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Exploring inhibitory potential of α–Glucosidase and α–Amylase activity and cytotoxic effect of *Glycyrrhiza glabra* traditionally used in Ayurveda for Diabetes management

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ABSTRACT

The herbs plant based medicine system utilized for various diseases treatment in Ayurveda medicine for many decades due to its great and broad medicinal values. The present study aimed at carrying out phytochemical analysis and evaluates its in-vitro antidiabetic activities of traditional medicinal plant Glycyrrhiza glabra. The plant root powder was collected from the local site of Davangere city, Karnataka, India. The Extraction of plant material was followed by the cold extraction method. An in vitro antidiabetic activity was evaluated by alpha-amylase method and α–Glucosidase inhibition assay method and estimated the rate of glucose uptake using 3T3L-1 cells. The Methanolic extracts of Glycyrrhiza glabra root showed significant inhibition of alpha-amylase and Alpha Glucosidase inhibition activity as compared with standard drug Acarbose and IC50 value was calculated. The phytochemical analysis revealed that Methanolic extract of Glycyrrhiza glabra root showed presence of secondary metabolites Alkaloid, Tannin, Terpenoids, Steroid, Saponin, Flavonoids, Mucilage, Volatile oil and these different phyto-compounds which might be responsible for significant in vitro antidiabetic activity. Further identification and isolation of bioactive substances and secondary metabolites from such medicinal plants and there in vivo efficacy evaluation will help in further validation of pharmacological activities in future prospective days.

Keywords: Glycyrrhiza glabra, Secondary metabolites, Antidiabetic, Acarbose, Cytotoxicity, 3T3L-1 cell lines.

INTRODUCTION:

Traditional medicine is an important part of healthcare system and the plants are the one of the most important sources of medicines of Ayurveda and other traditional system of medicines. Ayurveda is an ancient medical science, deals with the diagnosis of disease and its treatment with herbal and plant based medications (Jitendra *et al.,* 2020). *Glycyrrhiza glabra* commonly known as Yashtimadhu which has been used worldwide in various systems of medicine Ayurveda, Allopathic and other traditional system of medicine. It is mainly a perennial herb (Figure 1) with sweet root (Figure 2). It about 120cm in height, stolon is almost cylindrical, up to 2cm in diameter. The root is without pith, coarsely fibrous in bark and splintery in wood, odor and sweet in taste (Anilkumar, D *et al.*, 2012).The dried, peeled or unpeeled underground stems and roots constitute the drug, it bears compound pinnate leaves. Outer surface of yashtimadhu is yellowish-brown or longitudinally wrinkled with patches of cork. Flowers have pale blue appearance and flowering generally occurs from 2-3 years of planting onwards. The fruit is pod, 1-2cm long and 4-6mm in wide. The plant distribution is generally in a dry, sunny climate and is cultivated in the sub-tropical and warm temperate regions (Nidhi *et al.,* 2022).Plants and their secondary metabolite constituents are traditionally used for curing many diseases and have a long history of use in modern medicine such as atropine, morphine, quinine, vincristine, codiene, digoxine, etc. The pharmacological treatment of disease began long ago with the use of medicinal herbs. The chemical constitute of liquorice are glycyrrhizin, glycyrrhetinic acid, iso-flavonoids, chalcones, triterpenoids, coumarins and sterols, amines, gums, lignans, amino acids and volatile oils. These are found to be responsible for its various activities like antiulcer activity, wound healing activity, antiviral, anti-inflammatory, antioxidant, anti-diabetic, anti-arthritic etc (Teltumbde *et al.,* 2013). In old chines pharmacy it was considered to belong to drugs as a rejuvenating property when consumed for long period. It is the most prescribing herb after Ginseng in chines medicine used for aliments related Spleen, liver and Kidney (Anilkkumar *et al.,* 2012).

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with abnormal carbohydrate, fat, and protein metabolism due to defects in insulin secretion, insulin action, or both. The estimated number of people aged between 20 and 79 years with diabetes worldwide in 2015 was 415 million. The global prevalence of DM is expected to rise to 552 million by 2030.To overcome this serious problem the root of *Glycyrrhiza glabra* known as liquorice has various medicine uses it belonging to the herb plant that reduce blood sugar levels.

Liquorice can help to treat diabetes. It possesses hypoglycemic properties and its consumption helps in lowering glucose or sugar in blood. Invitro Alpha amylase and Alpha glucosidase method is used to demonstrate the Anti-diabetic activity. The Inhibition of Alpha amylase and Alpha glucosidase enzyme activity leads to reduce blood sugar level .Developing new antidiabetic medications from plant derived compounds which are easily accessible seems highly attractive research area as currently available medications have limitations in terms of safety, efficacy, and cost Globally, there are more than 1000 plant species that are being used as folk medicine for DM (Karthikeson *et al.,* 2017).

MATERIALS AND METHODS:

Collection and Extraction of Plant material

Glycyrrhiza glabra root powder was collected from local site of Davangere city, Karnataka, India. And it was authenticated by Department of Botany, Davangere University. Further extraction of plant material carried out by Cold Extraction method.

Cold Extraction

In the present study cold extraction was performed by using a beaker covered in aluminium foil, weighed out 10g of dried sample powder and dissolved it in 50ml of methanol. The extract was then filtered using Whatman filter paper and the filtrate was collected in a 250 ml beaker after the beaker was placed on a hot water bath at 50^{o}C for four hours. The filtrate was removed and stored at 50ºC for a few hours until the extract totally dried and changed into a semisolid state. Any residue that remained on the filter paper was disposed of, and the filtrate was taken for further use. The weight of the powder is recorded before and after drying. Further dried extract were used for phytochemicals screening, characterization and pharmacological activities (Harborne J.B. 1998).

Phytochemical Screening

Methanolic extract of *Glycyrrhiza glabra* root were successfully screened for the presence primary and secondary metabolites like Alkaloids, Carbohydrates**,** Tannins, Terpenoids, Glycosides, Steroids, Volatile oil were qualitatively determined as per the standard procedure with minor modifications to Vishnu Balamurgan *et al.,* 2019.

Phytochemicals are substances produced mainly by plants and these substances have biological activity. In the pharmaceutical industry, plants represent the main source to obtain various active

ingredients; they exhibit pharmacological effects applicable to the treatment of bacterial and fungal infections and also chronic-degenerative diseases such as diabetes and cancer. The following tests are conducted for qualitative analysis of secondary metabolites (Yadav *et al.*, 2011).

Test for Alkaloids

Dragendoff's test:

0.2ml of sample was taken and 0.2ml of HCl was added. To this 2-3 drops of Dragendoff's reagent was added and the appearance of orange or red precipitate and turbid solution indicates the presence of alkaloids (Nagaraju *et al.,* 2019)

Test for Carbohydrates:

Molisch's test: 0.2 ml of sample was mixed with few drops of Molisch's reagent (α- napthol dissolved in alcohol). 0.2 ml of sulphuric acid was added along the sides of the test tube and observed for the appearance of a purple colour ring for positive test

Test for Tannins:

Braymer's test: 0.2 ml of plant extract was mixed with 2 ml water and heated on water bath for 10 minutes. The mixture was filtered and ferric chloride was added to the filtrate and observed for dark green solution which indicates the presence of tannin.

Test for Terpenoids:

Salkowki's test:

0.2 ml of plant extract was taken in a test tube with 0.2 ml of chloroform. To this, concentrated sulphuric acid was added carefully to form a layer. Presence of reddish brown colour at the interface would show would show the presence of terpenoids.

Test for Glycosides:

0.2 ml of sample was mixed with 0.2 ml of chloroform.0.2ml of acetic acid was added to this solution and the mixture was cooled on ice. Sulphuric acid was added carefully and the colour change from violet to blue to green indicates the presence of steroidal nucleus (A glycone portion of glycoside.

Test for Steroids: Lieberman Burchardt tests: 0.2 ml of sample was mixed with 0.2 ml of chloroform. To this 0.2ml of concentrated sulphuric acid was added. The appearance of red colour in the lower layer of chloroform indicates the presence of steroids.

Test for Saponins:

Test for Saponins (Foam test): 0.2 ml of extract was added to 0.6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Flavonoids:

Alkaline reagent test: 0.2 ml of plant extract was taken in a test tube and mixed with dilute sodium hydroxide solution. To this diluted hydrochloric acid was added. Observation of yellow solution that turn colorless later would indicate the presence of flavonoids.

Mucilage test: 0.2ml of extract was taken in a test tube and 0.2ml of absolute alcohol was added and allowed to dry. If the precipitation occurs then mucilage is present.

Volatile oil:

0.2ml of extract was treated with few drops of dilute hydrochloric acid. The appearance of white precipitate indicates the presence of volatile oils.

α-Amylase inhibition assay:

The α-amylase catalyzes the hydrolytic degradation of polymeric carbohydrates such as amylase, amylopectin and glycogen by cleaving 1, 4, α-glycosidic bonds. In polysaccharides and oligosaccharides, several glycosidic bonds are hydrolyzed simultaneously; maltotriose is converted to maltose and glucose. Two types of α-amylases can be distinguished, the pancreatic type and salivary type. In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrate into shorter oligomers. Later on in the gut these are further hydrolyzed by pancreatic α-amylase into maltose, maltotriose and small malto-oligosaccharides. The digestive enzyme (α-amylase) is responsible for hydrolyzing dietary starch to maltose, which breaks down to glucose, prior to absorption. Inhibition of the α-amylase should reduce the unfavorable high postprandial blood glucose peak in diabetics (Roux & Perrier *et al.,* 1998).

The percentage inhibition of α-amylase is calculated as follows:

Percentage inhibition = Absorbance (control) – Absorbance (test) / Absorbance (control) X 100

α–Glucosidase inhibition assay:

Intestinal α-Glucosidase inhibitors are reported to be powerful therapeutic agents in carbohydrate metabolic disorders, especially diabetes mellitus and obesity. Postprandial hyperglycemia and hyperinsulinemia are expected to be diminished by inhibition of poly and oligosaccharide digestion in the intestinal-tract. Practically, a few α-Glucosidase inhibitors of microbial origin viz., Acarbose are clinically used for the treatment of diabetes mellitus (Matsuo *et al*, 1992).

The percentage inhibition of α - glucosidase is calculated as follows:

Percentage inhibition = Absorbance (control) – Absorbance (test) / Absorbance (control) X 100

Estimation of rate of glucose uptake using 3T3L-1 cells:

Cell lines and culture medium

3T3L-1 cell line was procured from ATCC, stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO2 at 37°Cuntil confluent. The cell was dissociated with cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells are checked and centrifuged. Further, 20,000 cells/well were seeded in a 96 well plate and incubated for 24 hrs at 37oC in CO2 incubator (5%) (Mary Shoba Das *et al,* 2015).

Differentiation of 3T3L-1 myoblasts

On Day 1: 5,000 cells were seeded per 100µl in DMEM with10% fetal bovine serum in a 96-well plate. The medium was replaced every 2–3 days. On Day 5: The spent medium was removed and differentiation of cells was initiated by adding 100µl DMEM with 2% horse serum. The medium was replaced daily. The myotubes reach maturity after 3 days of low serum further assay of 3T3l-1 myotubes assay was carried out.

Assay of 3T3L-1 myotubes:

One day before the assay, the medium was removed and 100µl DMEM without serum was added for the cells. On the day of the assay, the medium was replaced with 100µl DMEM ± 1µM insulin or Samples without serum or glucose and incubate for 2 hour at 37°C in 5% CO2.The medium was removed and 50µl of 0.1mM 2DG in PBS was added. Incubate for 30 minutes at 25°C. 25µl of Stop Buffer was added and briefly the sample was shaken.25µl of Neutralization Buffer was added later and shaken briefly. 100µl of 2DG6P Detection Reagent was added and shake briefly. The mixture was incubated for 1 hour at 25°C. The luminescence with 0.3–1 second integration on a luminometer was recorded.

Calculating the Glucose Uptake Rate

Rate of glucose uptake = ([2DG6P] × (volume of sample)) ÷ ((number of cells) × (time of uptake)

Cytotoxicity studies for 3T3L-1cell line:

Diabetes mellitus is a severe threat to human well-being across the world due to the rapidly increasing incidence of diabetes. In vitro cytotoxicity, glucose uptake activities of plant extract were examined using 3T3L-1cell line (Skehan *et al.,* 1990).

RESULTS:

Extraction of plant material

The 10g of *Glycyrrhiza glabra* powder was weighed and dissolved in 50ml of methanol, incubated at 50℃ for 4 hours. After filtered with whatman's filter paper and residue is kept few hours for evaporation of methanol and collected sample was weighed. The obtained yield of *Glycyrrhiza glabra* was 746mg as shown in (Table 1).

Phytochemical Screening:

Qualitative phytochemical analysis revealed that methanolic extract of *Glycyrrhiza glabra* and exhibited the presence of Alkaloid, Tannin, Terpenoid, Steroid, Saponin, Flavonoids, Mucilage, Volatile oil in Considerable quantity and the absence of Carbohydrate, Glycoside (Table 2).

Alpha Amylase inhibition assay:

The methanolic extract of Glycyrrhiza *glabra* was tested for α-amylase inhibition activity and IC50 value was calculated and measured at 58.73µg/ml and the results were compared with standard Acarbose the inhibition of α-amylase was measured with IC50 value of 3.844µg/ml as shown in (Table 3 & Figure 3). As compared to standard drug acarbose, the methanolic extract of *Glycyrrhiza glabra* showed significant antidiabetic property with α-amylase inhibition.

Alpha Glucosidase inhibition assay:

Further methanolic extract of *Glycyrrhiza glabra* was tested for α-glucosidase inhibition activity and IC50 value was calculated and measured at 71.92µg/ml and the results were compared with standard acarbose, the inhibition of α-glucosidase was measured with IC50 value of as shown in 7.22µg/ml (Table 4). As compared to standard drug acarbose the methanolic extract of *Glycyrrhiza glabra* showed significant antidiabetic property with α-glucosidase inhibition.

Estimation of rate of Glucose uptake using 3T3L-1cells

The sample shows the significant potential of glucose uptake in 3T3L-1 cell line at the concentration of 0.41 fold for 80µM, uptake was measured after cell lyses using luminometer. Sample extract and insulin is treated with 3T3L-1 cell line for the concentration for the glucose uptake.

Cytotoxicity studies for 3T3L-1cell line:

Cytotoxicity of *Glycyrrhiza glabra*, was tested by using 3T3L-1 cell line. The tested methanolic extract of *Glycyrrhiza glabra* shown inhibition with IC50 value of 7.22µg/ml. And the standard Doxorubicin shown inhibition of α-glucosidase with IC50 value of 71.92µg/ml. This report indicates that *Glycyrrhiza glabra* having less cytotoxic effect on 3T3L-1 Cell lines (Table 6).

DISCUSSION:

Glycyrrhiza glabra is used throughout the world as a traditional herbal remedy. The earliest record of it was used in medicine. The actions like memory enhancing activity, enhancement of skin complexion, Anti-ulcer activity etc. The regulation of gastrointestinal mobility, Modulating cardiac performance are appears to be new for Ayurveda which desires to be considered as per Ayurvedic point of view. Several reports suggested that presence of primary metabolites and

secondary metabolites in this plant and which are responsible for biological activity. The phytochemical screening is crucial step in the plant based therapeutic research (Nesar *et al.*, 2016).

In our present studies focused on qualitative phytochemical analysis and the study revealed that methanolic extract of *Glycyrrhiza glabra* and exhibited the presence of Alkaloid, Tannin, Terpenoids, Steroid, Saponin, Flavonoids, Mucilage, Volatile oil in Considerable quantity and the absence of Carbohydrate, Glycoside as shown in (Table 2).

Glycyrrhiza glabra methanolic root extract were tested for positive α-amylase and α-glucosidase enzyme inhibitory activity. Management of the blood glucose level is a critical strategy in the control of diabetic complications. Inhibitors of hydrolyzing enzymes (α-amylase and α-glucosidase enzyme) have been useful as oral hypoglycemic agents for the control of hyperglycemia especially in patients with Type-2 Diabetes mellitus. α-amylase is one of the main enzymes in the human body which is responsible for the breakdown of starch to more simple sugars. α-amylase hydrolyzes complex polysaccharides to produced oligosaccharides and disaccharides. The inhibitory properties of α-amylase and α-glucosidase by this plant extracts is minimizing the various side effect. *Glycyrrhiza glabra* plant extract shows α-amylase inhibition activity with IC50 values of 58.73µg/ml and α-glucosidase inhibition activity with IC50 value of 71.92µg/ml of Plant extract comparison with the reference Anti-diabetic drug Acarbose. It shows IC50 value of 3.844µg/ml and 7.22µg/ml respectively. Whereas, the methanolic leaf extracts of Lindernia ciliate shows α-amylase inhibition activity with IC50 value of 6.11mg/ml. and α-glucosidase inhibition activity with IC50 value of 6.10mg/ml. Comparison with the reference Anti-diabetic drug Acarbose. The mechanism of α-amylase and α-glucosidase mechanism enzyme inhibition activity could be exploited in the management in the treatment of Type-1 diabetes mellitus (Momina *et al*., 2020).

The methanolic root extracts of *Glycyrrhiza glabr*a shows Glucose uptake activity on 3T3L-1 cell. Transporting of glucose across the cell membrane of Yeast cells are mediated by the process. *Glycyrrhiza glabra* shown maximum glucose uptake with 0.41 fold was declined in 80µg/ml of concentration by using 3T3L-1 cell. Whereas, The glucose uptake in Withanaia sominifera root of methanolic extract active on 3T3F442A fibroblast cells shown maximum glucose uptake with 160.08% was declined in 40µg/ml of concentration by using 3T3F442A fibroblast cells.

The Cytotoxicity study of methanolic root extraction of *Glycyrrhiza glabra* shows a IC50 value of 82.40µg/ml in 3T3L-1 cell lines. Which is quite safe and used as non-toxic drug? Whereas, Solanum nigrum aqueous plant extract shows a IC50 value of 34.67µg/ml in Hela cell lines. Generally accepted that any extract with an IC50 value below or equal 100µg/ml can be considered to cytotoxic activity (Mohan *et al.,* 2020).

In our study cytotoxic activity of methanolic extract of *Glycyrrhiza glabra* shown inhibition with IC50 value of 7.22µg/ml and the standard Doxorubicin shown inhibition of α-glucosidase with IC50 value of 71.92µg/ml. This report indicates that *Glycrrhiza glabra* having very less cytotoxic effect on 3T3L-1 Cell lines.

CONCLUSION:

Glycyrrhiza glabra is most commonly used herb in various Ayurvedic medicines applicable in drug discovery and it's also helpful to easily understand the drug action by both the way traditional as well as modern. It's also lead to rise the probability of drug interaction means there is necessity to evaluate individual herbal therapy. It has strong ethno-botanical history, root and rhizomes shows various medicinal properties like antimicrobial, antidiabetic, antioxidant, anti-inflammatory etc. Recent researches proved vast clinical action as it is having therapeutic properties like anti-ulcer activity, wound healing activity, cognitive function enhancing activity, anti-arthritic activity etc. due to its major constituents is inhibition of α-glucosidase and α-amylase enzyme activity leads to a reduction in disaccharide hydrolysis which has beneficial effects on glycemic index control in diabetic patients.

This becomes a novel inhibition in the treatment of various diseases. The roots of this plant are used as a medicine and possess lot of medicinal properties and bioactive compounds. The chemical constituents *of Glycyrrhiza glabra* hold a strong new molecule, which could be immense medicinal applications in the drug discovery process for the development of new drugs which would be potential benefits in all age group of patients. The present attempt was made to understand the chemical properties, traditional use, bioactive constituents and pharmacologic activities and medicinal aspects of *Glycyrrhiza glabra* to undertake further studies on *Glycyrrhiza glabra* for exploring its potential in preventing and treating diseases. And also,

further investigations to carry out research in discovering potential therapeutic effect and developing new formulations.

CONFLICT OF INTEREST

No conflict of interest

Sample	Sample Taken for extraction	Solubility	Yield
Glycyrrhiza glabra	10g	Methanol	746. mg

Table 1: Shown: Yield of *Glycyrrhiza glabra* after crude extraction

Sample	Qualitative analysis of Primary and secondary Metabolites	Result
Glycyrrhiza glabra	Alkaloid	+
	Carbohydrate	-
	Tannin	+
	Terpenoid	+
	Glycoside	-
	Steroid	+
	Saponin	+
	Flavanoid	+
	Mucilage Test	+
	Volatile Oil	+

Table 2: Shown: Phytochemical Screening of *Glycyrrhiza glabra*

Note: (+) : Positive (Presence) and (-) : Negative (Absence)

Table 3: Alpha Amylase inhibition in Acarbose and *Glycyrrhiza glabra*

Acarbose(standard)			
Con.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	1.33	0.00	3.844
0.78125	0.95	28.57	
1.5625	0.84	36.84	
3.125	0.69	48.12	
6.25	0.55	58.65	
12.5	0.42	68.42	
25	0.34	74.44	
50	0.26	80.45	
***Glycyrrhiza glabra* (sample)**			
Con.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	1.33	0.00	58.73
7.8125	0.97	27.07	
15.625	0.81	39.10	
31.25	0.75	43.61	
62.5	0.58	56.39	
125	0.44	66.92	
250	0.25	81.20	
500	0.15	88.72	

Table 4: Alpha Glucosidase inhibition after treated with Acarbose v/s *Glycyrrhiza glabra*

Acarbose (standard)			
Conc.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	0.820	0.00	7.22
1.5625	0.774	5.62	
3.125	0.655	20.09	
6.25	0.426	48.11	
12.5	0.329	59.93	
25	0.254	69.02	
50	0.112	86.34	
***Glycyrrhiza glabra*(sample)**			
Con.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	0.831	0.00	71.92
7.81	0.753	9.39	
15.625	0.712	14.32	
31.25	0.621	25.25	
62.5	0.475	42.86	
125	0.316	61.93	
250	0.198	76.16	
500	0.157	81.11	

Acarbose(standard)			
Conc.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	0.820	0.00	7.22
1.5625	0.774	5.62	
3.125	0.655	20.09	
6.25	0.426	48.11	
12.5	0.329	59.93	
25	0.254	69.02	
50	0.112	86.34	
***Glycyrrhiza glabra*(sample)**			
Con.(µg/ml)	**OD**	**% of inhibition**	**IC_{50}(µg/ml)**
0	0.831	0.00	**71.92**
7.81	0.753	9.39	
15.625	0.712	14.32	
31.25	0.621	25.25	
62.5	0.475	42.86	
125	0.316	61.93	
250	0.198	76.16	
500	0.157	81.11	

Table 5: Rate of glucose uptake using 3T3L-1 cells in Insulin and *Glycyrrhiza glabra* and Insulin

sample	Concentration (µM)	RLU	2D6G µM	Glucose Uptake	Glucose Uptake fmol/cell/min	Fold change
Control	0.00	696	0.78	0.0002608	0.26077	0.00
Insulin	0.1	5574	5.51	0.0018364	1.83635	1.58
	1	9189	9.01	0.0030040	3.00399	2.74
Glycyrrhiza glabra	10	987	1.06	0.0003548	0.35476	0.09
	20	1256	1.32	0.0004417	0.44165	0.18
	40	1687	1.74	0.0005809	0.58086	0.32
	80	1963	2.01	0.0006700	0.67001	0.41

Table 6: Cytotoxicity studies for 3T3L-cell line treated with Doxorubicin and *Glycyrrhiza glabra*

***Glycyrrhiza glabra*(sample)**				
	Conc.µg/ml	**RFU**	**% inhibition**	**IC_{50}µg/ml**
Control	0	2841256398	0	
3T3L-1 cell line	10	2570986200	9.51	82.4
	20	2222658961	21.77	
	40	2011452632	29.21	
	80	1615487964	43.14	
	160	1213675484	57.28	
	320	864156635	69.59	
Doxorubicin				
	Conc.µg/ml	**RFU**	**% inhibition**	**IC_{50}µg/ml**
Control	0	2845562451	0	
3T3L-1 cell line	3.125	2615687954	8.80	23.56

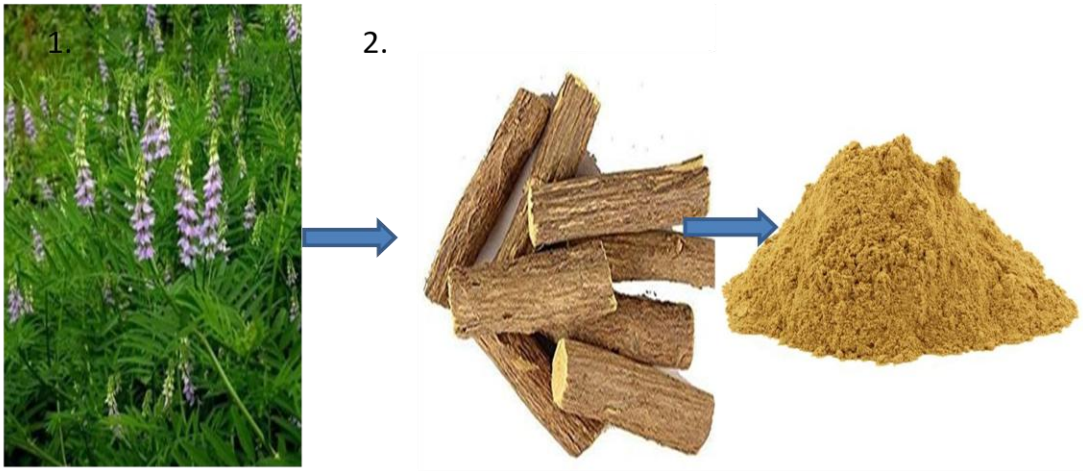

Fig.1: Plant of *Glycyrrhiza glabra* **Fig.2: Root and grinded powder**

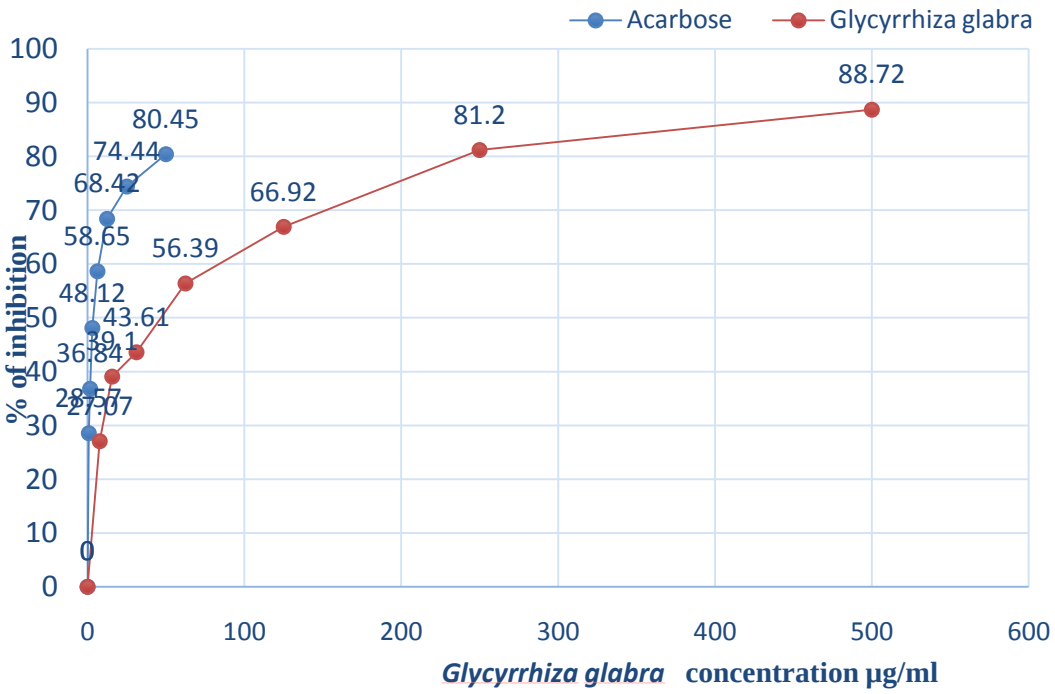

Figure 3: Graphical representation of Alpha amylase inhibition % after treated with Acarbose and *Glycyrrhiza glabra* .

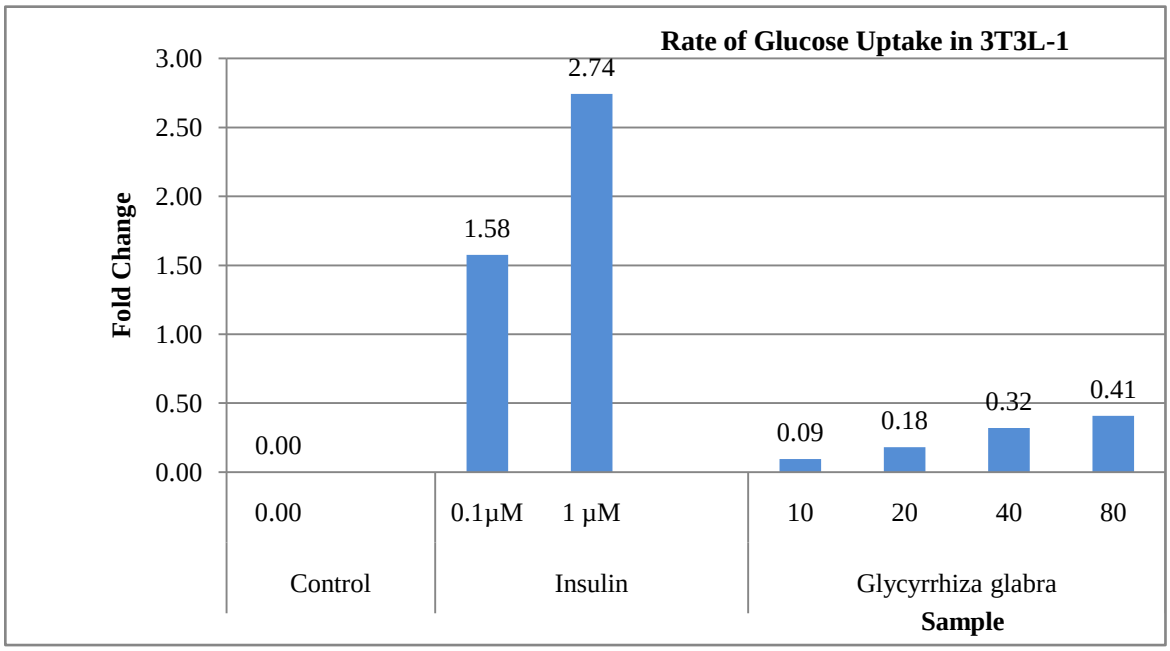

Figure 4: The Estimation of rate of glucose uptake in cells treated with various concentrations of test sample (*Glycyrrhiza glabra*).

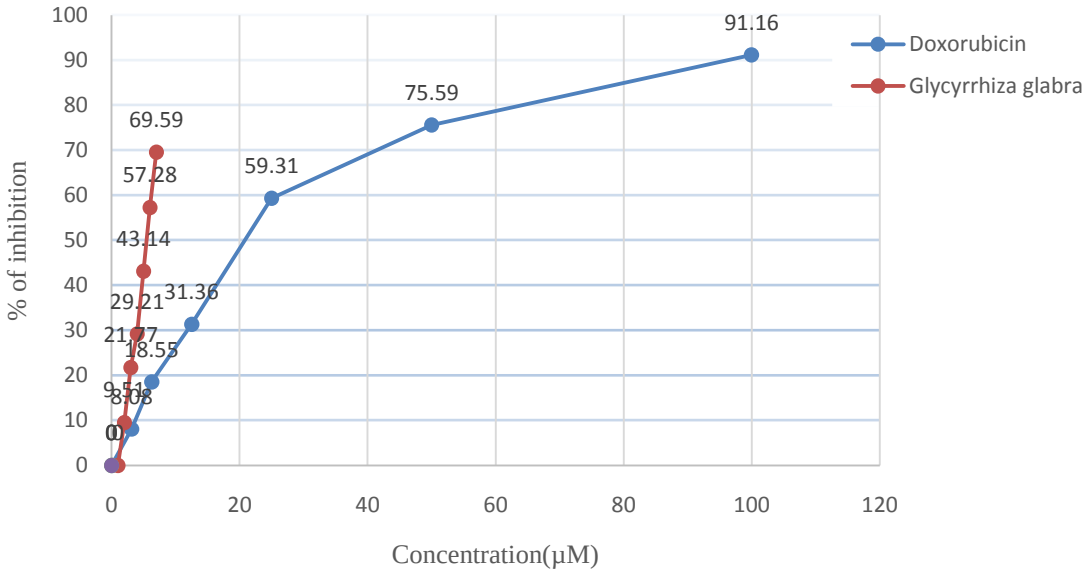

Figure 5: Shown Graphical representation of Cytotoxicity studies for 3T3L-1cell line treated with *Glycyrrhiza glabra (IC50*-7.22µg/ml) and Standard drug Doxorubicin (IC50-71.92µg/ml) Compared to standard drug *Glycrrhiza glabra* less toxic on 3T3L-1 cell line.