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Cytotoxicity analysis of anti-diabetic activity spectra of a polyherbal formulation

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Abstract

Since there is a very huge development in the use of traditional medicine, assurance and potency as well as quality control of herbal medicines and traditional therapies are becoming more important. Hence, to determine the active principal compounds and its possible biological and pharmacological activities of a polyherbal formulation (PHF) was made up of 11 plants namely *Moringa oleifera* Lam., *Aegle marmelos* (L.) Correa, *Murraya koenigii* (L.) Spreng, *Annona reticulata* L., *Piper betle* L., *Psidium guajava* L., *Momordica charantia* L., *Syzygium cumini* (L.) Skeels, *Gymnema sylvestre* R.Br, *Nelumbo nucifera* Gaertr., *Pterocarpus marsupium* Roxb. DMSO method used and got the cytotoxicity results.

Key words : Anti-diabetic, Cytotoxicity.

Abbreviations :
PHF – Polyherbal formulation

Diabetes mellitus is one of the most common metabolic disorders affecting millions of people worldwide. It occurs when the body is unable to properly regulate blood sugar (glucose) levels due to problems with insulin production, insulin function, or both. Insulin is a hormone produced by the pancreas that helps glucose move from the bloodstream into the body's cells, where it is used for energy. When this process is disrupted, glucose accumulates in the blood, leading to a condition known as hyperglycaemia[1,14].

Diabetes is generally classified into three main types: Type 1 diabetes, Type 2 diabetes, and gestational diabetes mellitus[1,11]. Type 1 diabetes develops when the body's immune system mistakenly attacks the insulin-producing cells of the pancreas, resulting in little or no insulin production. Type 2 diabetes, the most common form, occurs when the body becomes resistant to insulin or does not produce enough insulin to maintain normal blood glucose levels. Gestational diabetes develops during pregnancy and may increase the risk of

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developing Type 2 diabetes later in life[1].

The disease has been recognized since ancient times and was often identified by the presence of "sweet urine," a result of excess glucose being excreted from the body through urine. Under normal conditions, insulin helps maintain blood glucose levels within a healthy range. However, in people with diabetes, either insufficient insulin production or the body's inability to respond effectively to insulin causes blood glucose levels to remain elevated[4].

Over the past few decades, the prevalence of diabetes has increased significantly, making it a major global health concern. It is estimated that around 250 million people worldwide are affected by diabetes, with nearly 50 million cases reported in India alone [7,13]. The number of people living with diabetes is expected to continue rising, placing a substantial burden on healthcare systems and society[6].

Although considerable progress has been made in diabetes management, controlling the disease remains a challenge. Modern treatments, including insulin produced through recombinant DNA technology, have improved the quality of life for many patients. However, these treatments may not completely eliminate the complications associated with diabetes. Therefore, researchers continue to explore natural and alternative approaches that may offer safer, more effective, and long-term solutions for the management of both Type 1 and Type 2 diabetes mellitus[3,8].

Types of Diabetes :

1. Diabetes insipidus (DI)
2. Diabetes mellitus
3. Gestational

Diabetes Mellitus :

Diabetes mellitus refers to a group of metabolic disorders characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both[1]. Common symptoms associated with hyperglycaemia include polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased hunger)[14]. If left untreated, diabetes mellitus can lead to numerous complications. Acute complications include diabetic ketoacidosis (DKA) and hyperosmolar hyperglycaemic state (HHS), while long-term complications encompass cardiovascular disease, stroke, chronic kidney disease, diabetic foot ulcers, neuropathy, and diabetic retinopathy[15]. The condition develops either because the pancreas fails to produce sufficient insulin or because the body's cells do not respond effectively to the insulin produced[4].

Impact of Diabetes :

Over time, diabetes can lead to blindness, kidney failure, and nerve damage due to microvascular complications. Diabetes also accelerates atherosclerosis, increasing the risk of cardiovascular disease and stroke, which are major macrovascular complications [1,14]. The global prevalence and economic burden of diabetes continue to rise, imposing substantial healthcare costs and productivity losses[6].

Polyherbal formulation (PHF) :

Polyherbal formulation (PHF) is composed of eleven medicinal plants, namely *Moringa oleifera*, *Aegle marmelos*, *Murraya koenigii*, *Annona reticulata*, *Piper betle*, *Psidium guajava*, *Momordica charantia*,

Syzygium cumini, *Gymnema sylvestre*, *Nelumbo nucifera*, and *Pterocarpus marsupium*, which are well known for their therapeutic and antidiabetic properties[12].

Cytotoxicity Analysis :

Following treatment, cell viability was assessed using the MTT assay as described by Mosmann (1983).

1. PANC-1 cell line (passage no.) were grown in 10% complete media (10% FBS + 90% DMEM) and were incubated in humidified conditions at 37° C (Carbon dioxide incubator by Eppendorf Cellxpert) until the confluency was achieved. Media and Fetal Bovine Serum were supplied by Gibco.
2. Approximately 1×104 cells were seeded in each well of 96 well plate (Tarsons).
3. A well was used for addition of media and cells only (Untreated or without treatment) and a well was seeded with cells and treatment with the solvent was given to check the effect of solvent solely on cell lines.
4. After seeding, the treatment of samples FA, FB, FC, FD, FE, FF was given according to the concentrations provided and the plate was incubated for 24hours .
5. As it was single time point study, MTT reagent was added to the wells after 24 hours. MTT with concentration (5mg/ml) was used at volume of 50 µl was added after 24 hours of incubation. [MTT reagent was dissolved in 1x PBS buffer]
6. After 3-4 hours of incubation with MTT reagent, the wells were observed for the presence of formazan crystals which were later dissolved in DMSO (Hi Media) at volume of 100 µl. (Entire process of MTT assay was performed in dark conditions and the multi-well plate was incubated with wrapped foil).
7. Absorbance of all the wells were scored at 570nm using Epoch multiplate reader supplied by Bio Tek.

Sample FA treatment on PANC-1 cell line:

Table-1. Effect of Sample FA Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm%	Cell viability	% Cell inhibition
10	1.074	57.93	42.07
25	1.132	61.06	38.94
50	1.043	56.26	43.74
75	1.01	54.48	45.52
100	0.948	51.13	48.87
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Sample FB treatment on PANC-1 cell line :

Table-2. Effect of Sample FB Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm	% Cell viability	% Cell inhibition
10	1.143	61.65	38.35
25	1.222	65.91	34.09
50	1.162	62.68	37.32
75	1.171	63.16	36.84
100	1.185	63.92	36.08
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Sample FC treatment on PANC-1 cell line:

Table-3. Effect of Sample FC Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm	% Cell viability	% Cell inhibition
10	1.108	59.76	40.24
25	1.172	63.21	36.79
50	1.104	59.55	40.45
75	1.118	60.30	39.70
100	1.394	75.19	24.81
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Sample FD treatment on PANC-1 cell line:

Table-4. Effect of Sample FD Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm	% Cell viability	% Cell inhibition
10	1.182	63.75	36.25
25	1.210	65.26	34.74
50	1.075	57.98	42.02
75	1.106	59.65	40.35
100	1.133	61.11	38.89
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Sample FE treatment on PANC-1 cell line :

Table-5. Effect of Sample FE Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm	% Cell viability	% Cell inhibition
10	1.075	57.98	42.02
25	1.020	55.02	44.98
50	1.075	57.98	42.02
75	1.253	67.58	32.42
100	1.326	71.52	28.48
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Sample FF treatment on PANC-1 cell line:

Table-6. Effect of Sample FF Treatment on PANC-1 Cell Line Viability and inhibition at 24 Hours

Concentration (µg/mL)	Absorbance at 570nm	% Cell viability	% Cell inhibition
10	1.312	70.77	29.23
25	1.431	77.18	22.82
50	1.800	97.09	2.91
75	1.545	83.33	16.67
100	1.271	68.55	31.45
Untreated (Only cells + media)	1.854	——	——
Blank (DMSO)	0.178	——	——

Calculation:

% Cell viability = Absorbance (Treated)/ Absorbance (untreated)* 100

% Cell inhibition = 100 - % Cell viability

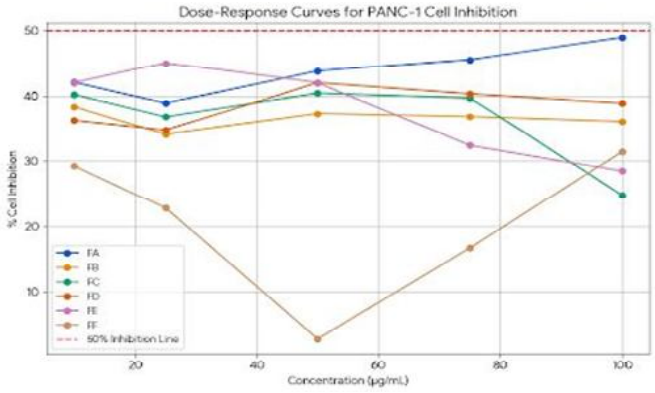

Fig. 1. Dose -Response Curves for PANC-1 Cell Inhibition

All samples were tested against a common untreated control, which had an absorbance of 1.854. This value serves as the reference point for calculating 100% cell viability.

Among all tested samples, Sample FA demonstrated the highest cytotoxic activity against PANC-1 cells, exhibiting the greatest percentage of cell inhibition across the tested concentration range. Further studies are required to identify the active constituents responsible for this activity and to evaluate their potential therapeutic applications.

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