VOLUME-8, ISSUE-9, SEPTEMBER-2019 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Medicine

IN VITRO ANTI ADIPOGENESIS ASSAY OF KARIVEPPILAI CHOORANAM ON 3T3-L1 CELL LINE

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ABSTRACT The adiposeness is a chronic metabolic disorder to affecting people worldwide, with significant morbidity and mortality caused by its micro vascular and macro vascular complications, affecting various important target organs and structures in humans. Recent scientific studies and newer trial drugs have been proved that early detection of hyperlipidaemia will decrease the number of severity in hyperlipidaemic complications. According to ICMR-INDIA B (phase I) study restricted to urban and rural study populations in 4 states in India was hyperlipidemia in 13.9%, low HDL cholesterol in 72.3% and high LDL Cholesterol in 11.8% (Gupta 2010 &Joshi2014) and NCEP-ATP III is observed the prevalence of hyperlipidemia is affected higher in males then in females (Sawant et al). The siddha medicines are well management of reducing weight loss and other complications. Aim: The present study was carried out to determine the anti adipogenic activities of kariveppillai choornam using Mouse Embryo Fibroblast 3T3-L1 cell line.

Methods and Material: The study is performed to assess the kariveppilai chooranam for its anti-hyperlipedemia by oil red o and triglycerides estimation methods. The test drug kariveppillai choornam is a poly herbal formulation containing eight plants.

Results: the kariveppilaichooranam is inhibiting lipid accumulation (anti adipogenesis) by 25.1% and 16.6% over control at 200 μg/ml and 100μg/ml respectively. simultaneously, glycerol released was found to be 119.82±0.90% and 134.53±1.38% over control at 200μg/ml and 100 μg/ml respectively.

KEYWORDS : Hyperlipidemia, Anti-adipogenisis, Mouse Embrio Fibroblast, Siddha drug

1. INTRODUCTION:

The hyperlipedemia is a metabolic condition which it was increased risk of coronary vascular disease or peripheral vascular disease. More deposit lipids in vascular bed resulted in narrowing blood vessels and it is leads to above complications. secondary effects of hyperlipedemia can alter liver functions, resulting fatty liver or Cirrhosis.in this situation various treatment methods are available in modern medicine.All the medicines are produced toxic side effects like SAS,SAMS and SINAM (statin induced necrotizing auto immune myopathy) Rhabdomyolysis (Paul D.Thompsonetal 2016).the anti hyperlipidemic drug Simvastatin and Provastatin was worsening condition in CKD and refractory dermatological lessions (BanchaSatirapoj etal.2015 and MicalP.Salna etal.2017).so,the above reasons the newer drug discovery is a most important and necessary to developing a new generation to treat hyperlipedemia patients. The many herbal products are available in siddha text books. in this study kariveppilai choornam is highly contains Poly phenols (Nilma S Rajurkar and S.M.Hande 2011),Tannin (Kannan p etal,2009) and Terpenoids (Choudhary and VijayaKanth M.S,2005) the above herbal Phytochemicals are response to reduced MTP inhibitions via DGAT and ACAT mechanism.

2. MATERIAL AND METHODS:

The eight individual ingredients was collected from south zone of Tamilnadu, India.which it was identified by medicinal plant experts and siddha pharmacologist at government siddha medical college, palayamkottai,Tirunelveli. Equal ratio in Sl.no1 to7and Sl.no 8 is seven per cent increase in total weight of 1to 7 (Table no1).the eight ingredients are,

Tableno1;Ingridents of kariveppillai choornam

sl.no	Ingritents	Important Alkaloids
1	Murrayakoenjii(l)sperng	Mahanimbine,koenimbine,koengicine
2	Gossypium herbaceum	Gossypin,Quercetin-3-0-glucoside
3	Curcuma longa (l)	Curcumin,Demethoxycurcumin
4	Coscinium fenestratum(gaertn.)colebr	Berlambine,Berberine,Sitosterol,Stigmasterol
5	Terminalia chebula (retz)	Chebulin,phenoliccompounds,Gallic acid
6	Terminalia bellerica gaertn.roxib	Gallic acid,Ellagicacid,Chebulagic acid,Cardio glycoside
7	Emblica officinalis (gaertn)	Terchebin,Ellagicacid,Phyllembic acid
8	Salaciareticulata (wall.ex)	Leucopelargonidin,Friedelan

Test solution preparation:

The *in-vitro* Cytotoxicity was performed for test extract on mouse embryo fibroblast cells to find toxic concentration of kariveppilaichooranam. based on this concentration the adipogenesis activity of kariveppilaichooranam by performed by oil o red staining and free triglyceride level was measured. preparation of test solution for Cytotoxicity studies, 10mg of test drug was separately dissolved in DMSOand volume was made up with DMEM - high glucose supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by 0.22μ syringe filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxicity studies.

Cell line and Culture medium

3T3-LI (Mouse Embryo Fibroblast) cell line was procured from national centre for cell sciences (NCCS), pune, India. stock cells were cultured in DMEM high glucose supplemented with 10% inactivated Foetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin b (5 μg/ml) in humidified atmosphere of 5% co2 at 37°c until confluent. thecells were dissociated with TPVG solution (0.2% trypsin, 0.02% edta, 0.05% glucose in pbs). the stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 well Microtitre plates. (Trypsin, 0.02% Edta, 0.05% glucose in PBS). the stock cultures were grown in 25 cm2 culture flasks and all experiments were carried out in 96 well

Micro titre plates. in- vitro anti-adipogenesis assay

culture and differentiation

Day 0: 3T3-L1 pre-adipocytes (NCCS, India) were culture din 4.5 g/l glucose-DMEM with 10% calf serum, antibiotic solution in 30 mm plastic petridishes until they reached 100% confluence.

Day 1: For differentiation, 2-day post-confluent cells were incubated for 48 h in DMEM with 10% FBS, antibiotics, and a differentiation cocktail termed MDI, which contained 0.5mm isobutyl methyl xanthine, 1μm dexamethasone and 100 nm insulin along with extracts.

Day3: After 48 h, cells were maintained in DMEM with 10% FBS, insulin and antibiotics for 6 days.

Day9: After 6 days, the cells were maintained in DMEM with 10% FBS along with test samples for 24 hours.

Oil Red O Staining

3T3-L1 adiposities were washed with Phosphate-buffered saline (Pbs) and fixed with 10% formalin for 30 min. after two washes with distilled water, cells were stained for at least 1 h at room temperature in freshly diluted oil red o containing 0.5% oil red o in isopropanol. finally, the dye retained in the 3t3 and quantified by measuring the optical absorbance at 500 nm.

Measurement of Triglyceride (TG)

The supernatants were assayed for TG according to the manufacturers instruction. The results are expressed in mg/dl of TG.

RESULTS :

The percentage of inhibition over control in test concentration (μg/ml)200,100 was 24.4±2.21,16.2±4.97 in N1 and N2 was showed 25.8±1.57,17.1±2.63 respectively. The average control is 25.1 and 16.6 (Table 2 &Fig.1).

Table 2.Result of percentage of inhibition over control in test concentration

Sl.no	Test Concentration (μg/ml)	Percentage Inhibition over control (AVG± SD)		
		(N1)	(N2)	(N3)
1	200	24.4±2.21	25.8±1.57	25.1
2	100	16.2±4.97	17.1±2.63	16.6

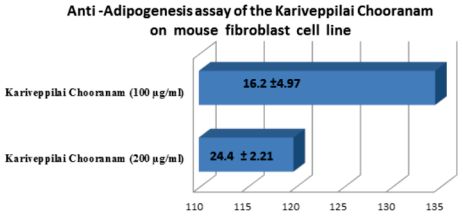

Table 3.Level of released triglyceride in cell supernatant

sl.no	test concentration (μg/ml)	triglyceride mg/dl (% control)
1	200	119.82±0.90
2	100	134.53±1.38

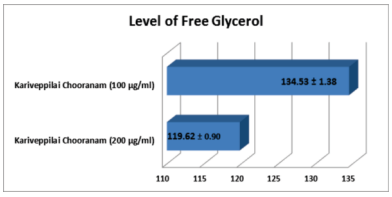

Figure 2. Level of released Triglyceride in cell supernatant

According to Table No3 and Fig 2.showed test concentration in 200 and 100μg/ml ,the percentage of triglyceride was 119.82±0.90 and 134.53±1.38 respectively.

DISCUSSION AND CONCLUSION

In-vitro cytotoxicity studies on kariveppilaichooranam against mouse embryonic fibroblast cell line by MTT assay exposing the cells to different concentrations of kariveppilaichooranam showed moderate anti-adipogenesis activity in dose dependent manner by inhibiting lipid accumulation by 25.1% and 16.6% over control at 200 μg/ml and 100μg/ml, respectively. simultaneously, glycerol released was found to be 119.82±0.90% and 134.53±1.38% over control at 200 μg/ml and 100 μg/ml, respectively.

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