Methodology: Kamalaiku chooranam has its reference in literature of Anubhava vaiddya murai by C.N. kuppuswami. The present study was undertaken to evaluate hepatoprotective activity of kamalaiku chooranam by using serial dilution of test formulation KMC [50, 100, 200 and 400ug/ml] were prepared using DMSO. Result: The study result was concluded that the drug kamalaiku chooranam has got significant hepatoprotective activity.***

Keywords: Jaundice, kamalaiku chooranam, hepatoprotective activity

1. Introduction

Jaundice is not a disease but rather a sign that can occur in many different diseases. Jaundice can occur in babies, children, and adults. Jaundice is not an illness, but a medical condition in which too much bilirubin a compound produced by the breakdown of hemoglobin from red cells is circulating in the blood .The excess bilirubin causes the skin, eyes, and the mucus membranes in the mouth to turn a yellowish colour. Jaundice is common in newborn babies and will usually clearuo without treatment. However for adults the symptoms of jaundice may indicate damage to the liver. If the cause is not treated, it can lead to liver failure, jaundice is a condition in which yellow discoloration of the skin and mucus membrane occurs due to an increase in the bile pigments, namely bilirubin in the blood. In many cases yellowness in the white part of eyes is more obvious than in the skin. Bilirubinis a byproduct of red blood cells in the body .The hemoglobin molecule that is released into the body by this process is split, with the heme portion undergoing a chemical conversion to bilirubin .Normally the liver metabolizes and excreates the bilirubin in the form of bile. However, if there is a disruption in this normal metabolism and /or production of bilirubin, jaundice may result.

2. Materials and Methods

2.1 Drug Selection

The Siddha formulation Kamalaiku Chooranam taken from the literature of Anubhava Vaiddya Murai by Dr. C. N. Kuppuswami and its indicated for kamalai.the herbal ingredients from faculty of Department of Gunapadam, Government Siddha Medical College, Palayamkottai.

2.2 Ingredients of Kamalaiku Chooranam

Keezhanelli[phyllanthus amarus]…… 2.5 grams
Seeragam[cuminum cyminum]……. 2.5 grams

2.3 Methods of Purification

Keezhanelli
Wash the whole plant and dry it in sunshade and powdered

Seeragam
Take one part of cuminum cyminum srrds and dry it in sunshade and then powdered

2.4 Method of Purification

The above purified ingredients were powdered individually and mixed together and stored in air tight container

2.5 Cell Culture and Maintenence

Chang liver cells, a human hepatocyte cells were obtained from National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in Minimum Essential Media (MEM) supplemented with 10% Fetal Bovine Serum (FBS), with 100units/ml penicillin and 100µg/ml streptomycin. Cells were cultured in 75cm^2 culture flask and incubated at humidified atmosphere with 5% co2 at 37c

2.6 CC14 Induced Hepatotoxicity in Change Liver Cells

Chang liver cells were seeded in 6 well plates at a density of 1X10^5 cells/well and allowed to grow for a period of 24 h. Test drug was administered at a concentration of 50 µg, 100

µg , 200 and 400 µg / ml. Standard silymarin 200 µg / ml for three hour following test drug exposure, 0.1% CCl4 was added to all the wells except control and incubated for a period of 24 h. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line.

$$\text{Survival rate (\%)} = \frac{A_{sample} - A_b}{A_c - A_b} \times 100$$

MTT Assay

The *in vitro* determination of hepato protective effect of the test formulation have been performed by counting viable cells after staining with a vital dye. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component is (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt upon incubation MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. The resulting colored solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material.

2.7 Biochemical Analysis of Kamalaiku Chooranamm

Preparation of the Extract

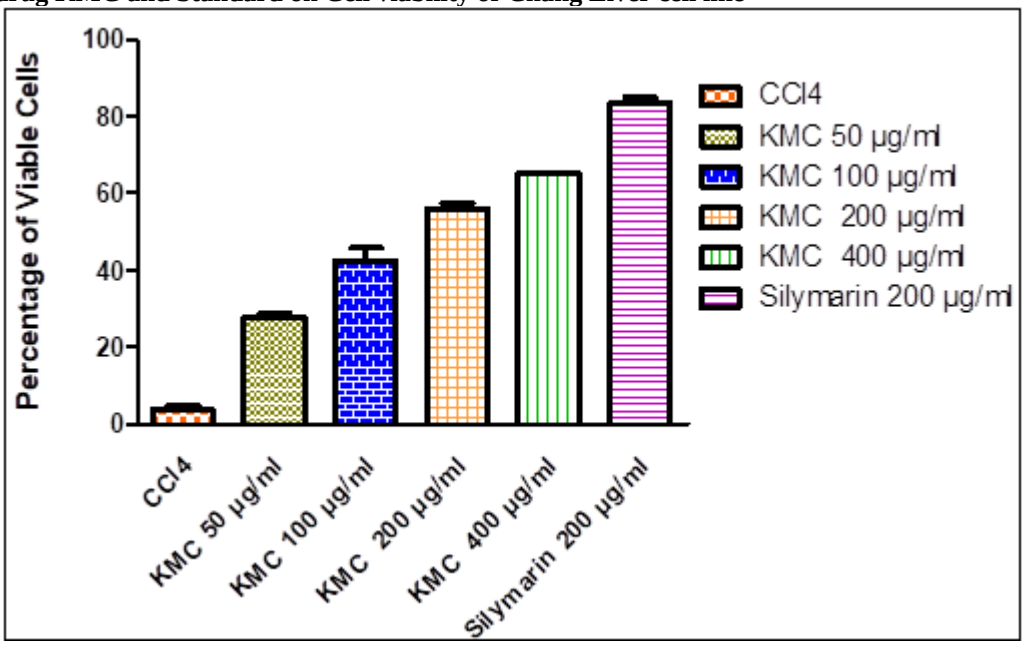
5gms of the test drug was weighted accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes .It is cooled and filtered in a100ml volumetric flask and then it is made to 100 ml with distilled water. This fluid is taken for analysis.

3. Results

Effect of Test drug KMC and Standard on Cell viability of Chang Liver cell line

S. No	Concentration in µg/ml	% cell Viability
1	CCl4 Control	3.8 ± 0.99
2	50	27.98 ± 0.89
3	100	42.54 ± 3.21
4	200	56.28 ± 1.54
5	400	65.96 ± 0.40
6	Silymarin 200 µg	83.21 ± 1.87

Effect of Test drug KMC and Standard on Cell viability of Chang Liver cell line



Phytochemical Analysis

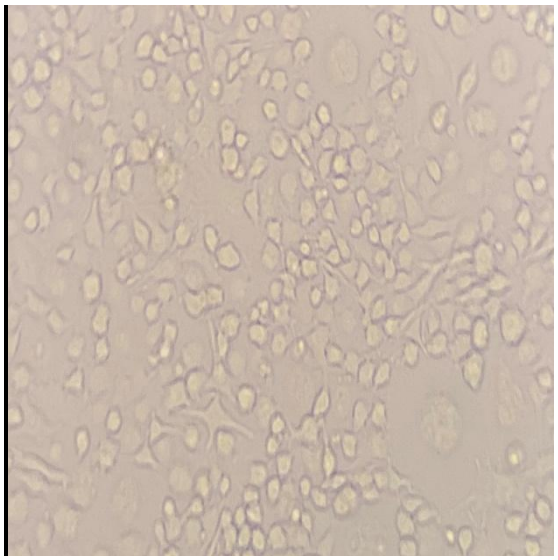
S. No	Procedure	Observation	Inference
1.	**Test for calcium:** 2 ml of the above prepared extract taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution.	No White precipitate is formed	Absence of calcium
2.	**Test for sulphate:** 2ml of the extract is added to 5% barium chloride solution.	A White precipitate is formed	**Presence of sulphate**
3.	**Test for chloride:** The extract is treated with silver nitrate solution.	No White precipitate is formed	Absence of chloride
4.	**Test for carbonate:**	No brisk effervescence	Absence of carbonate

	The substance is treated with concentrated HCL.	is formed	
5.	**Test for Starch:** The extract is added with weak iodine solution.	Blue colour is formed	**Presence of starch**
6.	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium ferro cyanide.	No blue colour is formed	Absence of ferric iron
7.	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	No blood red colour is formed	Absence of ferrous iron
8.	**Test for phosphate**: The extract is treated with ammonium molybdate and concentrated nitric acid.	No Yellow precipitate is formed	Absence of phosphate
9.	**Test for albumin:** The extract is treated with esbach's reagent.	No yellow precipitate is formed	Absence of albumin
10.	**Test for tannic acid:** The extract is treated with ferric chloride.	Blue black precipitate is formed	Indicate the presence of tannic acid
11.	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolourised	**Presence of unsaturated compound**
12.	**Test for the reducing sugar:** To 5 ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes.	No Colour changes occurs	Absence of reducing sugar
13.	**Test for amino acid:** One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	**Presence of amino acid**
14.	**Test for zinc:** The extract is treated with Potassium Ferro cyanide.	No white precipitate is formed	Absence of zinc

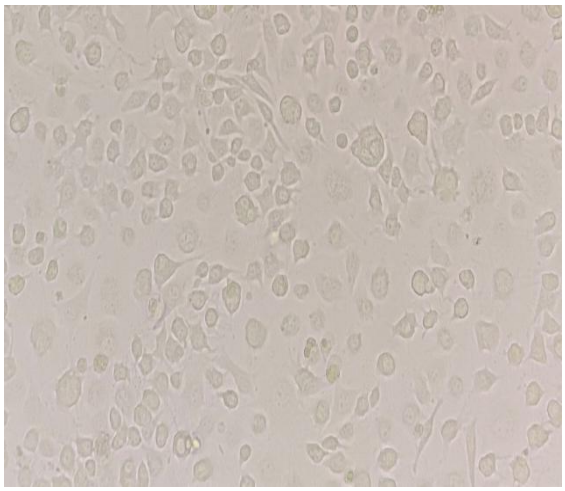
4. Result and Discussion

In-vitro hepatoprotective activity of test drug KMC on the cell viability of human liver hepatocytes (Chang liver cell line) against CCl4 induced hepatotoxicity was performed at varying concentration ranges from 50 to 400 µg/ml .The reuslt obtained from the study reveals that the percentage of cell viability of chang liver celline increases with increase in concentration of the test drug KMC. Highest viability of cell was observed at the concentration of 400µg/ml shows 65.96 ± 0.40 %, followed by this 200µg/ml shows 56.28 ± 1.54 similarly 100 and 50 mcg shows 42.54 ± 3.21 and 27.98 ± 0.89 % along with standard silymarin with the cell viability of 83.21 ± 1.87 % in MTT assay. It was concluded from the result of the present study that the formulations KMC possess promising hepatoprotective activity.

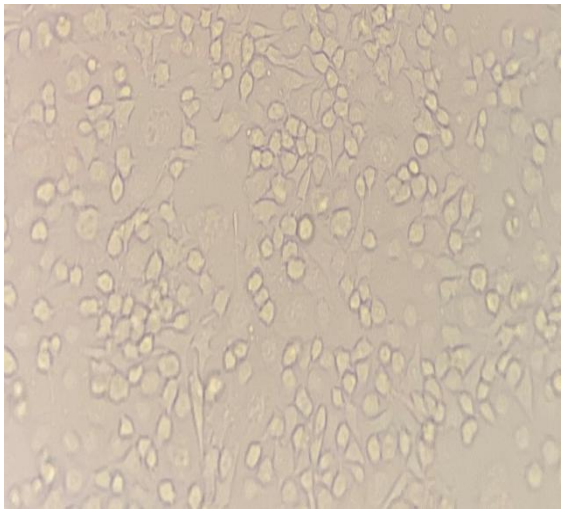
Chang Liver cells – Control group



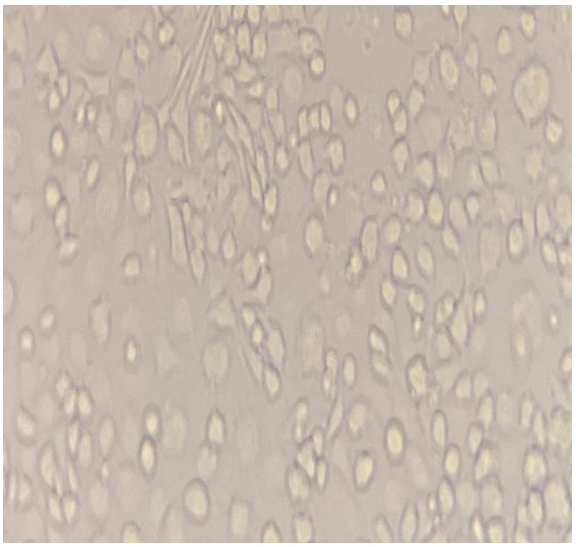
Chang Liver cells Incubated with CCl4



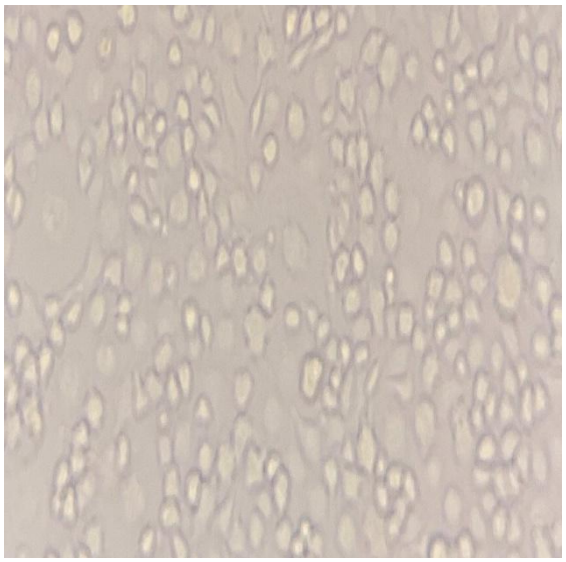
Chang Liver cells Incubated with Test Drug KMC - 50 µg



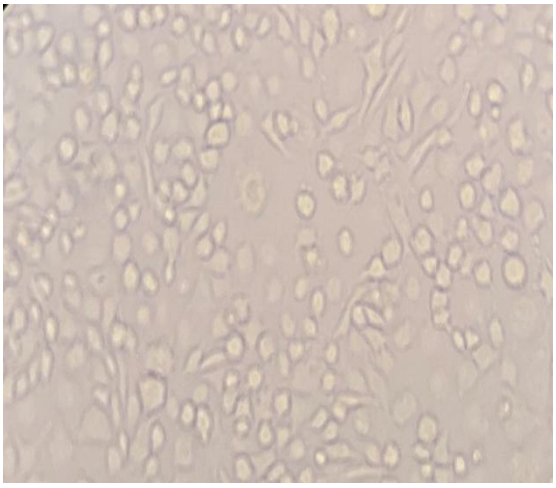
Chang Liver cells Incubated with Test Drug KMC - 100 µg



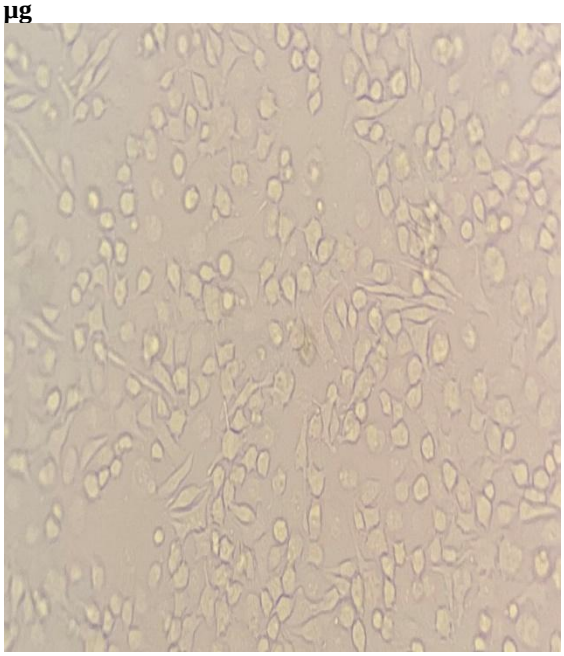
Chang Liver cells Incubated with Test Drug KMC - 200 µg



Chang Liver cells Incubated with Test Drug KMC - 400 µg



Chang Liver cells Incubated with Standard Silymarin - 200 µg



5. Conclusion

From this study it was concluded that test drug" KAMALAIKU CHOORANAM" possess promising Hepatoprotective property. This may further contribute in the management of jaundice.

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