FINAL REPORT OF MINOR RESEARCH PROJECT

Titled

"Role of Growth Factors of *Tinospora cordifolia* in Lymphocyte culture"

UGC Reference No. : F.No.MS-28/202057/XII/14-15/CRO. Dated- 09Feb2015

Submitted by

Mrs. Chanchal Chaturvedi

Asstt. Professor-Botany

Dr. Radhabai Govt. Navin Girls College, Raipur (C.G.)

Acknowledgement

I would like to express my special thanks and gratitude to our college principal, Dr. Aruna Palta, who motivated me to do this wonderful project on the topic **"Role of Growth Factors of *Tinospora cordifolia* in Lymphocyte culture."**

Secondally I would like to thanks my guide Dr. Suneeta Patra Asstt.Prof.(Botany),and co-guide Dr. P.K. Patra, Dean, Medical College,Bilaspur, who helped me a lot in finalizing this project within limited time frame, supporting and motivating me.

The project work aims for screening of *Tinospora cordifolia* for identification of bioactive molecules which could be involved in lymphocytes proliferation which play an important role in immunity processes.

I would like to extend a very special thanks to Dr.Jagannath pal, who helped me in lymphocyte isolation and culture techniques.

I would also like to a very special thanks to Dr.Pankaj Mishra, who helped me in phytochemical screening processes.

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I want to thank all the people associated with my project, directly or indirectly.

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ENCLOSURE-1

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Abstract

This study evaluates the effect of extracts of *Tinospora cordifolia* on *in vitro* proliferation of human mononuclear cells. Lymphocyte proliferation assay was carried out by incubating human peripheral blood mononuclear cells from healthy donor with different solvents extracts of *Tinospora cordifolia* stem, root and leaves at 100 µg /mL and 200 µg /mL, for 24 hours. The quantification of cells proliferation assay was performed by Trypan blue. Cells incubated, with only the culture medium, were used as control for cell proliferation, while the cells with plant extracts were evaluated. The result suggests, that the extract of *Tinospora cordifolia* was found to significantly enhance the proliferation of lymphocytes, as compared with control, while some solvents extracts performed cytotoxicity as compared to control. The proliferation of lymphocyte indicated the increase in the number of B and T cells, which release cytokines and growth factors that regulate other immune cells and secretion of antibodies in the blood.

Keywords: *Tinospora cordifolia,* Lymphocyte, proliferation, extract, cells, blood.

Introduction

Tinospora cordifolia (family-Menispermaceae) is a medicinal plant, commonly known as Giloy or Guduchi, a glabrous climbing shrub, is widely used in many Ayurvedic formulations. The use of plants, their extract and pure isolated compounds provide the base for modern pharmaceutical compounds. Plants

produce wide range of bioactive molecules provide defense against various diseases and infections.

A variety of active component derived from *Tinospora cordifolia* like alkaloids, flavonoids, phenols, tannins, terpens, sterols, glycosides and saponins have been isolated from the different parts of the plant body including stem, root and whole plant.(Upadhyay,2010).

Recently, the plant is of great interest to researcher across the globe because of its reported medicinal properties like anti-diabetic, anti-inflammatory, anti-cancer, anti-oxidant, anti-allergic, anti-arthritic, anti-spasmodic, anti-leprotic, anti-malarial, hepatoprotective and immune modulatory activities. (Sharma,2012). Determination of biological active compounds of *Tinospora cordifolia* is highly dependent on the type of solvent used in extraction procedure. This emphasizes the need to try as much solvents as possible in screening plant parts for phytochemicals. In the present study therefore different solvents like water, chloroform, ethanol, ethyl acetate and petroleum ether were used to obtain extracts of *Tinospora cordifolia* to screen for the presence of phytochemicals.

Collection and preparing plant materials:

The plant *Tinospora cordifolia* was collected from different areas of Raipur city and neighbouring towns. The plant was identified by flora of Hains (1961).The entire plant was washed and then dried in the shade at room temperature until all the plants parts became well dried. After drying, the plant material were powdered well by using grinder and placed into a well closed container.

Soxhlet apparatus and Shaker device were used for the extraction of phytoconstituents from the plant powder. Double beam UV-Visible Spectrophotometer was used for measuring the absorbance. Rotary vacuum evaporator and water bath were used for evaporation, Refrigerator was used for storage, Centrifuge machine and weighing balance were also used.

The phytochemical screening showed that *Tinospora codifolia* plant extracts contain a mixture of phytochemicals as alkaloids, flavonoids, saponins, phenols,

tannins, sterols, terpenes, glycosides and carbohydrates. The quantitative estimation indicated that the aqueous extracts had the highest contents of secondary metabolites. The secondary metabolites present in this plant are known to possess medicinal properties. The phytoconstituents like alkaloids have antioxidant, anti-asthmatic and immunomodulatory property and generally flavonoids have antioxidant and anti-inflammatory property. Tannin exhibit remarkable toxicity against bacteria and fungi. Phenolic compounds have antioxidant and anti carcinogenic properties. Saponins are known to possess inhibitory effects on inflammation and have antibacterial property. Therefore this plant can be potentially used for therapeutic purposes.

Lymphocytes :

Lymphocytes are a type of WBC generated by the immune system to defend the body against cancerous cells, pathoges and foreign matter. They circulate in blood and lymph fluid and are found in body tissues including the spleen, thymus, bone marrow, lymph node, tonsils, and liver. Lymphocyte provides a means for immunity against antigens. This is accomplished through two types of immune responses, humoral immunity and cell mediated immunity, humoral immunity focuses on indentifying antigens prior to infection, while cell mediate immunity focus on the active destruction of infected or cancerous cell.

There are three main types of lymphocytes, T cell, B cells and natural killer cells. Two of these lymphocytes are critical for specific immune responses. They are B cells and T cells.

Isolation of lymphocyte from whole blood :

Three ml of blood is taken from normal healthy individuals and collected in heparinised test tube. Three ml of Phosphate Buffered Saline (PBS) is added and, mixed well, 2 ml of ficoll hypaque solution is taken and carefully blood PBS mixture is layered on to the ficoll hypaque solution. It is centrifuged at 2700 rpm for 30 min. The opaque interface containing mononuclear cells is collected, mixed with PBS and centrifuged at 2000 rpm for 10 min and supernatant is discarded.

The centrifugation is repeated thrice and normal lymphocytes are resuspended in RPMI medium with 10% fetal bovine serum.

The research study will be designed to see the effect of growth factors of *Tinospora cordifolia* at cellular level. In our proposed work, lymphocyte will be isolated from whole blood, after that serum free medium i.e. RPMI-1640 will be prepared and in that medium lymphocyte will be cultured using different dilutions of *Tinospora cordifolia* extract, the effect of growth factors of *Tinospora cordifolia* on the lymphocyte cells proliferation will be observed.

Result and Discussion-

Treatment of varying doses of *Tinospora cordifolia* extract was significantly increased the total lymphocyte concentration. Result of these studies also revealed that *Tinospora cordifolia* has dose dependent effects.

Table1: Role of *Tinospora cordifolia* stem growth factors on Lymphocyte culture

SN.	SOLVENTS	CONCENTRATION	VIABLE % OF LYMPHOCYTES
1	Water	Control	89%
	Water	100µg/mL	91%
	Water	200µg/mL	87%
2	Chloroform	Control	98%
	Chloroform	100µg/mL	96%
	Chloroform	200µg/mL	93%
3	Ethyl acetate	Control	97%
	Ethyl acetate	100µg/mL	97%
	Ethyl acetate	200µg/mL	97%
4	Ethanol	Control	97%
	Ethanol	100µg/mL	99%
	Ethanol	200µg/mL	98%

The immune stimulatory activity of *Tinospora cordifolia* stem aqueous extract on *in vitro* culture of human mononuclear cells observed ,in this study indicates the high concentration of plant extract cytotoxic to lymphocyte proliferation, but low

concentration of plant extract significantly improved the lymphocyte proliferation. Aqueous extract of *Tinospora cordifolia* stem exhibited lymphocyte proliferation at 100µg/mL and the above extract show cytotoxic activity at 200µg/mL as compared to control. It indicates that the viability of lymphocytes was in the rank order;

100µg/mL > control >200µg/mL (In aqueous extract of stem)

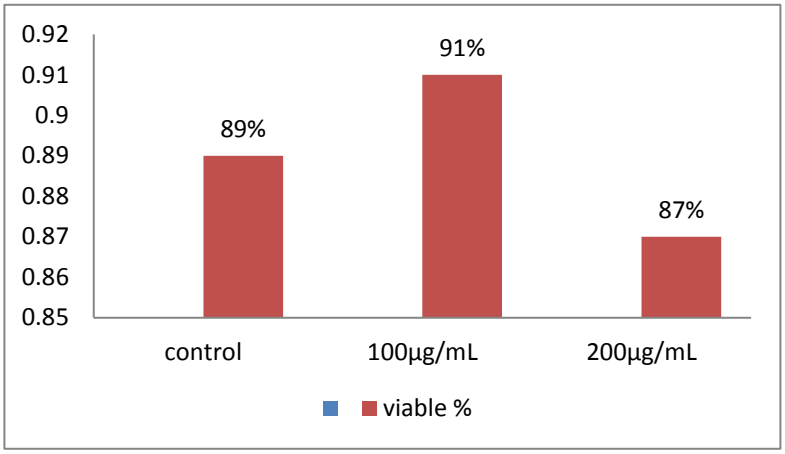

Fig. 1: Presentation of viable % of Lymphocytes in Aqueous extract of stem.

Tinospora cordifolia stem chloroform extract did not show any stimulating activity at 100µg/mL and 200µg/mL as compared to control, the above extract show cytotoxic activity as compared to control. It indicates that the viability of lymphocytes was in the rank order; Control > 100µg/mL >200µg/mL

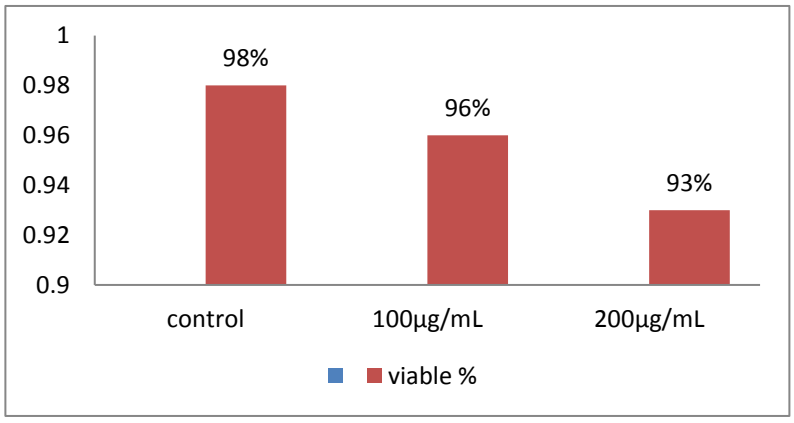

Fig. 2: Presentation of viable % of Lymphocytes in Chloroform extract of stem.

Tinospora cordifolia stem ethylacetate extract did not show any stimulating activity at 100µg/mL and 200µg/mL as compared to control, the above extract was similar to the control.

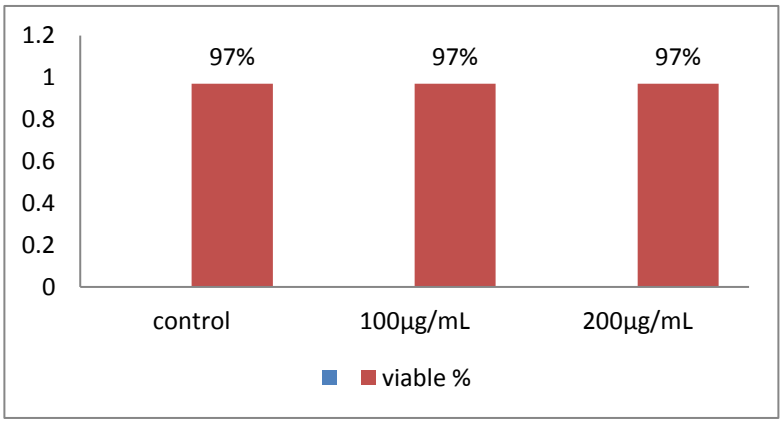

Fig. 3: Presentation of viable % of Lymphocytes in Ethylacetate extract of stem.

The immune stimulatory activity of *Tinospora cordifolia* stem ethanol extract exhibited boosting effect of lymphocyte on in vitro culture at 100µg/mL and 200µg/mL as compared to control. The result showed that viable cells percentage was higher at extract concentration of 200µg/mL and 100µg/mL than for control. It indicates that the viability of lymphocytes was in the rank order;

100µg/mL > 200µg/mL > control.

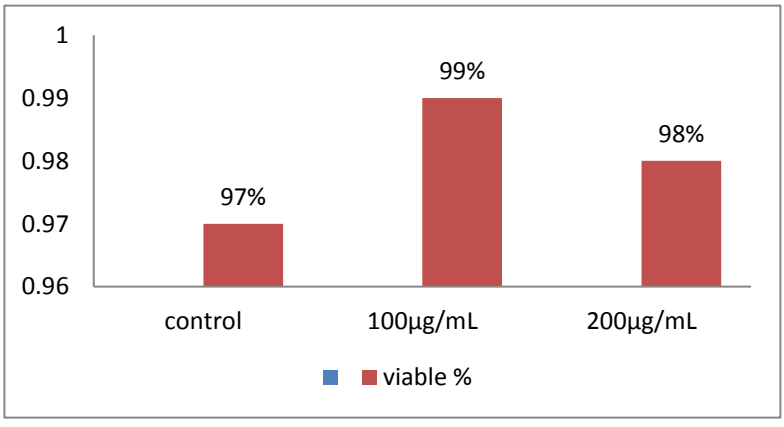

Fig. 4: Presentation of viable % of Lymphocytes in Ethanol extract of stem.

Table2: Role of *Tinospora cordifolia* leaves growth factors on Lymphocyte culture

SN.	SOLVENTS	CONCENTRATION	VIABLE % OF LYMPHOCYTES
1	Water	Control	94%
	Water	100µg/mL	95%
	Water	200µg/mL	96%
2	Ethanol	Control	97%
	Ethanol	100µg/mL	97%
	Ethanol	200µg/mL	98%
3	Petroleum ether	Control	98%
	Petroleum ether	100µg/mL	97%
	Petroleum ether	200µg/mL	97%

The immune stimulatory activity of *Tinospora cordifolia* leaves aqueous extract exhibited boosting effect of lymphocyte on in vitro culture at 200µg/mL and 100µg/mL as compared to control. The result showed that viable cells percentage was higher at extract concentration of 200µg/mL and 100µg/mL than for control. It indicates that the viability of lymphocytes was in the rank order;

200µg/mL > 100µg/mL > control.

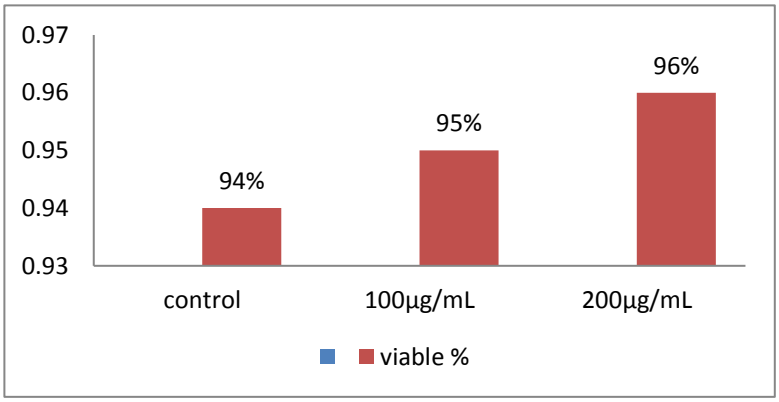

Fig. 5: Presentation of viable % of Lymphocytes in Aqueous extract of leaves.

The immune stimulatory activity of *Tinospora cordifolia* leaves ethanol extract exhibited boosting effect of lymphocyte on in vitro culture at 200µg/mL as compared to control, while 100µg/mL concentration exhibited similar effect as control.

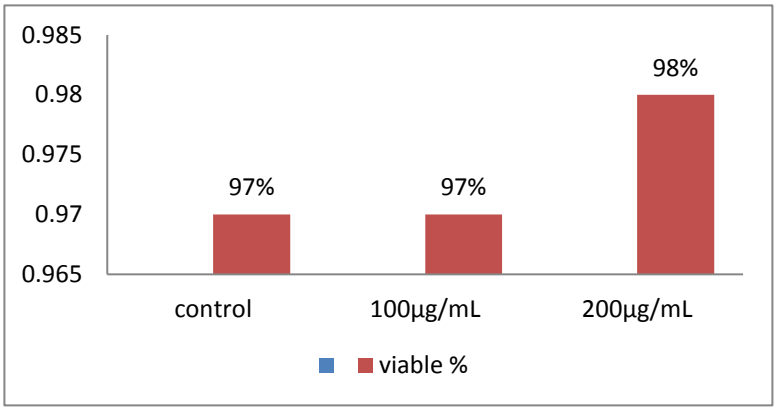

Fig. 6: Presentation of viable % of Lymphocytes in Ethanol extract of leaves.

Tinospora cordifolia leaves petroleum ether extract exhibited cytotoxic effect of lymphocyte on *in vitro* culture at 200µg/mL and 100µg/mL as compared to control.

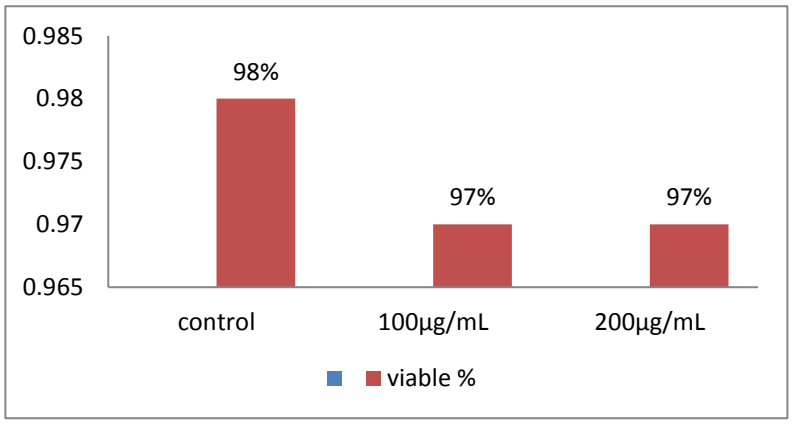

Fig. 7: Presentation of viable % of Lymphocytes in Petroleum ether extract of leaves

Table3: Role of *Tinospora cordifolia* root growth factors on Lymphocyte Culture

SN.	SOLVENTS	CONCENTRATION	VIABLE % OF LYMPHOCYTES
1	Water	Control	98%
	Water	100µg/mL	96%
	Water	200µg/mL	97%

Tinospora cordifolia root extract on *in vitro* culture of human mononuclear cells observed in this study did not show any activity at 100µg/mL and 200µg/mL as compared to control, the above extract show cytotoxic activity as compared to control.

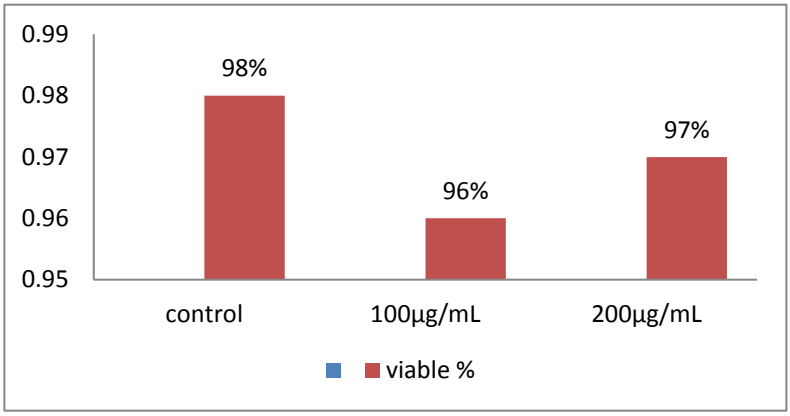

Fig. 8: Presentation of viable % of Lymphocytes in Aqueous extract of root.

Conclusion-

From the above results, it can be concluded that *Tinospora cordifolia* plant has potent several secondary metabolites such as, alkaloids, phenols, tannins, flavonoids, saponins, terpenes, glycosides, sterols etc. which plays an important role in immunity and whole plant parts growth factors with different solvents at different dilutions are responsible for lymphocytes proliferation.

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