Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400

https://doi.org/10.46798/ijam.2021.v24i01.4

Volume 24 Number 1
April - June 2022, pp. 32-39

Anti-diabetic and anti-proliferative potential of Siddha polyherbal formulation Tulasi oil (SPFTO) – An *in-vitro* evaluation

Dr. S. Yavanarani[1*] and Dr. R. Selvakumar[2]

[1]Yavanarani Siddha Clinic, Pillaiyarpalayam, Kanchipuram, Tamilnadu, India – 631501.
[2]Siddha Government Hospital, Tiruttani, Tiruvallur, Tamilnadu, India - 631209

Abstract

Background: There are many health hazards associated with diabetes mellitus, characterized by high blood glucose levels caused by defects in insulin secretion or insulin action, or both. Among the most life-threatening diseases, cancer presents many health hazards both in developed and developing countries, characterized by irregular cell proliferation. The prevalence of diabetes and cancer are escalating day by day and this shows the urgently require for the management of diabetes and cancer. **Methods:** In this study was aimed to evaluate the antidiabetic and anticancer potential of Siddha polyherbal formulation Tulasi oil (SPFTO) by glycosylation of hemoglobin assay, glucose uptake by yeast cells, alpha amylase inhibition and MTT assay method, respectively. **Results:** The inhibition rate in all concentrations of Tulasi oil exhibited higher inhibition of glycosylation, glucose uptake in yeast cells assay and α amylase as compared with the standard drug. The cytotoxic activity for MCF7 cell line was 65.75% at 50 µg/mL concentration by MTT assay method. **Conclusion:** The results of this study demonstrate that the Siddha polyherbal formulation Tulasi oil has significant antidiabetic and antiproliferative activities.

Keywords: Siddha, Tulasi oil, antidiabetic, antiproliferative, MCF7 cell line

*Author for Correspondence. E-mail: dr.yavana25@gmail.com

Introduction

A person with diabetes mellitus experiences changes in hormones, metabolic enzymes, and lipid peroxidation which results in liver and kidney damage. Diabetes mellitus is treated with oral hypoglycemic agents and insulin, which are available in different types [1]. A major concern to mankind is cancer, the second most common cause of death after cardiovascular diseases [2]. Breast cancer is extremely common in developing countries like India, and the number may rise alarmingly in the future because of environmental pollution, lifestyle changes, and genetically modified foods [3]. There are a number of genes involved in breast cancer development, and histology reveals a heterogeneous population of cells [4,5]. As a result, compounds that prevents the cell division, reduce the level of cell proliferation, induce cell death, and/or stimulate apoptosis would be ideal for the treatment of cancer [6].

Since synthetic and commercial medicines are associated with a variety of side effects, herbal remedies are becoming increasingly popular. Furthermore, alternative medicines must be highly effective, affordable, and readily available to the public at nontoxic levels. For this purpose, it is necessary to test the molecules of plant products with the ability to act at different dose levels that aren't toxic. It is believed that Siddha and Ayurveda treat a wide variety of diseases with raw and lyophilized herbal extracts, including cancer and diabetes [7].

Thulasi oil is a polyherbal formulation prescribed in the management of childhood asthma. Literature review of the ingredients of Tulasi oil revealed that the drugs are good expectorant, analgesic, anti-allergic, antidiabetic agent, anti inflammatory, antioxidant, antidote, anti-pyritic, carminative and antimicrobial agent [7]. It contains various phytoconstituents i.e., Eugenol, Naphthalene, Oleic acid and Ricinoleic acid derivatives which possess the actions of bronchodilator, anti-inflammatory, anti-tussive, anti-asthmatics, anti-oxidant, anti-viral, antibiotics etc [8]. Since not much research had been done to evaluate the biological activity of the Tulasi Oil, thus the aim of the present study was to assess the anti-diabetic and anti-proliferative potential of Siddha polyherbal formulation Tulasi oil (SPFTO).

Materials and methods

a) Non-enzymatic glycosylation of haemoglobin method

SPFTO's antidiabetic activity was measured *in vitro* by measuring non-enzymatic hemoglobin glycosylation, measured colorimetrically at 520nm. In phosphate buffer 0.01 M, pH 7.4, glucose (2%), haemoglobin (0.06%), and Gentamycin (0.02%) solutions were prepared. One ml of each solution was mixed with each other. The concentrations, 100 – 500 µg/ml of SPFTO was added to above mixture. Incubation of the mixture was in the dark for 72 hours, and glycosylation of hemoglobin at 520nm was measured using a colorimetric method. Metformin was used as standard drug for the study, and percentage of inhibition was calculated [9].

$$\% \text{ inhibition} = \frac{As - Ac}{As} \times 100$$

b) Glucose uptake in Yeast cells method

Glucose uptake assays in yeast was mostly depends on the utilization of radioactive glucose or its derivatives, which experiences from numerous inherent problems in the use of radioactive substances. This hampers the development of facile methods for measuring glucose transport activity. Commercial baker's yeast was centrifuged several times (3,000 grams; 5 minutes) in distilled water until the supernatant fluids were clear, and a 10% (vol/vol) suspension was prepared. A mixture of SPFTO concentrations (100–500g/ml) was added to 1ml of glucose solution (5, 10, and 25 mM) and incubated for 10 minutes at 37°C. A yeast suspension was added and vortexed for 60 minutes at 37°C. After 60 minutes, the tubes were centrifuged (2,500 x g, 5 min), and glucose was measured in the supernatant, with Glynase taken as standard [10].

c) α- Amylase Inhibition method

One ml of potato starch (1% w/v), one ml of Glinil drug solution/ SPFTO at five concentrations of 100, 200, 300, 400 and 500µg/ml was taken. One ml of α- amylase enzyme (1% w/v) and two ml of acetate buffer (0.1 M, 7.2 pH) was added on the test and standard drug sample. An iodine-iodide indicator was added to the mixture after the above mixture had been incubated for 1 hour. The absorbance was measured at 565nm in UV-visible spectroscopy [9]. All the experiments were carried out in triplicates. The percentage of α- amylase inhibition was calculated using the following formula

$$\text{Increase in glucose uptake (\%)} = \frac{As - Ac}{As} \times 100$$

d) Anticancer Property of purified Molecules:

Preparation of MCF-7 cell suspension

MCF-7 cell line was obtained from NCCS, Pune and it was subcultured in Dulbecco's Modified Eagle's Medium (DMEM) and trypsinized separately. A flask containing disaggregated cells was then filled with 25 mL of DMEM containing 10% FCS. By gently passing the cells through the medium with the pipette, the cells were homogenized [11].

Seeding of cells

The homogenized cell suspension was added to 24 well culture plate along with SPFTO (0-500 g/mL) and incubated in a humidified CO_2 incubator with 5% CO_2 for 48 hours in an inverted tissue culture microscope (LARK Innovative Fine Technology, Chennai). Cytotoxicity assays were performed with 80% confluence of cells [11].

Cytotoxicity assay

An assay was performed using MTT (Sigma Aldrich, Bangalore) which, when cleaved by mitochondrial succinate dehydrogenase and reductase, yields a measurable purple dye formazan. During 48 hours of incubation, MTT was added to wells and left for 3 h at room temperature.

Formazan production is directly proportional to viable cell number, while cytotoxicity is inversely proportional. After removing the contents from all wells, 100ml SDS in DMSO was added to dissolve the formazan crystals, and absorbance was measured at 540 nm using a Lark LIPR-9608 microplate reader [12].

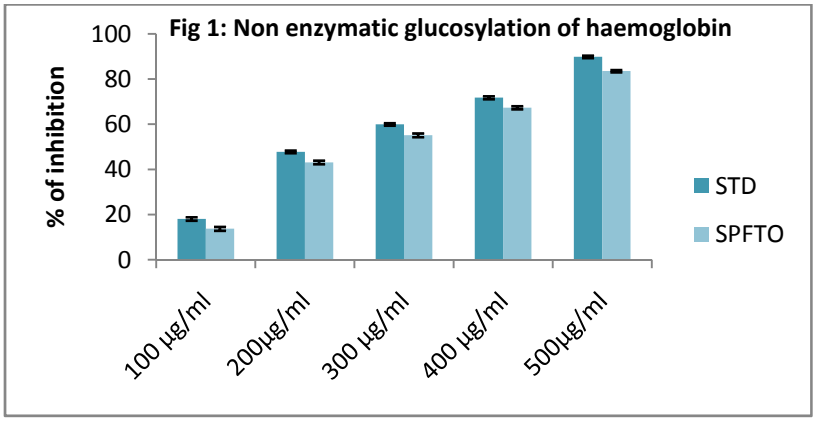
Statistical analysis

The experiments were repeated three times and the mean values are presented with mean ± Standard Deviation (SD).

Results and Discussion

a) Non enzymatic glycosylation of haemoglobin method

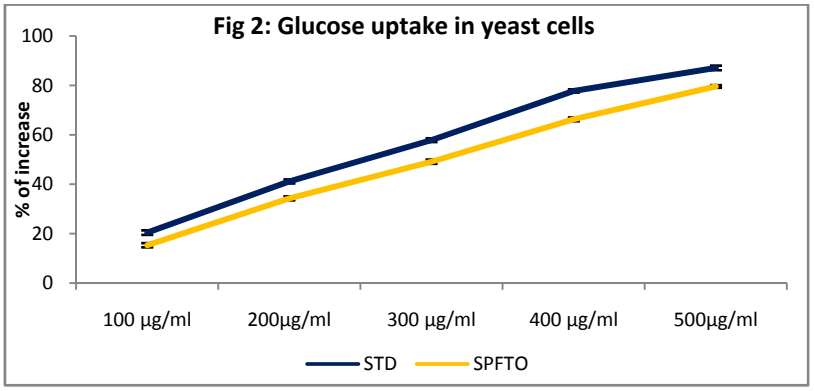
Inhibition of the haemoglobin glycosylation by SPFTO is evident from an increase in haemoglobin concentrations. The SPFTO at the concentration of 500 µg/ml exhibited higher inhibition of glycosylation (83.53±0.511%) as compared with the standard drug (89.84±0.529%) in same concentration. During the period of 72 hours, the SPFTO decreases hemoglobin glycosylation by decreasing the formation of the glucose-hemoglobin complex and amount of free hemoglobin increases. Similarly, According to Acharya [13] the stem bark of *Bauhinia purpurea* can act as a good antidiabetic herb. There is a dose dependence inhibition of glycosylation. As the drug concentration increases, the percentage inhibition increases because the formation of glucose-haemoglobin complex decreases [free haemoglobin increases], which indicates glycosylation inhibition. It is not important to perform this test in order to detect diabetes. It is more important to judge how diabetes is controlled. Red blood cells contain hemoglobin that is bound to glucose, which creates an abduct Alc. The higher level of the blood-glucose concentration is meant by the greater the amount of glucose-bound (called glycosylated) hemoglobin. Such glucose hemoglobin links are quite stable and can last for 60 to 120 days (the life span of red blood cells). So, glycosylated hemoglobin serves as a reliable indicator of glucose levels in the blood (the degree of control over the disease). It should not be more than 12%.



Values are expressed as mean ± SEM

b) Glucose uptake in yeast cells

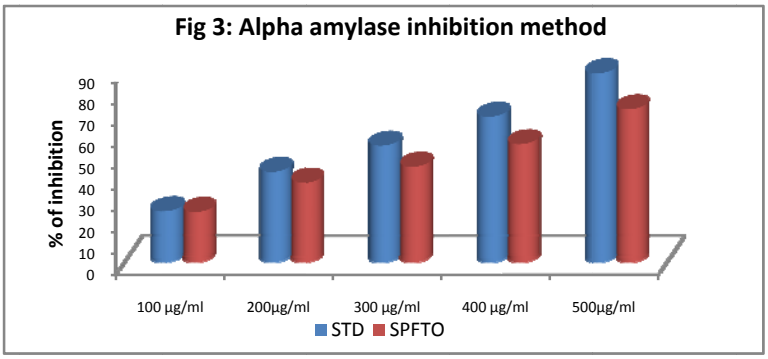
Assays like this use the SPFTO to detect glucose movement across yeast membranes. It was used as a suspension medium of yeast cells, suspended in SPFTO and glucose at various concentrations. A significant amount of glucose remained in the solution after incubation due to the effect of SPFTO on yeast cells. SPFTO significantly increased yeast cell glucose uptake after 500 µg/ml glucose concentrations by 79.57±0.525% and minimum uptake of glucose at 100 µg/ml glucose concentrations (15.34±0.851%). The result suggests that SPFTO exhibited maximum level inhibition (Fig 2). Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers. It is reported that in yeast cells (*Saccharomyces cerevisiae*) glucose transport is extremely complex and it is generally agreed that glucose is transported in yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose [14].



Values are expressed as mean ± SEM

c) Alpha amylase inhibition method

Amylase is an enzymatic protein that breaks down starches into simple sugars. As the concentration of SPFTO increased from 100 to 500µg/ml, the percentage inhibition of α amylase increased from 23.53 to 70.91% (Fig 3). Whereas, the standard anti-diabetic drug glinil showed the highest percentage of α amylase inhibitory activity (88.9%) at the dose of 500 µg/ml. The enzyme alpha amylase breaks down big alpha-linked polysaccharides like glycogen and starch to produce glucose and maltose. Alpha amylase inhibitors bind to the alpha-bond of polysaccharides to stop them from breaking down into mono- and disaccharides. [15].



Values are expressed as mean ± SEM

d) In vitro anti-proliferative assay

As shown in Table 1, SPFTO caused a dosage (3.125–400 µg/mL) dependent inhibition of cell proliferation towards MCF-7. Noticeably, the present investigation observed significant decrease in cell survival in these cancer cell lines when treated with the SPFTO. The percentage of maximum cell viability of SPFTO was 84.56% (minimum cell inhibition- 15.44%) observed at 3.125 µg/ml whereas minimum cell viability of SPFTO was 61.38% (maximum cell inhibition - 38.62%) observed at 400µg/ml. The IC50 of the SPFTO was 148.36 µg/ml against MCF-7 cell lines (Table 1). Similarly, Pradhan et al., [16] evaluated the different concentrations of aqueous extract of *Ocimum gratissimum* exhibited significant antiproliferation activity against MCF-7 cell line with an IC50 value 41.7 µg/ml [16]. Also, Nangia-Makker et al., [17] showed that crude extract of *Ocimum gratissimum* and its hydrophobic and hydrophilic fractions differentially inhibit breast cancer cell chemotaxis and chemo-invasion *in vitro* and retard tumour growth. It may concluded that tulasi oil have significant levels of bioactive components that play a major role in the cytotoxicity and deterioration of cancer cells.

Table 1: *In vitro* cytotoxicity effect of Compound against SPFTO

S. No	Sample Concentrations (µg/ml)	MCF-7 Cell Viability (%)
1.	0	100.00
2.	3.125	84.56
3.	6.25	82.10
4.	12.5	74.62
5.	25	71.52
6.	50	63.55
7.	100	66.10
8.	200	65.17
9.	400	61.38
10.	**IC50**	**148.386**

Conclusion

According to this study, the Siddha polyherbal formulation Tulasi oil shows strong anti-diabetic action (non-enzymatic glycosylation of haemoglobin, glucose uptake in yeast cells, and alpha amylase suppression methods), as well as anti-cancer activity. To project Tulasi oil's polyherbal formulation as a therapeutic target in research on the treatment of diabetes and cancer, further pharmacological and biochemical studies will be useful in elucidating the mechanism of action and providing important information.

Acknowledgement

The Authors gratefully acknowledge the Director and faculties, Govt. Siddha Medical College, Chennai for their extensive support and advice throughout this study.

Conflict of Interest: The authors have declared that there is no Conflict of Interest.

References

1. Tsang, M. W. (2012). The management of type 2 diabetic patients with hypoglycaemic agents. *ISRN endocrinology*, *2012*, 478120.
2. Nagai, H. & Kim, Y.H. (2017). Cancer prevention from the perspective of global cancer burden patterns. *Journal of thoracic disease*, *9*(3), 448–451.
3. Brody, J. G. & Rudel, R. A. (2003). Environmental pollutants and breast cancer. *Environmental health perspectives*, 111(8), 1007–1019.
4. Torre, L. A., Bray, F., Siegel, R. L. *et al*., (2015). Global cancer statistics, 2012, CA: *A Cancer journal for clinicians*, 65(2), 87–108.
5. Torre, L. A., Siegel, R. L., Ward, E. M. & Jemal, A. (2016). Global cancer incidence and mortality rates and trends-an update. *Cancer epidemiology biomarkers & prevention*, *25*(1), 16–27.
6. Deng, S., Shanmugam, M. K., Kumar, A. P., Yap, C. T., Sethi, G., & Bishayee, A. (2019). Targeting autophagy using natural compounds for cancer prevention and therapy. *Cancer*, *125*, 1228–1246.
7. Yavanarani, S., Sathiyabama, M., Mirunaleni, P., Suresh, K., Meenakshi Sundaram, M. & Banumathi, V. 2021. Therapeutic effectiveness of Siddha formulation Thulasi Ennai: A Review. *Int J Curr Res Chem Pharm Sci, 8*(4), 9-15.
8. Yavanarani, S., Mirunaleni, P., Sathiyabama, M., Suresh, K., Meenakshi Sundaram, M. & Banumathi, V. 2018. GCMS analysis of Thulasi ennai – A Siddha polyherbal formulation. *Int J Adv Multidiscip Res, 5*(7), 65-69.
9. Selvi, R. & Yogananth, N. 2016. *In vitro* evaluation of antidiabetic potential of leaf and stem extracts of *Solanum xanthocarpum* and *Solanum nigrum. Int J Adv Res Biol Sci*, *3*(12), 191-195.
10. Ramanathan, S., Vijaya, PP., Yogananth, N., Anuradha, V. & Syed ali, M. 2014. *In vitro* evaluation of antidiabetic potential of *Gymnema sylvestre* and *Dregea volubilis*. *International Journal of comprehensive research in biological sciences*, *1*(1), 9-12.

11. Abondanza, T. S., Oliveira, C. R., Barbosa, C. M. V., Pereira, F. E. G., Cunha, R. L. O. R., Caires, A. C. F., Comasseto, J. V., Queiroz, M. L. S., Valadares, M. C. & Bincoletto, C. 2008. Bcl-2 expression and apoptosis induction in human HL60 leukaemic cells treated with a novel organotellurium (IV) compound RT-04. *Food and chemical toxicology, 46*, 2540-2545.

12. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of immunological methods, 65*, 55-63.

13. Acharya, A. S. & Manning, J. M. 1980. Reactivity of the amino groups of carbon monoxy hemoglobin with glyceraldehydes. *Journals of biological chemistry*, *255*(4), 1406–1412.

14. Teysink, B., Jasper, A., Diderich, H. V., Westerhoff, D. K. V. & Walsh, M. C. 1998. Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%. *J Bacteriol*, *180*, 556–562.

15. Chan, E. W. C., Baba, S., Chan, H. T., Kainuma, M. & Tangah, J. 2016. Medicinal plants of sandy shores: A short review on *Vitex trifolia* L. and *Ipomoea pes-caprae* (L.) R. Br. *Indian journal of natural products and resources*, *7*(2), 107-115.

16. Pradhan, S., Pradhan, D. & Behera, B. 2018. Antiproliferation activity of *Ocimum gratissimum* aqueous extracts on human breast cancer MCF‐7 cell line. *World J Pharm Res*, *7*, 421‐8.

17. Nangia-Makker, P., Raz, T., Tait, L., Shekhar, M. P., Li, H., Balan, V., Makker, H., Fridman, R., Maddipati, K. and Raz, A. 2013. *Ocimum gratissimum* retards breast cancer growth and progression and is a natural inhibitor of matrix metalloproteases. *Cancer Biol Ther*, *14*(5), 417-427.