International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-4, 2016 ISSN No: 2277 - 7873

REVIEW ARTICLE

Tinospora cordifolia and Its Anti-Diabetic Activity: A Review

Debpratim Chakraborty*

Department of Pharmaceutical Technology, Jadavpur University, Jadavpur-700032, India.

Manuscript No: IJPRS/V5/I4/00151, Received On: 10/11/2016, Accepted On: 22/11/2016

ABSTRACT

Now a day Diabetes mellitus is one of the most popular diseases in research field. Diabetes is metabolic disease which is also the cause of various lives threatening disease. Medical science is very much advanced now but still we have no medicine without insulin for procurement of this disease. The medicines which are mainly using against diabetes have more side effect than that of efficiency. That's why we are going for natural or traditional medicine. *Tinospora cordifolia* has significant anti-diabetic activity in diabetic animals and has an efficacy of 50% to 58% compared to insulin. The alkaloid rich fraction of *tinospora* including palmatine has been reported for insulin-mimicking activity which ultimately helps to release insulin from corresponding cell in vitro and in vivo. Different animal model study has been done for reviewing the antidiabetic activity of Tinospora plant. In a study diabetes is induced by injecting alloxan monohydrate (180 mg/kg bw) intraperitoneally and *Tinospora cordifolia* extract (20ml/kg bw) was administrated orally twice a day. The whole plant extract of *Tinospora* very significantly ($p<0.001$) reduces the blood glucose level. In another study rats were injected with streptozotocin of 55 mg/kg b.w. intravenously to make them diabetic and the extract of tinospora was administered in different concentration. The extract of *Tinospora cordifolia* has significant ($P < 0.05$) anti-diabetic activity in diabetic animals and has an efficacy of 40% to 80% compared to insulin. In another study it has been found that the extract inhibits alpha glucosidase which ultimately show anti-diabetic properties. *Tinospora cordifolia* has various other biological activities but we have been focused on its anti-diabetic activity. The future prospect is to elucidate the structure of the chemical constituents which are mainly responsible for anti-diabetic activity. In future we may also modify the activity of those constituents by QSAR, pharmacophore study.

KEYWORDS

Diabetes Mellitus, Palmitine, *Tinospora Cordifolia*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease in which blood sugar level get high over a prolonged period. Symptoms include frequent urination, polydipsia and polyphagia [1]. Acute complications include diabetic ketoacidosis (when your body can't produce enough insulin) and Hyperosmolar hyperglycemic state (HHS) producing coma.

***Address for Correspondence:**
Debpratim Chakraborty,
Department of Pharmaceutical Technology,
Jadavpur University, Kolkata-700032, India.
E-Mail Id: debpratim008@gmail.com

Chronic complications include cardiovascular disease, stroke, kidney failure, foot ulcers, and damage to the eyes[1].

Diabetes is due to either the pancreas not producing enough insulin or the β cells not responding properly to produce the optimum insulin[2]. *Tinospora cordifolia* is an herb used in Ayurveda for its wide range of pharmacological activities. It is being researched for a variety of health effects, including its effect on diabetes, glucose metabolism, inflammation, immune

system support, and neurology etc. We have focused on its anti diabetic activity. It may decrease the blood glucose challenging with the marketed anti-diabetic drug. Now scientists are trying to identify and isolate that chemical or chemical which are mainly responsible for anti diabetic activity. If we may further go for chemical modification then we may get a potent drug and that will ultimately help to improve human health.

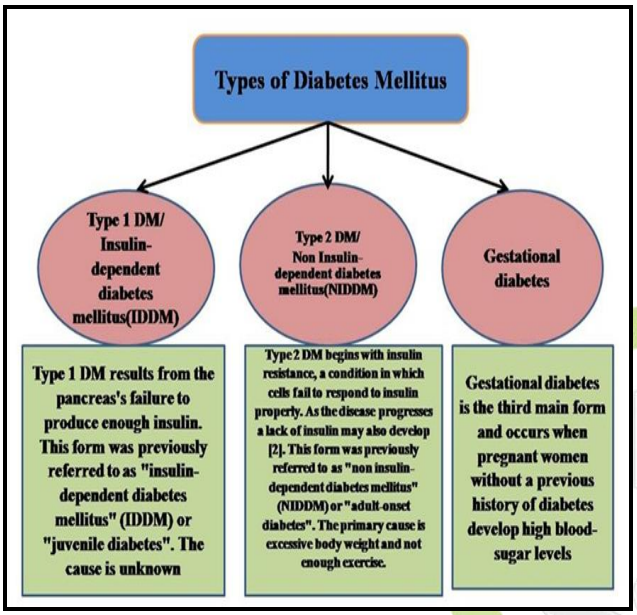

Figure 1: Types of diabetes Mellitus

Prevalence

Globally in 2013, it was estimated that approximately 382 million people is suffering from diabetes (8.3% of total population). In North America and the Caribbean Island it is 11% of total population (Approximately 37 million people) and in the Middle East and North Africa the percentage is 9.2 of total population (Approx 35 million people) and in Western Pacific the prevalence is 8.6% (138 million). In 2013, the top most countries with higher prevalence of diabetes are given in [Figure 3A].

35 out of 219 countries (16% of the total) [Figure 3B][4] is very much prone to diabetes and the percentage of diabetic people is nearly 12% or higher. These countries are located mainly in Western Pacific, Middle East and North Africa regions mainly[3].

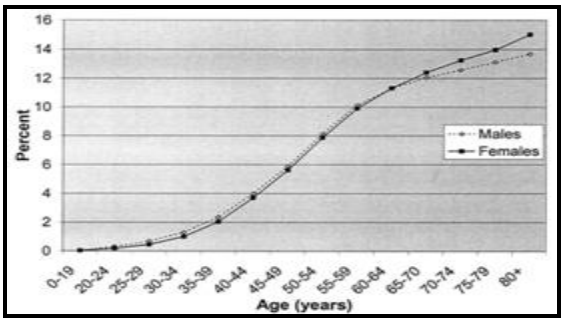

Figure 2: Chances of diabetes with age (WHO)

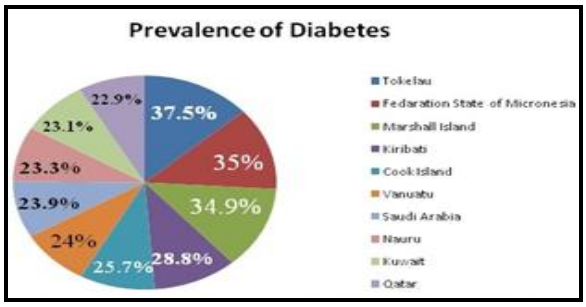

Figure 3A: The Top 10 Countries with higher prevalence of Diabetes

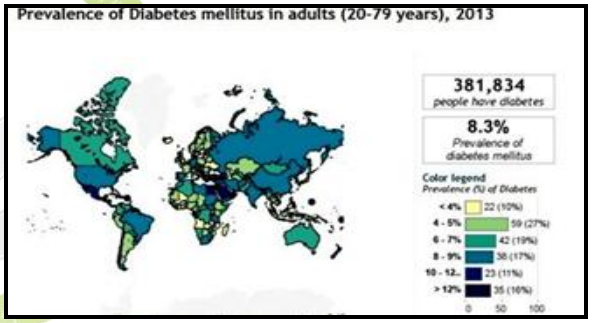

Figure 3B: Prevalence of Diabetes

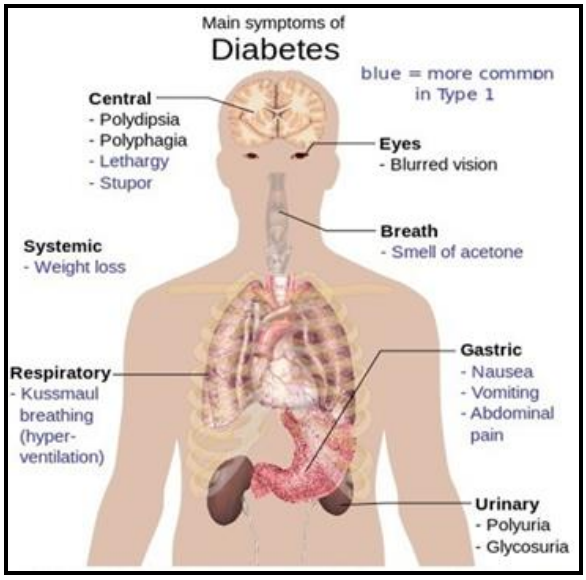

Figure 4: Main sign & symptoms of Diabetes

In 2014, the International Diabetes Federation (IDF) estimated that diabetes resulted in 4.9 million deaths. The World Health Organization (WHO) estimated that death due to diabetes was near about 1.5 million in 2012, Now a day in the whole world diabetes is the 8th leading cause of death. The IDF are trying to estimate the amount of deaths caused by diabetes. More than 75% of death due to diabetes is happening in developing countries [WHO estimation, 2013]. The prevalence of diabetes with different age of both sex shows in Figure 2[3].

Sign and Symptoms of Diabetes

The usual symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) [5]. Symptoms may develop rapidly (weeks or months) in type 1 Diabetes, while in type 2 Diabetes symptoms usually develop much more slowly.

Several other signs and symptoms (Figure 4)[5] are often observed which includes Irritation in eyes, headache, fatigue, unhealing of cut and bleeding from cuts, and itching. Prolonged high blood glucose may cause absorption of glucose in the eye lens, which resulting in eye sight problem. A number of skin rashes also associated with diabetes.

Pathogenesis: The Pathogenesis of Diabetes mellitus is as follows (Figure: 5)[6,7,8]

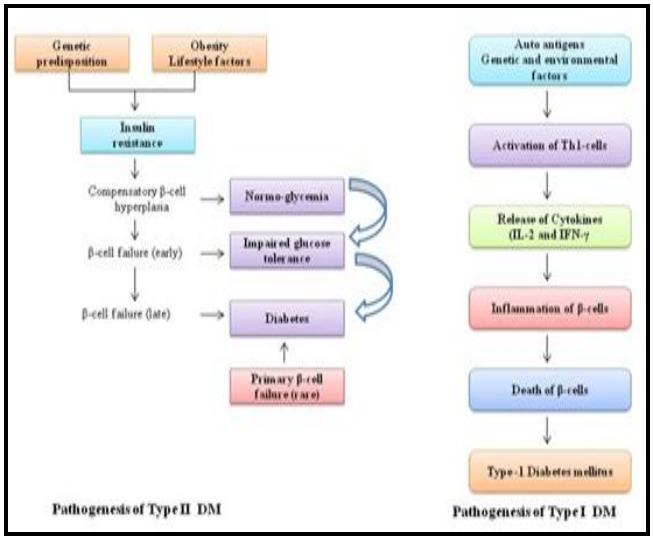

Figure 5: Pathogenesis of Type I & II Diabetes Mellitus

Why we are going for traditional medicine

In modern medicine there is no satisfactory effective therapy is still available to cure the metabolic disease (DM). Though insulin therapy is the ultimate choice of medication for the management of diabetes mellitus, but it has several drawbacks like insulin resistance, patient discomfort which leads to discontinue the medication and various side effects like[9] anorexia, Loss of appetite, Cerebral atrophy, Cerebral hypoxia and fatty liver[10]. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues (acarbose, miglitol and voglibiose) which down regulate gastric emptying which leads to decrease the glucose uptake, In the other hand it down regulate the postprandial glucagon level and it leads to decrease the glucose production from liver and the both ultimately decrease the plasma glucose level. Sulphonylureas, the most widely used class of drugs act by closure of ATP dependent channel. Biguanides (Metformin) helps to decrease hepatic glucose production and decrease glucose absorption from intestine. But there are several drawbacks of these drugs for prolong use at higher doses like liver problems, lactic acidosis and diarrhea, nausea, vomiting, flatulence. Till now we are unable to find a safe drug or chemical moiety with minimal adverse effects, which can be taken for long durations and which will have optimum potency. Though biguanides and sulfonylureas are very useful in treatment of diabetes mellitus but their use is restricted for unpleasant pharmacokinetic properties, failure in second order kinetic and accompanying side effects[11]. For these above mentioned reasons recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases. However today it is necessary to provide scientific justification for the use of plant or its active principles[12]. Throughout the world many traditional plants exist for the treatments of diabetes.

Chemical Constituent and the Active Chemical for Anti Diabetic Activity

From Pharmacological study report it can certainly said that various plant extract of Tinospora show anti-diabetic activity in vivo. It has been reported[13] that various medicinally potential phytoconstituents are isolated from tinospora like Alkaloid, Glycoside, Diterpenes, Sterols, Flavonoids, Saponins etc[14].

Table 1: Types of Chemicals

Type of Chemical	**Name of Active Principle**
Alkaloid	Berberine, Palmatine, Tembetarine, Magnoflorine, Choline , Tinosporin, Isocolumbin
Glycoside	Tinocordiside, Tinocordifolioside Cordioside, Cordifolioside A, Cordifolioside B, Syringin, Palmatosides.
Diterpenes	Tinosporon, Tinosporides
Sterols	b -sitosterol, d-sitosterol, b -hydroxyecdysone.

These different phyto chemicals have different activities and thus the plant enhance application in experimental and clinical research. Scientists assume that the alkaloids are mainly responsible for the anti-diabetic activity [Figure 6].

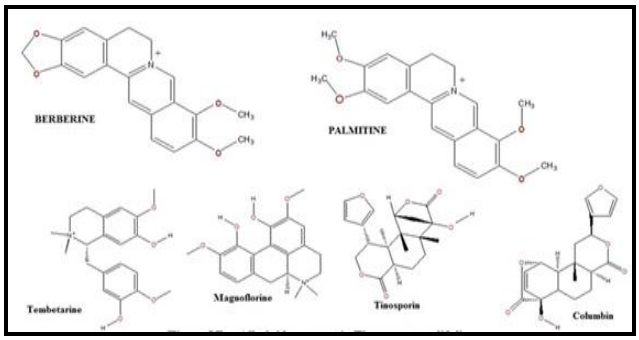

Figure 6: Alkaloids present in Tinospora Cardifolia

It is clear that the stem extract of *Tinospora cordifolia* has significant antidiabetic activity in diabetic animals and has an efficiency of 50% to 58% compared to insulin[14]. The high alkaloid containing fraction of stem, including palmatine (protoberberine alkaloid) has been reported for insulin-mimicking and insulin releasing effect both in vitro and in vivo [14]. From another study it has been found that Borapetoside C isolated from Tinospora (5 mg/kg, i.p.) obstruct the elevated plasma glucose in diabetic animal, increased glucose utility, delayed the development of insulin resistance and then enhanced insulin sensitivity. The activation of insulin induced IR-Akt-GLUT2 expression in liver and the enhancement of insulin sensitivity by borapetoside C may leads to hypoglycemic action[15]. The whole plant or just a selected part such as the bark or the stem is used for extracting and isolating the active constituents. The plant part with the highest alkaloid content is dried at room temperature and then cut them into small pieces (Make powder form if needed). The extraction of alkaloids from the finely cut dried plant material is based on maceration and percolation, or is performed by using a Soxhlet apparatus. The dried plant tissue is extracted using different solvents, and at the end of the extraction, if the extract contains some of alkaloid is identified by Dragendorff's reagent[20]. After extraction the next is Isolation. For Isolation the common methods are crystallization, column chromatography, TLC and thus we obtain the individual alkaloids from the plant extract. Afterwards highly sensitive and sophisticated methods are used for isolation (various chromatographic methods including HPLC, HPTLC) and identification (spectroscopic methods) of the active constituents. However, with HPLC many peaks cannot be assigned to the individual species (the same problem occurs with TLC). This complication can be resolved by using ion-pair HPLC[21] and utilizing sodium perchlorate.

TLC Pattern of Pamitine[16]

Currently, standard spectroscopic methods are used for investigating the structure of natural products are nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and ultraviolet spectroscopy (UV) and the most useful one is mass spectrometry (MS). Single-crystal Xray diffraction is a powerful technique used for

determining the molecular topology. However, this technique is not sufficient for obtaining high-quality single crystals. Applications of the above mentioned methods to the structural analysis and characterization of the chemical constituents[17]. For example if we consider Mass Spectroscopy, Mass spectrometry is a powerful tool for investigating the structures of complex molecules and natural products for a long time. It is frequently used in identification of the active constituents in crude mixtures of alkaloids or in plant extracts. The base peaks produced by ESI-MS[18] without CID (collision induced dissociation) are: berberine 336, palmatine 352, and Borapetoside C 537. Thus the molecular mass can be determined by Mass Spectroscopy and the molecular formula can be determined by NMR spectra i.e the molecular formula of Borapetoside C is $C_{27}H_{36}O_{11}$.

Table 2: TLC pattern of Pamitine

Component	Silica gel G(Activated)[70-230 mesh] + Solvent (Water: n-Butanol: Acetic Acid=5:4:1)	Fluorescence colour Under UV
Palmitine	35	Dark Yellow
Barberine	30	Yellow

Tinospora and Its Effect on Diabetes

In a study alcoholic and aqueous extract of *Tinospora cordifolia* was prepared. Healthy albino rats (9 months old) of either sex, weight 150-190gm were used for the experiment. They are kept in polypropelene cage in optimum condition (12 hours light and 12 hours dark condition with temperature 25±0.5ºC and relative humidity 50-70% with enough food pallet and water ad libitum). Food is withdrawal before 16 hours of starting experiment. Diabetes was induced in fasted albino rats with single intraperitoneal dose of alloxan monohydrate. Alloxan injection was prepared in 0.9% normal saline. Then measure the blood glucose level after 3 days. The blood glucose level is changed in Triphasic way.

1. Sudden Hyperglycemic condition after 1 hour of administration of diabetes inducing agent.
2. Hypoglycemic condition for 6-8 hours.
3. Stable Hyperglycemic condition after 48 to 72 hours). Rats with fasting blood glucose more than 220 mg/dl was considered for study and if less than that then they are discut out. During dose standardization study it was found that 180 mg/kg intraperitoneal dose of alloxan monohydrate was suitable for diabetes induction with the 6-12 month old rats. For this study the animals were divided into three groups (n=6).

Experimental diabetes was induced with alloxan 180 mg/kg intraperitoneal dose. In present study we have observed that the whole plant extract of *Tinospora cordifolia* very significantly ($p<0.001$) reduces the blood glucose towards the normal blood glucose level and that may be either due to insulin mimicking activity of the plant extract or it increase insulin secretion from β cell and this may leads to decrease of the blood glucose towards the normal value[19].

In another study it has been found that increased blood glucose in the diabetic condition is gradually reduced and almost nearer to normal after 90 days by the administration of *Tinospora cordifolia* extracts in which methanol extract shows better reduction. The glycosylated hemoglobin level in diabetic control is 5.11 ± 0.68%, which is reduced to 2.58 ± 0.65% in diabetic *Tinospora cordifolia* methanol extract treated rats. The glucokinase level is high in untreated rats and it decreased up to 50% in STZ induced rats. But the result has reversed in the *Tinospora cordifolia* treated rats where the activity of glucokinase is appreciably increased[20].

In another study the Aqueous and alcohol extracts of *Tinospora cordifolia* (TC) were prepared[21]. The yield of extracts was approximately 8.5% and 7%, respectively. Female albino rats (body wt. 180-210 g) were used in this study.

Table 3

Group No.	Name of Group	Diabetes inducing Agent	Drug	Food and Water
1	Normal Control	NO	NO	Yes
2	Diabetic Control	Alloxan monohydrate(180mg/kg B.W)	NO	Yes
3	Test	Alloxan monohydrate(180mg/kg B.W)	*Tinospora cordifolia* extract (20ml/kg bw) twice a day	NO

Table 4

Group No.	Group Name	Diabetes inducing agent	Drug	Food and Water
1	Normal control	NO	NO	Yes
2	Diabetic control	STZ	NO	Yes
3	Standard	STZ	Insulin	NO
4	Test(I)	STZ	Aqueous extract of TC ((200 mg/kg b.w.)	NO
5	Test(II)	STZ	Aqueous extract of TC (400 mg/kg b.w.)	NO
6	Test(III)	STZ	Alcohol extract of TC (200 mg/kg. b.w)	NO
7	Test(IV)	STZ	Alcohol extract of TC (400 mg/kg. b.w.)	NO

After 16 hours fasting condition, freshly prepared streptozotocin (STZ) were injected to induce diabetes. Control animals received citrate buffer alone. Diabetes status was changed in triphasic way and was measured after 72 h of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dL were selected for this study. Single dosage of either aqueous extract (dissolved in normal saline) was given orally for 10 days or alcohol extract (dissolved in gum acacia) was given orally for 30 days to specific groups through oral route of administration[22].

The present study clearly showed that *Tinospora cordifolia* has significant anti-diabetic activity in diabetic animals and has an efficacy of 40% to 80% compared to insulin. From this study we came to know that *Tinospora cordifolia* administration in diabetic animals did not show any increase in serum insulin levels or regeneration of pancreatic β cells but showed increased hepatic glycogen synthase and decreased glycogen phosphorylase activity[23].

In another study it has been found that alpha glucosidase inhibitors have been potential use in the treatment of diabetes mellitus. The stem extract of *Tinospora cordifolia* was evaluated for inhibition of that enzyme. The crude ethyl acetate, dichloromethane (DCM), chloroforms and hexane extracts of *Tinospora cordifolia* were studied. 15 mg of the DCM extract of tinospora was most effective which show 100 % inhibition of the alpha glucosidase. The DCM *Tinospora cordifolia* stem extract (0.3 mg / g bw) show activity in diabetic animals by 50 and 58 %

respectively as compared to the controls. The extract was found to inhibit alpha glucosidase in a non-competitive manner[24]. In another study it is demonstrated that different extract of Tinospora cordifolia show glucose uptake activity up to an optimum level in EAT cells. In their study they use three major extract of Tinospora cordifolia stem (Aqueous, Ethanol and Methanol) and check the glucose uptake activity at a lower dose with different concentration of each extract. They also marked that at higher doses the glucose uptake is inhibited by either attending the saturation level of GLUT or by feedback mechanism. Glucose uptake stimulating activities of extract of Tinospora (Aqueous, Methanol and Ethanol) were evaluated and it has been calculated that aqueous extract of stem show potent glucose uptake activity compare to other extract at higher dose. With increasing the dose glucose uptake gradually increased (Figure 7)[25]. For the transport of glucose through biological membrane, specific transport proteins are required. Passive transport of glucose is catalyzed by glucose transport protein GLUT1 & GLUT3. These proteins are abundantly present in human tissue and also EAT cells[25].

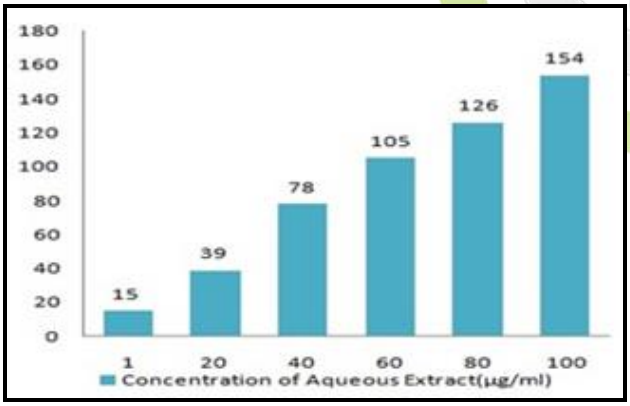

Figure 7: Glucose uptake with aqueous extract in different concentration

In another study it has been concluded that hydro-alcoholic extract of Tinospora at 10µg/ml enhance the glucose uptake via stimulating the Insulin receptor and signaling through P13K which mobilize glucose transport protein GLUT4[26].

Other Biological Activity of Tinospora Cordifolia

- Studies have shown that in rat groups, there is an enhancement in the bone marrow cells as well as α-esterase activity when treated with alcoholic extracts of *Tinospora cordifolia*. Thus it becomes evident that these drugs have immune-modulatory activity[27].
- *Tinospora cordifolia* have antispasmodic and antipyretic activity[28].
- The aqueous extract of the stem of *Tinospora cordifolia* show anti-inflammatory activity in albino rats. It has significantly inhibited acute inflammatory response introduced by carrageenin when administered orally or intraperitoneally[29].
- The plant extract helps to enhancement immune-stimulation and synthesis of acetylcholine. The plant extract may increase choline level which has memory enhancing property[30].
- Tinospora cordifolia plant material minimizes the effects of free radicals including the peroxy radicals and its antioxidant activity in association with the inhibition of lipid peroxidation, thereby *Tinospora cordifolia* plant extract may be considered as hepatoprotective agent[31].

Medicinal Application

1. *Tinospora cordifolia* helps in pro-healing processes like growth factor activation, angiogenesis and granulation tissue formation by using the plant extract[32]. It's also used as immune-modulators in diseases like obstructive jaundice, hepatic fibrosis, peritonitis and sepsis.
2. Studies have reported that *Tinospora cordifolia* extract actively or passively protect from CCl4 toxication, probably by the production of monocyte colony stimulating factor which ultimately helps to detoxification [33].
3. The leaves extract have shown anti-HIV 1 activity. Thus it can be said that plant extract might be helpful in protecting and treating various viral diseases in humans[34].

4. *Tinospora cordifolia* is widely used against monkey malaria. Studies have shown that mixture of herb and Tulsi leaves increases body resistance upto 3 times and serves as a powerful counter of Plasmodium virus attacks[35].
5. Crude extract of *Tinospora cordifolia* contains a polyclonal B cellmitogen which increases immune response in mice. An arabinogalactan-polysaccharide, G1-4A from the stem of T. cordifolia has been examined to affect induced immune-suppression[36].
6. Studies have shown that *T. cordifolia* used for treatment of asthma[37].
7. *Tinospora cordifolia* show tumor suppressive activity also. Studies have shown that the polysaccharide fraction of plant (i.p in mice) resulted in the inhibition of lung tumor[30].
8. It is used in jaundice[38] general debility, dyspepsia, fever and urinary diseases[39].
9. The powder of root and stem is used for treatment of cancer[40].
10. Whole plant extract is act as good antioxidant and one of the best anti-psychotic drugs[41].

CONCLUSION

From the above study it can be concluded that *Tinospora cordifolia* is an effective anti-hyperglycemic drug that can be used in the treatment of DM. Although its activity is feeble compared to insulin, it can be used as a supportive drug in the treatment of DM. If the chemical constituent can be indentified which is mainly responsible for anti diabetic activity then by molecular modification the therapeutic activity may be increased as much as insulin. Insulin has various side effect and patient incompatibility but it may be a potent drug with very less side effect in future.

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