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'Potentiality of *Pidangunaari Kudineer*(Polyherbal decoction) for Palmitic acid induced Hepatic Steatosis in the Chang Liver Cells'

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ABSTRACT

Fatty liver is a common clinical condition in this current scenario. Non alcoholic Fatty liver disease is nothing but accumulation of extra fat in the hepatic cells. Many research studies concluded that, Non alcoholic Fatty liver disease was directly connected with Non-Communicable diseases (NCD) like obesity, diabetes etc. Management and prevention of NAFLD (Non alcoholic Fatty liver disease) is quite a challenging one in currently available invasive options. To found a promising solution, we conducted the research on Traditional poly herbal formulation Pidangunaari Kudineer (Herbal decoction), which was classically indicated for the treatment of Splenomegaly. Review of Literatures concluded that ''both are having sisterhood relationships'' and occurrence will be hepatomegally followed by splenomegaly or viz versa. The aim of the study was to examine the effect of *Pidangunaari Kudineer* on Palmitic acid induced hepatic Steatosis in chang liver cells. The result of the studied formulation shows significantly reduced the FFA (Free Fatty Acid) Accumulation and further inflammations. It could be the right choice for the Non-invasive and potential therapy for the treatment of fatty liver diseases.

Keywords

Hepatomegaly, Pidangunaari Kudineer, Fatty liver, Siddha Medicine, Chang liver cells, Splenomegaly.

INTRODUCTION

In today's modern lifestyle, an increase in dietary fat intake, particularly saturated fat increases the prevalence of obesity and diseases caused by metabolic syndrome, diabetes and Steatosis. Fatty liver disease is also known as Hepatic Steatosis. Fatty liver disease is the most common form of liver disease worldwide, and is thought to be the hepatic manifestation of metabolic syndrome. It is a non discriminating disease affecting both children and adults and no socioeconomic class is spared. The incidence and prevalence of this condition is increasing day to day. Climbing prevalence of obesity and metabolic syndrome on the global scale, gradually increase in fatty liver disease. It is also estimated 30-40% of people in India having non alcoholic induced fatty liver diseases (NAFLD). It is assuming higher importance now because of high possible role in the development of cardiovascular disease, association with diabetes and impaired glucose tolerance, strong relationship with metabolic syndrome etc.

Pidangunaari Kudineer is a poly herbal formulation used for the treatment of Splenomegaly. The literature reviews clearly shows that, splenomegaly and hepatomegaly were having strong sisterhood relationships. Whenever hepatomegaly occurs, the person was more susceptible to splenomegaly might have more possibility on the occurrence and viz versa. The incidence of such conditions was followed one by one. The Correlation between the test drug and the proposed study were framed based on the indication and literature survey. Hepatosplenomegaly is the simultaneous enlargement of the liver (Hepatomegaly) and spleen(Splenomegaly). Hepatosplenomegaly is a sign seen in various disease processes. One of the major causes for hepatomegaly is fatty liver.

In Siddha literatures, *Manneral noi* (Splenomegaly) and *Kaleeral noi* (Hepatomegaly) has classified into four and three types respectively. Pathological cause for both the clinical condition would be the possible increase of *Pitha* humor and leading to digestion disorder. Yet now, no systems of medicine describes the diet advises and restriction for the affected diseases. One of the speciality of our Siddha lies in that dietary management (*Pathiyam*) and Vehicle selection (*Anubanam*).

Recent science explains, the pathological change of normal liver to that of steatosis is a complex process. Obesity, or more precisely increased visceral fat, has been shown to have a direct influence on glucose and lipid metabolism, with resultant liver Steatosis and

inflammation [van der Poorten et al. 2008]. Insulin resistance, leading to hyperinsulinemia, plays a significant, if not predominant role in the development of steatosis by increasing free fatty acid delivery to the liver, via increased intake and lipolysis, and increased de novo lipogenesis which contributing 60% and 26% of steatosis, respectively.

Epidemiological status in India

Non alcoholic fatty liver diseases can occur at all ages including childhood, thoughthe highest prevalence is described in those between 40-50 years of age.Absence of signs and symptoms and a lack of sensitive andspecific diagnostic tests for the easiest detection which are the barriers to estimate the prevalence of NAFLD.

The Prevalence of non alcoholic fatty liver disease is around 9% to 32% of general population in India with higher prevalence in those with overweight or obesity and those with diabetes or pre-diabetes. The current epidemics of obesityand diabetes among adults and children residing in bothdeveloped and developing countries suggest that prevalenceof NAFLD should have increased over time, and no doubt in increasing in future.

Need for hepato-protective agent from herbal origin

The major needs for hepato-protection are due to gradual increasing awareness and well know drastic effects of chemical drugs, turning the peoples towards herbal origins. . Drugs from Herbal origin have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. One of the important and well documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent. “Liver injury” is not a single entity; the lesion observed depends not only on the chemical agents involved, but also on the period of exposure.

MATERIALS AND METHODS

Collection and Authentication of Plant material

The Preparation of *Pidangunari kudineer* was started based on the Siddha literature ‘’Siddha Vaithya Thirattu’’. The major ingredients of Pidangunaari kudineer was Pidangu naari or Purankai naari (Premna tomentosa Wild), Manjal (Curcuma longa L.), Kadukai thol

(Terminaliachebula Retz). Premna tomentosa leaves were collected from Thenmalai hill station near Shenkottai, Tamilnadu. The plant leaves were identified and authenticated by Botanical experts of Walters' Siddha Research centre, Tirunelveli, and Department of Gunapadam (herbal pharmacology) staffs of Government Siddha Medical College, Palayamkottai. The ingredients were mixed in the ratio of 15 grams of Permna tomentosa leaf (exactly 4 leaves), Four grams of Terminalia chebula fruit (outer rind), Two grams of Curcuma longa (rhizome).

Cell lines and growth media

Cell lines and growth media Chang liver cell lines (Human normal liver cells) were cultured in DMEM (Dulbecco‟s modified eagles medium).

Culturing and maintenance of Chang liver cells

Chang liver cells were purchased from National Centre for Cell Science (NCCS), Pune and maintained in Dulbecco's Modified Eagles Medium (DMEM) containing L-glutamine with high glucose at 37°C and 5% CO2 in a humidified atmosphere in a CO2 incubator.

Cell culture and treatment

Chang liver cells were cultured in DMEM medium supplemented with 20% heat inactivated Foetal Bovine Serum (FBS). Antibiotics (Streptomycin and penicillin) were added to prevent bacterial contamination. The culture was then filtered and sterilized using 0.2 µm pore size cellulose acetate filter (Sartorious).

Sub-culturing of Chang liver cells

The subculturing involves transferring a small number of cells in to a new vessel. FBS is used to provide sufficient amount of nutrients for the proper growth of Chang cell line.

Trypsinization

It is the process of using trypsin, a proteolytic enzyme which breaks down proteins, to dissociate adherent cells from the vessels in which they are being cultured. The cell lines were washed with phosphate buffer saline and the fully confluent cells were trypsinized using 500 µl of trypsin (0.025% Trypsin in PBS/ EDTA solution) for 2 minutes at 370C and passed to T flasks in complete aseptic conditions. After disaggregation the cells are transferred to other flask and supplemented with media.

Screening of Palmitic acid induced steatotic activity

Oil red o (spectrophotometric method) (Anushaet al., 2006)

Procedure: Chang liver cells were grown at an initial density of 10^5cells/well in a 12 well plate and treated with different concentration of palmitic acid and fatty acid for 24 hrs. After 24hrs of incubation, the cells were washed three times with ice cold phosphate buffered saline and fixed with 4% paraformaldehyde for 30 min. After fixation cells were washed three times and stained with oil red o solution (0.5g in 60% ethanol) for 15 minutes at room temperature. The cells were washed again with phosphate buffered saline to remove unbound staining. Oil red o was eluted by adding 1ml of 100% isopropanol and incubated for 10 min with gently shaking such that oil red o was evenly mixed. Transferred these solutions to a 1.5ml eppendorf tube. Measured OD at 500 nm using 100% isopropanol as blank.

Oil red o (microscopic method) (Anushaet al., 2006)

Chang liver cells were grown at an initial density of 10^5cells/well in a cell plate and treated with 5µl of kamilari and fatty acid for 24hrs. After 24hrs incubation, cells were then washed three times with ice cold PBS and fixed with 4% paraformaldehyde for 30 minutes. After fixation cells were washed three times and stained with oil red o solution (0.5g in 60% ethanol) for 15 minutes at room temperature. The cells were washed again with

phosphate buffered saline to remove unbound staining. The image was acquired image under phase contrast microscopy (Olympus CKX 41). The captured images were further evaluated using image analysis software.

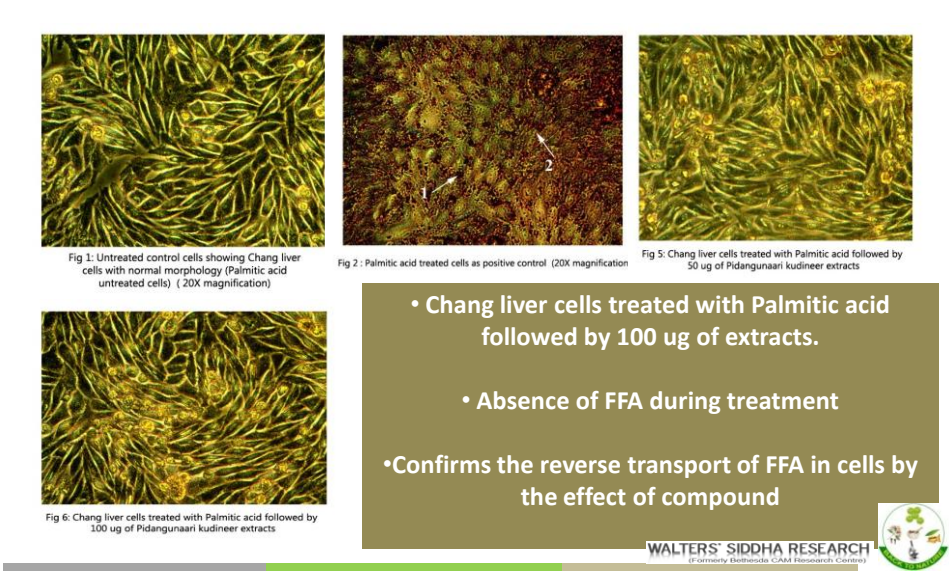
RESULTS AND DISCUSSION

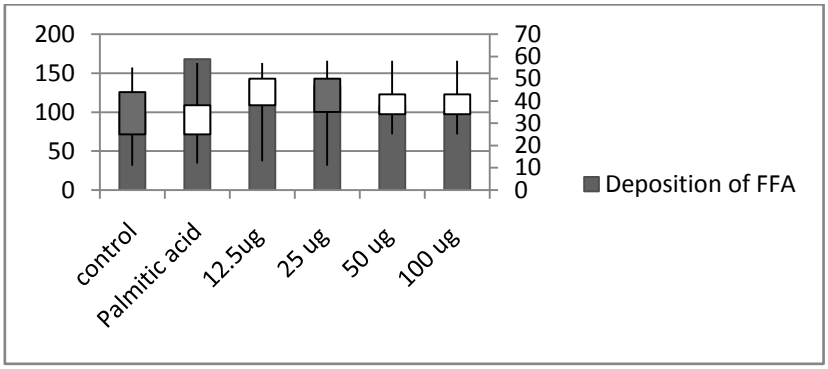
Concentration (µg)	OD (500 nm)	Percentage Increase in Oil red –O (Absorbance units)
Control	0.0691	
12.5	0.0977	141
25	0.0928	134
50	0.0752	108
100	0.0707	102
Palmitic acid	0.1162	168

At higher concentrations the drug effectively transported palmitic acid complexes across cell membrane which confirms anti steatotic activity, At 100ug/ml the Oil red O was almost completely transported across cell membrane having OD almost same as that of untreated control cells

Absorbance measured at 500nm

$$\% \text{ of FFA deposition} = \frac{\text{Standard - test}}{\text{Standard x 100}}$$





Graph. 1. Graphical representation of Free fatty acid deposition on Palmitic acid induced (Positive control) liver cells and reduction of such FFA in test drug induction

From the results it can be observed that the positive control Palmitic acid induced group is having an OD of 0.1062 which shows nearly 50% increase in Oil red O intensity when compared with normal cells (negative control) suggesting Fatty acid accumulation. Treatment with extracts produced a dose dependent decrease in OD units suggesting reverse transport of FA and 100ug effectively reduced the total FA accumulation reverting the

condition similar to that of normal cells. The study show significant anti steatotic effects on Pidangunaari kudineer extracts.

CONCLUSION

There are some very strong beliefs among the lay public regarding Siddha Medicine. One among them is the unquestionable efficacy of Siddha medicine in treating Hepatic disorders. Jaundice has always been treated with Siddha medicine as the first priority. This work is another effort from the Author's team to scientifically prove the Siddha literary claims. Pidangu Naari Kudineer, a poly-herbal decoction is in use for Centuries to treat Hepatic disorders. It has been scientifically proved for Palmitic acid induced Hepatic Steatosis in the Chang liver cells.

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