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A Biochemical Approach to Understand the Concept of *Snigdha Guna* of Ghee and *Ruksha Guna* of *Terminalia arjuna* on Behaviour of 3T3-L1 Cell Line with Respect to Adipocytic and Anti-Adipocytic Activity : In Vitro

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ABSTRACT

Ayurvedic medicine is a system of traditional medicine native to India. *Ayurveda* is based on peculiar fundamental principles like *Shatpadarth*, *Tridosha* Theory, *Panchamahabhuta* Theory, *Prakriti*, *Ojas*, *Dhatu*, *Mala*, *Agni*, *Manas*, and *Atma* etc. The concepts of *Ayurveda* are expressed with *Gunas*. The increased elements are treated by opposite *Guna*. In support of the above concept, an experimental study on the 3T3-L1 cell line is carried out for examine the adipocytic and anti adipocytic activity of *Snigdha* and *Ruksha Guna* respectively. The results were assessed with the help of evaluation of sublethal concentration by using XTT assay and lipid assay. The aim of basic research in *Ayurveda* through bioscience is to explore scientific innovation and opportunities in fundamental concept of *Ayurveda*. Fundamental research needs to be done to highlight the concept of *gurvadi guna* through the in vitro study.

Key words: Basic Principles of *Ayurveda*, *Gurvadi Guna*, *Snigdha Guna*, *Ruksha Guna*, 3T3-L1 cell line.

INTRODUCTION

Ayurveda is the science of healing living to treat disease and maintenance of good health and well-being.^[1] The concept of *Samanya Visheshha Siddhanta* is the foremost principle which advocates the usage of *Ahara* and *Aushada* in keeping the health and curing diseases based on its *Guna*.^[2]

Guna is one among the *Satkarana Padarthas* in

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Ayurveda. *Guna* is the character or property which remains in a *Dravya* with inherent relationship and is devoid of action. Among of them, *Gurvadi Guna* is a set of primary attributes of all substances associated always with substances but are without quality. The concept of *Gurvadi Guna* occupies important place in the principles of *Ayurveda*. *Snigdha* and *Ruksha Guna* are the type of *Gurvadi Guna* which is opposite of each other. *Snigdha Guna* produces moisture, unctiousness, oozing, softness and moistening with *Santarpana Karma*. *Ruksha Guna* reduces unctuousness and produces roughness and non-sliminess with *Apatarpana Karma*.^[3] *Gurvadi Guna* play an important role for nourishment of tissues. *Snigdha Guna* protects cellular lining and *Ruksha Guna* reduces wetness and accerates healing quality.

Everything in *Ayurveda* is a validated through observation, direct examination and knowledge derived from ancient text. But it requires translate concepts and practical application into idiom of modern biochemistry, correlation of *Dosha* and

genomic variation, correlation of *Guna* and cellular functions. *Guna* are used in various treatments, but we do not know how cellular function carried out by using various drugs of different *Guna*. Hence *Ayurveda* has to provide the much needed support of biochemistry science of cell line study. There is strong need to explain fundamental principles of *Ayurveda* in a modern context through cell culture for future aspect.

Cell culture is the process of growing cells artificially. Animal cell culture involves in vitro maintenance and propagation of animal cells in a suitable nutrient media. Cell based assay plays an important role in pharmaceutical industry, microbiological culture.^[4] Current knowledge on cells and in vitro if used along with concepts of regeneration in *Ayurveda* can contribute to the development of regenerative medicine with integrative approach for that knowledge. It is observed by the adipocytic and anti adipocytic activity of *Ruksha* and *Snigdha Guna* on 3T3-L1 cell line.

3T3-L1 cell line is a well-established pre-adipose cell line that was developed from murine Swiss 3T3 cells. The 3T3-L1 cells are derived from disaggregated 17 to 19 day old Swiss 3T3 mouse embryos, which display a fibroblast-like morphology that, under appropriate conditions, can acquire an adipocyte-like phenotype.^[5,6,7]

To see the adipocytic and anti adipocytic activity on 3T3-L1 cell line, we have taken drug Ghee (*Ghrta*) as a *Snigdha Guna* and *Terminalia arjuna* (*Arjuna*) as a *Ruksha Guna* respectively. Research is the prime need of contemporary *Ayurveda*. The aim of basic research in *Ayurveda* through bioscience is to explore scientific innovation and opportunities in fundamental concept of *Ayurveda*. Fundamental research needs to be done to highlight the concept of *Gurvadi Guna* through the cell line study.

AIM

To study the *Snigdha Guna* of Ghee and *Ruksha Guna* of *Terminalia arjuna* on Behaviour of 3T3-L1 Cell Line with respect to Adipocytic and Anti-adipocytic Activity: *In Vitro*

OBJECTIVES

Primary objective

- To established the in vitro cell culture models for studing the adipocytic and anti adipocytic activity of *Snigdha Guna* and *Ruksha Guna* using 3T3-L1 adipose cell line.
- To study the *Snigdha Guna* and *Ruksha Guna* of Ghee and *Terminalia arjuna* respectively on 3T3-L1 adipose cell line.

Secondary objective

- To calculate and report the 50% sublethal concentration (LC 50) value of Ghee and *Terminalia arjuna* on 3T3-L1 adipose cell line.

MATERIAL AND METHODS

Study design: This study is analytical and experimental type of study.

Inclusive criteria: Adipocytic cell lines of 3T3-L1 were taken for the study.

Exclusive criteria: Adipocytic cell lines of 3T3-L1 were included and other is Excluded.

To examine the adipocytic (*Snigdha*) and anti adipocytic activity (*Rukshata*) on 3T3-L1 cell line, intracellular lipid accumulation (*Snigdha*) and lipid absorption (*Rukshata*) were evaluated by-

Evaluation of sublethal concentration using XTT assay:

For examining the intracellular lipid accumulation (*Snigdha*) and lipid absorption (*Rukshata*) on 3T3-L1 cell line, viability of cells must be needed. The viability of cells is examined by toxicity of drugs. On this scale, Ghee of buffalo and *Terminalia arjuna* extract were calculated 50% sublethal concentration. XTT assay were done to determine the sublethal concentration of Ghee of buffalo and *Terminalia arjuna* extracts.

XTT Assay

The cells were treated with Ghee of buffalo and *Terminalia arjuna* at various Concentrations and incubated for 24 hours with untreated cells. Then solutions of XTT were added to detect the cell viability. Then tetrazolium salt XTT reduced to orange colored compounds of formazan. The dye form is

water soluble. The dye intensity can be read at a wavelength (450nm) with a spectrophotometer. The intensity of the dye is proportional to number of metabolically active cells. The greater the number of active cells in the well, the greater the activity of mitochondrial enzymes, and the higher the concentration of the dye formed, which can then be measured and quantitate.

Oil Red O staining

Oil Red O staining is a lysochrome diazo dye. It is used for staining of triglycerides and lipids. To examine the adipocytic and anti-adipogenic activity of Ghee of buffalo and *Terminalia arjuna* in 3T3-L1 cells, intracellular lipid accumulations were assessed by using ORO staining.

Lipid assay

It is used for total analysis of lipids. By using oil red o staining, the stained lipid droplets were quantified by using microplate reader at wavelength 520nm.

Chemicals and Reagents

XTT-(2,3-bis-[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-6-carboxanilide) inner salt from Himedia and PMS (Himedia)

Experiment designing and evaluation of the adipocytic and anti-adipocytic activity

3T3-L1 cells were cultured in 2×10^4 cell per well in a 24 well tissue culture plate. The cells seeded in the wells were divided in following groups.

1. Control (Cells only)
2. Cell + Adipocytic differentiation media (IBMX+ Dexa + Insulin)
3. Cell + differentiation media + *Terminalia arjuna* Conc-10 $\mu\text{g/ml}$
4. Cell + differentiation media + *Terminalia arjuna* Conc-50 $\mu\text{g/ml}$
5. Cell + Differentiation media + *Terminalia arjuna* Conc-100 $\mu\text{g/ml}$
6. Cell + Differentiation media +Ghee Conc-10 $\mu\text{g/ml}$
7. Cell + Differentiation media + Ghee Conc-50 $\mu\text{g/ml}$

8. Cell + Differentiation media + Ghee Conc-100 $\mu\text{g/ml}$

Oil Red O (ORO) staining

To examine the adipocytic and anti-adipogenic activity of Ghee of buffalo and *Terminalia arjuna* in 3T3-L1 cells, intracellular lipid accumulation in differentiated adipocytes were assessed by using ORO staining.

Statistical analysis

All the statistical analysis was performed using MedCalc (version10) by t test. P-value <0.05 was considered as statistically significant.

OBSERVATIONS AND RESULTS

Results of Cytotoxicity Assay

The cytotoxicity assay is based on the ability of metabolically active cells to reduce the tetrazolium salt XTT to orange colored compounds of formazan. The intensity of the dye is proportional to the number of metabolically active cells. A result of the XTT shows a non-significant difference in the cell viability of the PBCM after the treatment with Ghee (Fig no. 1.) while moderate toxicity was observed with *Terminalia arjuna* extracts treatment at higher concentration of 100 $\mu\text{g/ml}$ (Fig no. 2). LD 50 value for Ghee treatment was very favorable i.e. 13570 $\mu\text{g/ml}$ (Figure no.3); Similarly, LD 50 Value for *Terminalia arjuna* extracts treatment was also considerably high i.e.3327 $\mu\text{g/ml}$ (Figure no. 4). This suggests that both Ghee and *Terminalia arjuna* extracts are safe to be used for the treatment.

Figure 1: Effects of Ghee of buffalo on viability of the 3T3-L1 preadipocytic cell line.

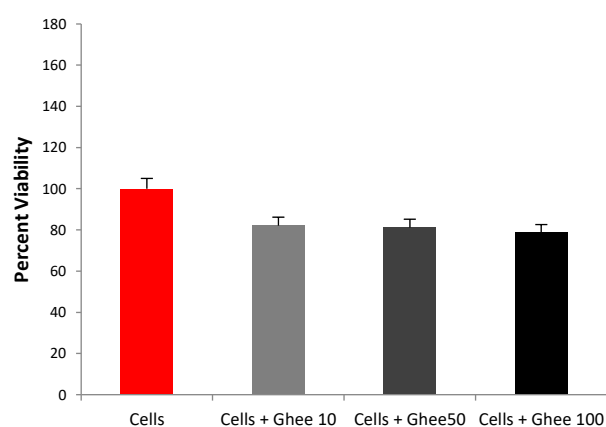


Figure 2: Effects of *Terminalia arjuna* extracts on viability of the 3T3 L1 preadipocytic cell line

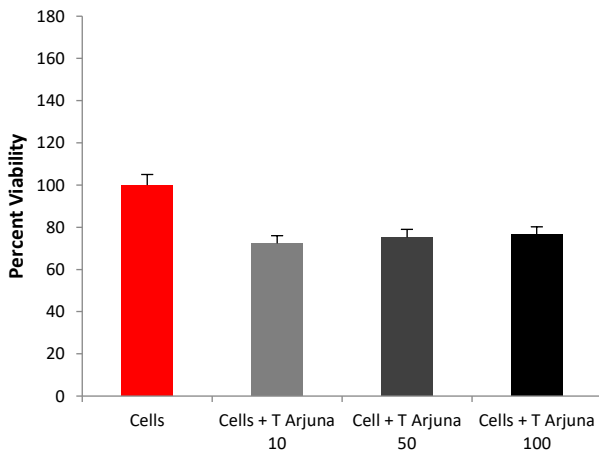


Figure 3: Calculation of the LD₅₀ Value of Ghee of buffalo.

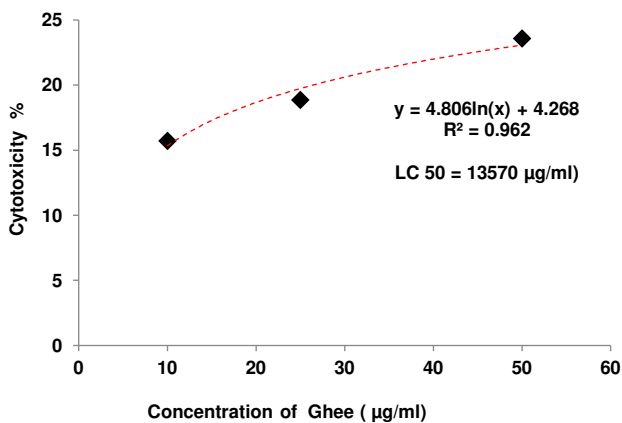
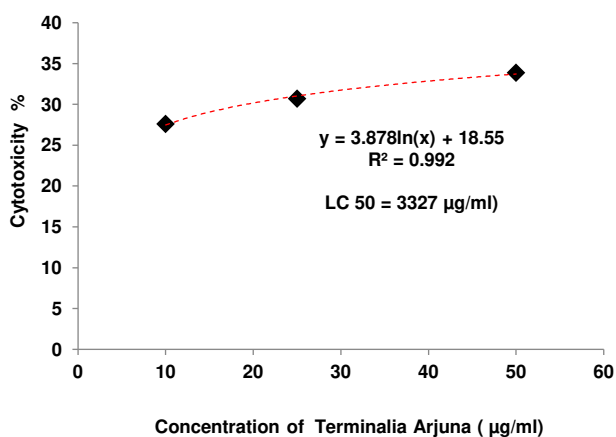


Figure 4: Calculation of the LD₅₀ Value of *Terminalia arjuna*



Results of evaluation of the adipocytic and anti-adipocytic activity of Ghee and *Terminalia arjuna* extracts.

We found that both ghee of buffalo do not show any adipogenic activity at in any of the evaluated concentration i.e. 10, 50 & 100 µg/ml. (fig no.5 and 7). Similarly, we had also observed the anti-adipogenic activity of *Terminalia arjuna* extracts in 3T3-L1 cells differentiated with IBMX +Dexa+Insulin at lower concentration of 10 µg/ml while found to support adipogenesis at higher concentration of 100 µg/ml (figure no.6 and 8).

When see the adipocytic activity of *snigdha guna* drug Ghee and anti adipocytic activity of *ruksha guna* drug *Terminalia arjuna*, we found that, the confidence interval (CI) of our study is 95%. So that here we found $p > 0.05$. Hence the null hypothesis is accepted with result is non significant.

Figure 5: Oil Red O staining of 3T3-L1 cell after induction with Ghee of buffalo (A) Control cells; (B) Differentiated 3T3-L1 cells; (C-E) 3T3-L1 cell induced with 10, 50 and 100 µg/ml Ghee

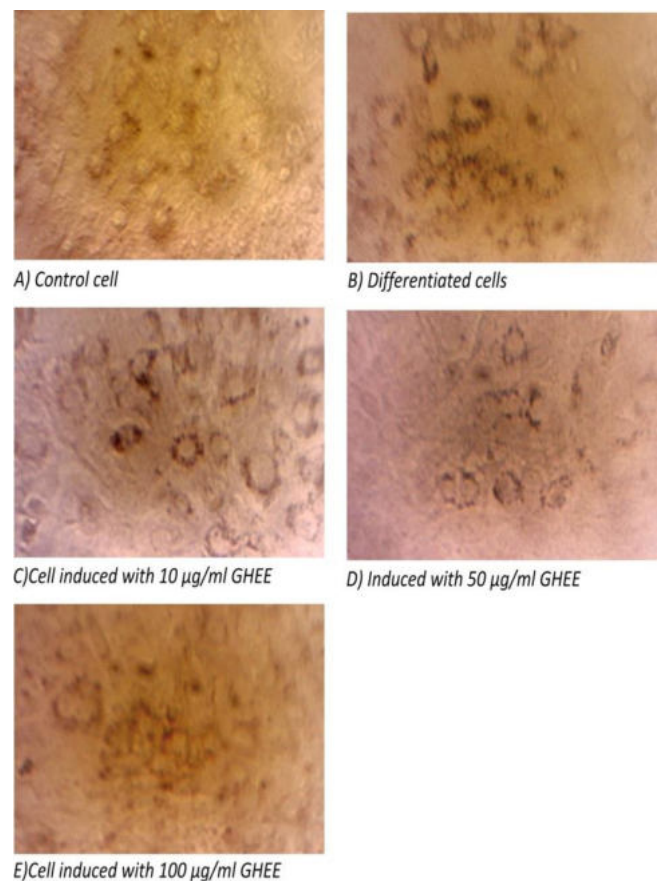


Figure 6: Oil Red O staining of 3T3-L1 cell after induction with *Terminalia arjuna* (TA). (A) Control cells; (B) Differentiated 3T3-L1 cells; (C-E) 3T3-L1 cell induced with 10, 50 & 100 µg/ml *Terminalia arjuna*.

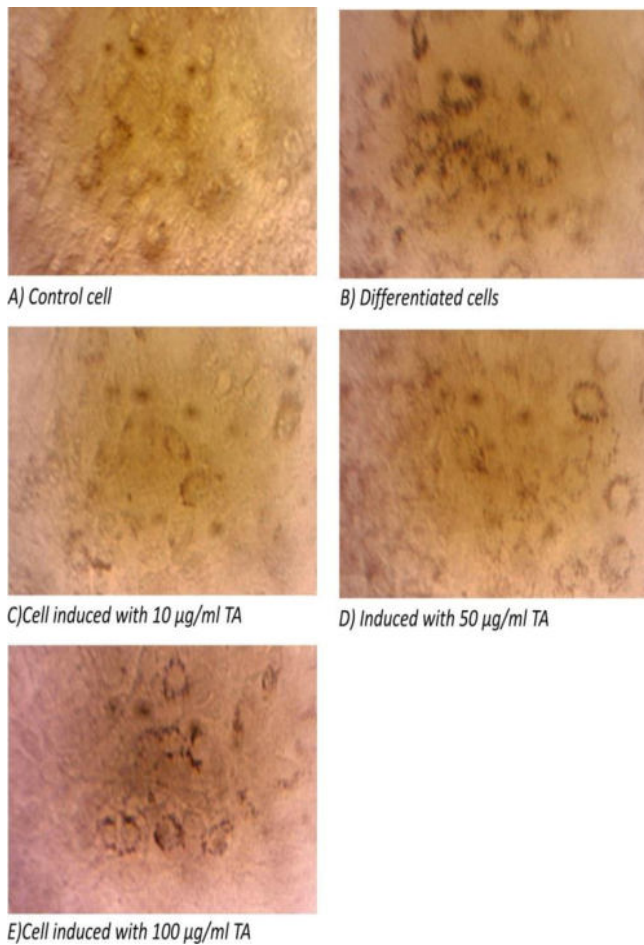


Figure 7: Depicts the changes in the total lipid content of 3T3-L1 cell after induction with Ghee of buffalo.

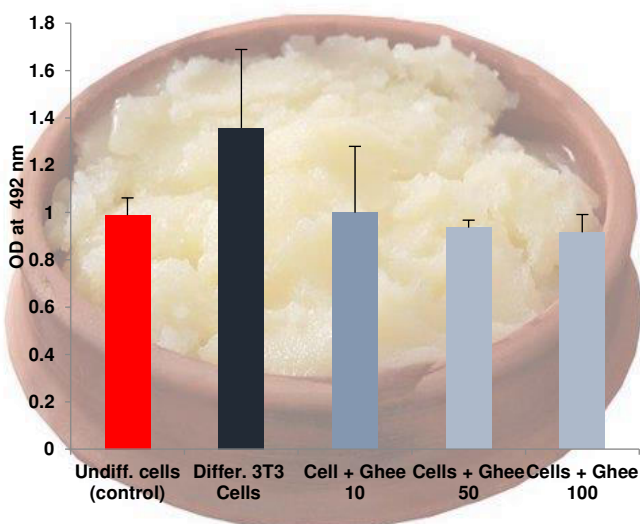
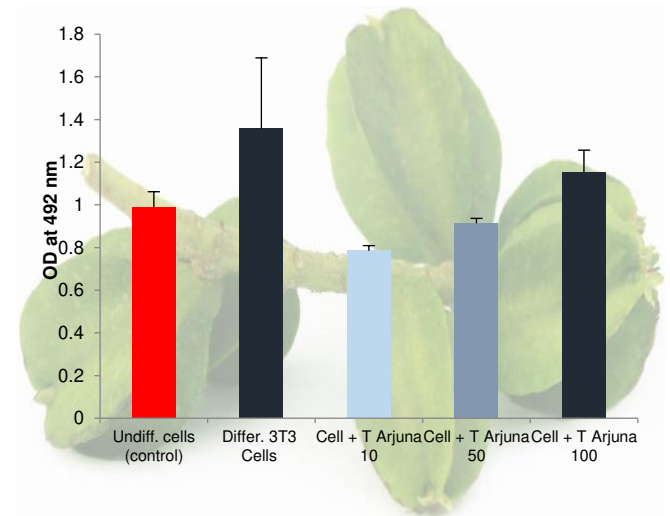


Figure 8: Depicts the changes in the total lipid content of 3T3-L1 cell after induction with *Terminalia arjuna* (TA).



DISCUSSION

In this study, an attempt was made to evaluate the adipocytic (*Snigdha*) and anti adipocytic activity (*Rukshata*) of *Snigdha* and *Ruksha Guna* by establishing the cell line model. Before evaluation of adipocytic and anti adipocytic activity of Ghee of buffalo and *Terminalia arjuna* extracts, we had seen the viability of cells by using cytotoxicity assays using different concentrations of 10 microgram /ml, 50 microgram /ml and 100 microgram /ml. These results showed that both Ghee of buffalo and *Terminalia arjuna* extracts are safe to be used for treatments.

Buffalo Ghee is *Snigdha Guna Dravya* which is *Madhur Rasa*, heavy for digestion. It is *Sheeta Virya* in property, increases the quantity of *Kapha Dosha* and decreases *Vata* and *Pitta Dosha*. According to *Charaka*, buffalo milk is heavier to digest and unctuous than that of cow’s milk. Along this concept, *Charaka* also said that ghee of goat, buffalo and sheep has similar properties like their milk properties.

Buffalo Ghee consists of 62% monounsaturated fats, rich in conjugated linoleic acids (CLA) vitamin A, D, E and K.^{[8][9]} Buffalo Ghee contains about 5% more saturated fats than cow’s Ghee.^[10] Due to enriched content of CLA, buffalo Ghee has been reported as an antioxidant, anticarcinogenic, antidiabetic, antiatherogenic and anti adipogenic properties.^[8] In

our present study, we found that buffalo Ghee does not show adipocytic activity in any of evaluated concentrations i.e. 10, 50, 100 microgram/ml. Because Ghee protects the body from various diseases and promotes longevity. Ghee is one of the best uncting (*Snigdha Dravya*) substance among all the unctious substances.^[11] Ghee alleviates *Pitta* and *Vata Dosha*, beneficial for *Rasa Dhatu*, *Sukra Dhatu* and *Ojas*, cooling, softening and improves voice and complexion.^[12] Ghee has also property of *Sanskarsya Anuvartanat*. *Meda Dhatu* consist of *Snigdha Guna*. Its main works as lubrication means providing softening to all body through its *Snigdha Guna* property.^[13] According to *Charaka*, the person with essence of *Meda Sarata* have particular unctuousness in complexion, voice, eyes, hand, hair, skin hairs, nails, teeth, lips, urine and faeces. This indicates wealth, power, happiness, simplicity, health, strength and longevity.^[14] According to *Sushruta*, who has urine, sweat are unctuous, voice pleasant, has body of big size and unable to tolerate exertion is to be understood as person of excellence of fat.^[15] *Meda Dhatu Sarata* indicates the healthy characteristic of the persons endowed with *Meda Dhatu*. When see the features of *Meda Vriddhi*, increase of *Meda* produces unctuousness of the body, increase of the abdomen and flanks, cough, dyspnoea, bad smell etc.^[16] According to *Vagbhata*, increases *Meda Dhatu* causes fatigue, increased breathing even after little work, drooping of buttocks, breast and abdomen.^[17] *Meda Dhatu Vriddhi* indicates the unhealthy state of body.

The difference between the features of the *Meda Sarata* and *Meda Vriddhi* is the *Meda Sarata* is the strong state and *Meda Vriddhi* is the unhealthy state of the body. Like that, the buffalo Ghee also the *Snigdha Guna Dravya*. Even if buffalo Ghee does not show adipocytic activity at various concentrations i.e. 10, 50 and 100 microgram/ml. It does not mean *Snigdha Guna* do not works as adipogenesis. It works as adipogenesis by providing unctuousness, softness and cooling to all body through its *Snigdha Guna*. Buffalo Ghee has also been reported for prevention of cardiovascular diseases, strengthening immune

function and modifying body composition to treat obesity or build lean body mass.^{[18][19]} It is also found that it is useful in reducing adiposity in humans.^[20] When in present study buffalo Ghee does not show adipocytic activity at various concentrations. It means *Snigdha Guna Dravya* buffalo Ghee provides unctuousness (*Snigdhatata*) to all body for proper functioning in obese person. It maintains excess *Meda Dhatu* through unctuousness property (*Snigdha Guna*) in obese person and also maintain the diminish *Meda Dhatu* in lean body person through unctuousness property (*Snigdha Guna*).

About the *Ruksha Guna Dravya Terminalia arjuna*, we had observed the anti adipogenic activity of *Terminalia arjuna* on 3T3 L1 cell line at lower concentrations of 10 microgram /ml. It found to support adipogenesis at higher concentrations of 100 microgram/ml.

It has been reported from in vivo study, some mice are treated with *Terminalia arjuna* for see the lipid lowering activity. The results had shown that *Terminalia arjuna* had a marked reduction in total cholesterol, triglycerids, LDL cholesterol and VLDL cholesterol.^[21] Also in another study, it happens *Terminalia arjuna* extract was found to be a powerful anti-diabetic component that can inhibit the growth of 3T3 L1 cell line. The results suggest that *Terminalia arjuna* have more proliferative capability and dose dependent inhibition of growth of 3T3 L1 cell line. That things conclude that *Terminalia arjuna* is a good radical scavenger.^[22] In our present study, we had observed the anti adipogenic activity of *Ruksha Guna Dravya Terminalia arjuna* on 3T3-L1 cell line at lower concentration of 10 microgram/ml. Because *Kashaya Rasa* of *Terminalia arjuna* is pacifying, astringent, absorbing, pacifies *Kapha*, *Rakta* and *Pitta Dosha*, utilizes the body fluid, rough cold and slightly light. If it is used excessively, causes flatulence, impotency, thirst, stiffness, lean body mass, produces *vata dosha* disorders.^[23] When see the side effects of *Ruksha Dravya Terminalia arjuna*, it has been used in the dose of 3-6 gm in *Churna* form in various clinical studies.^[24] This has been found that the optimum dose of coronary artery disease. At this dosage, it is well

tolerated. If higher dose is given than optimum dose, there has side effects like gastritis, headache and constipation.^[25]

Another study on adipocytic activity of *Terminalia arjuna* proved that dried bark powder of *Terminalia arjuna* have potent hypolipidemic and cardioprotective activity.^[26-30] But we found that *Terminalia arjuna* support adipogenesis at higher concentration of 100 microgram/ml. Because *Madhura Rasa* of *Terminalia arjuna* is unctuous, heavy and cold, promotes strength and lustre alleviates pitta, bulk promoting and stabilizer. If used excessively, produces *Kaphaj* disorders such as obesity, laxity, over sleep, heaviness.^[31] Due to this, *Terminalia arjuna* shows adipogenesis at higher concentration of 100microgram /ml through its *Madhura Rasa*.

Taking the present result into consideration, it is possible to say that *Snigdha Guna Dravya* i.e. Ghee of buffalo does not increase the fats or lipids in the body. It is useful for longevity, generates immunity system, protects the body from various diseases and nourishes all the tissues of the body. According to our study report, Ghee of buffalo does not show lipidemic activity on 3T3-L1 cell line.

Similarly, about *Ruksha Guna Dravya Terminalia arjuna*, further clinical study will be need to say that it is safe to be used at lower concentration according to severity of heart diseases. Because in our study *Ruksha Guna Dravya Terminalia arjuna* show anti adipocytic activity at lower concentration while found increases adipogenesis at higher concentration.

CONCLUSION

In this study the facts are depicted with the help of “t” test. It implies that by using XTT assay shows a non-significant difference in the cell viability of the PBCM after treatment with ghee of buffalo while moderate toxicity was observed with *Terminalia arjuna* extracts treatment at higher concentrations. This suggests that Ghee of buffalo is safe to be used for the treatment in higher concentration since it has very highly favourable LD50 value of 13570 microgram/ml. The

experimental data of present study suggest that ghee of buffalo has non adipogenic activity at 10, 50 and 100 microgram/ml concentrations. *Terminalia arjuna* is safe if used in lower concentration 50 microgram/ml or less since it has LD value of 3327 microgram /ml. The results of evaluation of the anti adipocytic property of *Terminalia arjuna* showed anti lipidemic activity at lower concentrations while found to increase the adipogenesis at higher concentrations. However, mechanism of adipogenic property of *Terminalia arjuna* at higher concentrations needs to confirm with the help of in vivo study.

Biochemistry science gives knowledge of cells, tissues and their nutrients, organisms and molecular science. It is useful to investigate normal physiology and biochemical of cells. There is scope for future study to signify *Gurvadi Guna, Guru and Laghu Guna* by using different biochemical analysis. We can also distinguish the *Guru and Laghu Guna* properties of *Dravya* through biochemical way. This review and analysis is carried out with a caveat that the methods of cell culture study and evidence of cell line approach of biochemical science is need of Ayurveda. Further additional work will be needed for understanding the properties and utility of *Guna* in modern concept through biochemical way. The advent of Ayurveda science and modern biochemistry view have turned attention of scientists increasingly from different physiological view to in vitro or in vivo study.

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