Haya: The Saudi Journal of Life Sciences (SJLS) **ISSN 2415-623X (Print)**
Scholars Middle East Publishers **ISSN 2415-6221 (Online)**
Dubai, United Arab Emirates
Website: http://scholarsmepub.com/

Antihyperglycemic and Antilipidemic Activity of *Solanum torvum* Roots

Jitendra Debata[1*], H.K Sundeep Kumar[2]
[1]GNITC-School of Pharmacy, Ibrahimpatnam, Rangareddy-501506, Telangana, India
[2]Institute of Pharmacy and Technology, Salipur, Cuttack-754202, Odisha, India

Original Research Article

***Corresponding author**
Jitendra Debata

Article History
Received: 11.05.2018
Accepted: 16.05.2018
Published: 30.05.2018

DOI:
10.21276/haya.2018.3.5.4

Abstract: Ethanolic root extract of *Solanum torvum* Sw (Family: Solanaceae) was evaluated for its antihyperglycemic and antilipidemic activity in streptozotocin induced diabetic rats. The extract was given orally in two different doses (200 and 400 mg/kg) for 28 days. Metformin (2.5 mg/kg) was used as a standard drug for activity comparison. Various parameters studied were blood glucose concentration, serum lipids, glycosylated haemoglobin and liver glycogen. The extract showed significant antihyperglycemic activity in dose dependent manner. Further, the extract was favourably and significantly corrected the alterations in the values of the lipid parameters, organ weights, liver glycogen and glycosylated haemoglobin content in diabetic rats. Therefore, it may be suggested that the ethanolic root extract of *Solanum torvum* has potential ability to prevent the secondary complications of diabetes mellitus like atherosclerosis.

Keywords: *Solanum torvum*, Streptozotocin, Metformin, Antihyperglycemic, Antilipidemic.

INTRODUCTION

The World Health Organisation (WHO) has been promoting a movement for "Saving Plants for Saving Lives". This is because of the growing understanding of the pivotal role medicinal plants play in providing herbal remedies to health maladies [1]. Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the entire plant species, at one time or others were used for medicinal purposes.

It is estimated that world market for plant derived drugs may account for about Rs.2,00,000 crores. Presently, Indian contribution is less than Rs.2000 crores. The annual production of medicinal and aromatic plant's raw material is worth about Rs. 200 crores. This is likely to touch US $5 trillion by 2050 [2-4]. Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder. It is estimated that more than 300 million people in the world will have diabetes by the year 2025 [5-7]. At present, approximately 18-20 million people are diabetic in India, and it is projected that by 2025, there will be 20-60 million diabetics in India, and it will have the second largest number of diabetics in the world [8].

Diabetes mellitus (DM) is a complex and multifarious group of disorders that disturbs the metabolism of carbohydrates, fats and proteins [9]. Hyperglycemia in the diabetics is associated with alteration of glucose and lipid metabolism and modification in liver enzymes level [10, 11]. Liver is an important insulin dependent tissue which plays a pivotal role in glucose and lipid metabolism and is severely affected during diabetes [12]. Liver participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. In DM, lipid abnormalities are almost the rule. Typical finding are elevation of total and VLDL cholesterol, triglyceride concentration, lowering of HDL cholesterol and a predominance of small, dense LDL particles [13]. Lipid abnormalities in patients with diabetes are likely to play important role in the development of atherogenesis [14]. Medicinal plants have always played an important role in the management of DM especially in developing countries since time immemorial. From the beginning of last century, evidence of lipid lowering properties of medicinal plants has also been documented [15]. In recent years, many traditionally used medicinal plants have been tested for their antidiabetic potential in experimental animals model [16]. Few of the traditional plant treatments for diabetes has received scientific scrutiny and the WHO has recommended that this area warrants immediate attention and there is still an unmet need for medicinal plants and phytopharmaceuticals with scientifically proven antidiabetic activity.

Solanum torvum Sw, syn. *Solanum ficifolium* Ortega (Solanaceae) is found in moist localities in West Bengal, Bihar, wider part of Odisha and peninsular India. Spreading or sprawling shrubs 2-3 m tall, prickles 3-7 mm long, slightly hooked, laterally flattened, scattered on stems, both leaf surfaces, and main veins, sparse on aged and mature growth, all parts pubescent with stellate hairs, sparse on upper leaf surface, dense on lower surface [17-19,20].

The dried powder of whole plant is used as folk medicine in the treatment for asthma and inflammation [21]. Fresh fruits are used as sedative, expectorant and anthelmintic [22,23]. Leaves are used as sedative and diuretic [24] and also possess antibacterial, analgesic, antipyretic, anti-malarial and antidiarrheal properties [25-29]. The fruits are reported to have hypoglycemic and antitumor-promoting effect [30, 31] and possess antibacterial, antioxidant, antifungal activity [3, 33]. The leaves of the plant are reported to be use as antiviral, antidiabetic [34, 35]. Presence of campesterol, beta-sitosterol, stigmasterol [23,29], chlorogenin, chlorogenon, torvonin A, torvonin B [23, 36-40] and solasodiene [41] in the leaves have been reported earlier. In the present investigation, we report the Antihyperglycemic and antihyperlipidemic activities of the methanol extract of the roots of *Solanum torvum*.

MATERIALS AND METHODS

Plant material

The plant material (root) was collected from the forests of Ganjam district of Odisha during June 2017 and identified by the taxonomists of the Botanical Survey of India, Shibpur, Howrah, and West Bengal, India. A voucher specimen has been kept in our research laboratory for further reference. The collected materials were washed with water and shade dried for one week. The dried plant materials were pulverized using a mechanical grinder to obtain a coarse powder.

Preparation of extracts

The powdered plant material (600 gram) was defatted with petroleum ether and extracted with 1.5 litres of ethanol (90% v/v) for 48 hrs using a Soxhlet extractor. The extract obtained was evaporated under vacuum to remove the solvent completely and concentrated to obtain a dark colour semisolid residue (8.68 g). Preliminary phytochemical screening of the ethanolic extract was performed for determination of major phytochemical constituents using standard methods [42, 43].

Animals

Wistar rats (150-200 g) of either sex were maintained in the animal house at Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha, India under standard environmental conditions of temperature (25°C) and light/dark cycles (12/12 h). All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy and Technology (Regd. No. 1053/PO/Re/S/07/CPCSEA). All standard drugs and ethanolic extract were suspended in normal saline solution using sodium carboxy methyl cellulose (0.5% w/v) for pharmacological studies. All control groups animals received 0.5% w/v sodium CMC in normal saline as vehicle (3 ml/kg body weight, per os) through oral route.

Evaluation of antidiabetic activity [44-46]

The acclimatized animals were kept fasting for 24 hours with water *ad libitum* and then intraperitoneal injection of a dose of 150 mg/kg of streptozotocin in normal saline was given. The animals were provided standard laboratory diet *ad libitum* after one hour. Under mild anesthesia the blood was withdrawn from the tip of the tail of each rat and the blood glucose level was checked before and after 24 h of introduction of streptozotocin. Rats having the blood glucose level above 225 mg/dl were selected for antidiabetic study. Metformin (2.5 mg/kg) was used as reference standard for activity comparison. The test samples were suspended in 0.5% sodium carboxy methyl cellulose in normal saline. All the test samples were fed to the animals through oral route. Group I comprised of normal rats and Group II as the diabetic rats (controls) which received only vehicle (3 ml/kg). Animals of Group III and IV (diabetic rats) received the ethanol extract of at 200 and 400 mg/kg. Group V (diabetic rats) served as positive control and received metformin (2.5 mg/kg). Treatment with the test samples (twice daily) were carried out for 28 days. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was measured on 0, 7, 14, 21 and 28th day of treatment.

Biochemical studies

At the end of 28 days of treatment with the test extract, the animals were sacrificed by decapitation under ether anesthesia and blood samples were collected from test, standard and solvent treated groups including normal animal as reference. The serum supernatant was separated out by centrifugation and was subjected for the determination of the lipid profile studies such as total cholesterol, triglycerides, HDL, LDL, VLDL and free fatty acids [47]. The major organs like the liver, kidney and pancreas were taken out and weighed [48]. For the estimation of liver glycogen content, the liver was homogenized in 5% w/v trichloroacetic acid and its glycogen content was determined by the method of Caroll *et al.* [49]. Glycosylated haemoglobin content was estimated by the method of Gabbay *et al.* [50].

STATISTICAL ANALYSIS

Data from the experiments were analyzed using one way-Analysis of Variance (ANOVA) followed by Dennett's Multiple Comparison test. Values were expresses as mean ± SEM. $p<0.05$ was

considered as the minimal level of statistical significance.

RESULTS AND DISCUSSION

Preliminary phytochemical tests of the ethanol extract revealed presence of alkaloids, flavonoids, terpenoids, saponins and sugars.

Screening for antidiabetic activity

Single dose of administration of streptozotocin showed rise of blood sugar level more than two times from the normal level within 24 h. The results of effect of ethanol extract of *S. torvum* are presented in Table 1. As shown in the table, the extract showed significant decrease in blood glucose level in a dose dependant manner. In diabetic rats, the blood glucose level was reduced from 289.55 to 193.22 mg and from 277.4 to 184.3 mg with 200 and 400 mg/kg doses of the extract respectively on the 28th day. Metformin (2.5 mg/kg) caused a reduction of blood glucose level from 284.46 to 112.35 mg.

streptozotocin is known to cause direct and selective cytoxicity to the pancreatic β-cells by causing cell membrance disruption after its intracellular accumulation [51], resulting in a decrease in endogenous insulin secretion and release, which leads to decreased glucose utilization by the tissues [52]. In the present study, the dose of streptozotocin (150 mg/kg, i.p.) was selected in order to partially destroy the pancreatic β-cells. In these conditions, insulin was secreted but not sufficiently to regulate the blood glucose [53], thus leading to the significant increase of fasting blood glucose level in streptozotocin induced diabetic rats. In our present study, we have observed that the ethanol extract of *S. torvum* could reverse the hyperglycaemic condition in diabetic rats and brought about hypoglycaemic action because blood glucose once lowered by the extracts did not increase again throughout experiment as compared to untreated streptozotocin control, where the blood glucose level was always remaining above the initials. The possible mechanism of action of the test extracts may be due to by promoting the insulin release from the undestroyed β-cells or its action may be insulin like [54].

Organ weights

Table 2 reveals the organ weights of experimental animals. It was observed that the weights of the isolated organs (liver, kidney and pancreas) of the sample treated groups were higher than the diabetic control group of animals. Induction of diabetes with streptozotocin is associated with a characteristic loss of tissue proteins [55]. The treatment with *S. torvum* resulted in an improvement in the body weight as compared to the diabetic rats which may be due to their protective effect in controlling muscle wasting, i.e. reversal of gluconeogenesis.

Biochemical studies

As shown in Table 3, the diabetic rats showed elevated levels of serum cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol. A decreased value of HDL-cholesterol was also noticed. Oral treatment of the extract at tested doses caused significant alterations of the above lipid parameters and the effect appeared to be comparable to that of metformin. Diabetes mellitus is known to cause hyperlipidemia through various metabolic derangements amongst which insulin deficiency has been known to stimulate lipolysis in the adipose tissues and give rise to hyperlipidemia and fatty liver. Thus in diabetes hyperlipidemia and hypertriglyceridaemia often occur [46]. The subsequent hyperlipidemia shown by diabetic rats can be used as an index for hyperglycemia.

Table 4 reveals the effect of the ethanol extract on liver glycogen and glycosylated haemoglobin. It is observed that the extract significantly improved the liver glycogen and glycosylated haemoglobin contents at the tested doses and the activity were found to be comparable to the reference standard.

Table-1: Effect of ethanol extract of *S. torvum* on blood glucose concentration in streptozotocin induced diabetic rats

Experimental groups	Fasting Blood Glucose (mg/dl)				
	0 Day	7 Day	14Day	21Day	28Day
Control	79.26±8.6	77.15±10.24	78.16±12.17	80.15±11.27	79.54±10.53
Diabetic control	290.35±17.84	292.19±18.53	294.54±20.06	298.37±22.64	299.27±23.22
Diabetic + 200mg extract	289.55±19.29	277.86±18.35	257.35±19.27	239.41±22.43	193.22±24.91**
Diabetic + 400mg extract	277.4±15.57	261.75±18.21	236.5±21.12	207.2±22.58*	184.3±24.17**
Diabetic + Metformin	284.46±12.45	246.7±16.15	208.43±18.13*	148.24±12.33**	112.35±12.15**

Data expressed as mean ± SEM. Evaluation by One Way -Analysis of Variance (ANOVA) followed by Dunnett'sMultiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.

Table-2: Effect of ethanol extract of *S. torvum* on organ weights in experimental rats

Experimental groups	Organ weight (g)		
	Liver	Kidney	Pancreas
Control	6.35±0.14	1.13±0.02	0.6±0.03
Diabetic control	4.18±0.13	0.75±0.01	0.48±0.06
Diabetic + 200mg extract	5.56±0.14**	0.9±0.07	0.54±0.03
Diabetic + 400mg extract	6.17±0.13**	1.13±0.08*	0.64±0.06
Diabetic + Metformin	7.00±0.07**	1.12±0.05*	0.62±0.05

n=6; Data expressed as mean ± SEM. Evaluation by One Way-Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control

Table-3: Effect of ethanol extract of *S. torvum* on lipid profile in experimental rats

Experimental groups	T-CH	TG	HDL-CH	LDL-CH	VLDL-CH
Control	75.23±6.7	43.4±4.25	24.35±3.14	42.4±4.12	8.36±0.71
Diabetic control	196.3±6.23	140.25±6.33	16.8±2.31	161.7±14.15	32.4±2.37
Diabetic + 200mg extract	166.14±8.25**	121.35±5.12**	21.07±2	112.36±11.17*	20.6±2.35**
Diabetic + 400mg extract	115.4±12.62**	83.52±10.7**	23.17±2.39*	77.47±10.16**	18.02±2.04**
Diabetic + Metformin	95.77±9.65**	77.25±4.48**	34.3±3.26**	58.4±8.12**	16.47±2.61**

T-CH: Total cholesterol; TG: Triglycerides; HDL-CH: HDL cholesterol; LDL-CH: LDL cholesterol; VLDL-CH: VLDL cholesterol

n=6; Data expressed as mean ± SEM. Evaluation by One Way-Analysis of Variance (ANOVA) Followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.

Table-4: Effect of methanol extract of *S. torvum* liver glycogen and glycosylated hemoglobin in experimental rats

Experimental groups	Liver glycogen (g/100g)	Glycosylated hemoglobin (%)
Control	3.46±0.23	6.13±0.16
Diabetic control	0.89±0.05	10.23±0.12
Diabetic + 200mg extract	2.51±0.15*	8.22±0.14**
Diabetic + 400mg extract	2.76±0.05**	7.12±0.17**
Diabetic + Metformin	3.14±0.11**	6.75±0.14**

n=6; Data expressed as mean ± SEM. Evaluation by One Way-Analysis of Variance (ANOVA) followed by Dunnett's Multiple Comparison test. *P<0.05, **P<0.01 as compared to diabetic control.

CONCLUSION

In the present study, antidiabetic activity of ethanol root extract of *S. torvum* was evaluated in Wister rats using streptozotocin induced diabetes model. The study revealed significant improvements in different biochemical parameters we have studied. Rats treated with the extract showed improvement in liver glycogen, HDL cholesterol and has shown its ability to enhance the glycogenesis process in the liver of the diabetic rats. Further, the extract significantly reduced the levels of LDL cholesterol and increased that of HDL cholesterol. The facilitation of atherogenesis by LDL cholesterol is due to its role in depositing cholesterol in the vascular bed. HDL cholesterol however carries out the reverse transport of excess cholesterol from cells of tissues to the liver. Thus along with antidiabetic activity, the ethanol root extract of *S. torvum* has the potential to prevent formation of atherosclerosis and coronary heart disease which are the secondary complications of diabetes mellitus.

REFERENCES

1. Anonymous. (1999). Ancient-modern concordance in Ayurvedic plants: some examples. *Environ Health Perspect.* 107 (10): 783–789.
2. Thomas, J. (1997). Medicinal and aromatic plants research in India. In UNDP.
3. Mathur, A. (2003). Who owns Traditional Knowledge, Working Paper No. 96: *Indian Council for Research on International Economic Relations*, 1-33.
4. Raskin, I., Ribnicky, D.M., Komarnytsky, S., & Poulev, A. (2002). Plants and human health in the twenty-first century. In: *Trends in Biotechnology*, 20(12): 522-531.
5. Verma, N.P., Mehta, S.P., Madhu, S., Mather, H.M., & Keen, H. (1986). Prevalence of known diabetes in an urban Indian environment: the Darya Ganj diabetes survey.*Br Med J*, 293: 423-27.
6. Ramchandran, A., Snehlatha, C., Daisy, D., & Viswanathan, M. (1992). Prevalence of glucose intolerance in Asian Indian: Urban-rural difference and significance of upper adiposity. *Diabetes Care*, 15: 1348-1355.
7. Ramaiya, K.L., Kodali, V.R., & Alberti, K.G. (1990). Epidermiology of diabetes in Asians of the Indian subcontinent, *Diabetes Metab Rev*, 6: 125-46.
8. Akhilesh, K.T., Pravin, K.B., Jagdish, R.B., Dinesh, M.B., & Khalique, M. (2011). Herbal antidiabetics: A Review, *Int. J. Res. Pharm. Sci.*,

2(1): 30-37.
9. Eliza, J., Daisy, P., Ignacimuthu, S., & Duraipandiyan, V. (2009). Chemico-Biological Interactions, 182(1), 67-72.
10. Jain, S., Katare, Y., & Patil, U.K. (2011). Der Pharmacia Lettre, 3(3), 183-193.
11. Farswan, M., Mazumder, P.M., & Percha, V. (2009). Indian J Pharmacol, 41(1), 19-22.
12. Moller, D. (2001). Nature, 414, 821-827.
13. American Diabetes Association (ADA), (2007). Diabetes Care, 30, 4-41.
14. Otamere, H.O., Aloamaka, C.P., Okokhere, P.O., & Adisa, W.A. (2011). British Journal of Pharmacology and Toxicology, 2(3), 135-137.
15. Kritchevsky, D. (1995). J. Nutr., 125(Suppl.3), 5589-5593.
16. Rajasekaran, S. K., Ravi, K., Sivagnanam, K., & Subramanian, S. (2006). J. Clinical and Experimental Pharmacology and Physiology, 33, 232- 237.
17. Adjanohoun, J., Aboubakar, N., Dramane, K., Ebot, E., Ekpere, A., & Enoworock, G. (1996). Traditional medicine and pharmacopeia-contribution to ethnobotanical and floristic studies in Cameroon, In: CNPMS. Porto-Novo, Benin, 50–52.
18. Forest, S., Kim, S., & Lloyd, L. (2003). *Solanum torvum*, United States Geological Survey-Biological Resources Division Haleakala Field Station, Maui, Hawai, 1-4.
19. Wagner, W.L., Herbst, D.R., & Sohmer, S.H. (1999) Manual of the Flowering Plants of Hawai. Vols. 2, Bishop Museum Special Publication 83, University of Hawai and Bishop Museum Press, Honolulu, HI, 14-17.
20. Oppenheimer, H.L. Meidell, J.S., & Bartlett, R.T. (1999). New plant records for Maui and Moloka. Bishop Mus. Occ. Pap. 59(2): 7-11.
21. Panthong, A., Kanjanapothi, D., & Taylor, W.C. (1986). Ethnobotanical review of medicinal plants from thai traditional books, part 1: plants with antiinflammatory, antiasthmati and antihypertensive properties. J. Ethnopharmacol, 18 (3): 213-228.
22. Alam, M.K. (1992). Medical ethnobotany of the marma tribe of Bangladesh. Econ Bot, 46 (3): 330-335.
23. Adesina, S.K. (1982). Studies on some plants used as anticonvulsants in amerindian and african traditional medicine. Fitoterapia, 53: 147-162.
24. Mahmood, U., Shukla, Y.N., & Thakur, R.S. (1983). Non-alkaloidal constituents from *Solanum torvum* leaves. Phytochemistry, 22 (1): 167-169.
25. Coee, F.G. & Anderson, G.I. (1996). Ethnobotany of the garifuna of eastern Nicaragua. Econ Bot, 50 (1): 71-107.
26. Holdsworth, D. (1993). Medicinal plants of the gazele peninsula, New Britain Island, Papua New Guinea. Part I. Int. J. Pharmacog, 31 (1): 19-22.
27. Holdsworth, D. (1975). Traditional medicinal plants used in the treatment of malaria and fevers in papua new guinea. Papua New Guinea Med J, 18:142-148.
28. Coee, F.G. Anderson, G.I. (1996). Screening of medicinal plants used by the garifuna of eastern nicaragua for bioactive compounds. J Ethnopharmacol, 53: 29-50.
29. Dominguez, X. A. Alcorn, J. B. (1985). Screening of medicinal plants used by huastec mayans of northeastern mexico. J Ethnopharmacol, 13 (2): 139-156.
30. Mueller-Oerlinghausen, B., Ngamwathana, W., & Kanchanapee, P. (1971). Investigation into thai medicinal plants said to cure diabetes. J. Med Ass Thailand, 54: 105-111.
31. Maurakami, A., Nakamura, Y., Ohigashi, H., & Koshimizu, K. (1997). Cancer chemopreventive potentials of edible thai plants and some of their active constituents. Mem Sch Biol Oriented Sci Technol Kinki Univ, (1): 1-23.
32. Valsaraj, R., Pushpangadan, P., Smitt, U.W., Adsersen, A., & Nyman, U. (1997). Antimicrobial screening of selected medicinal plants from india. J Ethnopharmacol, 58 (2): 75-83.
33. Kannan, M., Dheeba, B., Gurudevi, S., & Ranjit Singh, A. J. A. (2012). Phytochemical, antibacterial and antioxident studies on medicinal plant Solanum torvum. Journal of Pharmacy Research, 5(5), 2418-2421.
34. Roychoudhury, R. (1984). Virus inhibitor from *Solanum torvum*. Indian Phytopathol, 37 (4): 665-668.
35. Gopalsamy, R. G., Savarimuthu, I., Michael, G.P., & Ponnusamy, S. (2011). Antihyperglycemic activity and antidiabetic effect of methylcaffeate isolated from *Solanum torvum* Swartz. fruit in streptozotocin induced diabetic rats, European Journal of Pharmacology, 6 (2–3): 623–631.
36. Cuervo, A. C., Blunden, G., & Patel, A.V. (1991). Chlorogenone and neochlorogenone from the unripe fruits of *Solanum torvum*. Phytochemistry, 30 (4): 1339-1341.
37. Dopke, W., Nogueiras, C., & Hess, U. (1975). On the steroid alkaloid and saponin contents of *Solanum torvum. Pharmazie*, 30: 755.
38. Mahmood, U., Agrawal, P.K., & Thakur, R.S. (1985). Torvonin-a, a spirostane saponin from *Solanum torvum* leaves. Phytochemistry, 24 (10): 2456-2457.
39. Agrawal, P.K., Mahmood, U., & Thakur, R.S. (1989). Torvonin-b, a spirostane saponin from *Solanum torvum*-1. Heterocycles, 29 (10): 1895-1899.
40. Yahara, S., Yamashita, T., Nozawa, N., & Nohara, T. (1996). Steroidal glycosides from *Solanum torvum.* Phytochemistry, 43 (5): 1069-1074.
41. Smith, S.W., Giesbrecht, E., Thompson, M., Nelson, L.S., & Hoffman, R.S. (2008). Solanaceous steroidal glycoalkaloids and poisoning by *Solanum torvum*, the normally edible susumber

berry. Toxicon, 52(6): 667-76.
42. Trease, G.E., Evans, W.C. (1989). Pharmacognosy, ELBS Publication, New Delhi, India. 171-175.
43. Harborne, J.B. (1984). Phytochemical method, A Guide to modern techniques of plant analysis, Edited by Chapman and Hall, New York. 76-85.
44. Ainapure, S.S., Arjaria, P.D., Sawant, V.R., Baid, P.S., & Varda, A.B. (1985). Indian J. Pharmacology, 17, 238-239.
45. Acharyya, S., Dash, G.K., Mondal, S., Acharyya, A. & Dash, S.K. (2010). Studies on the hypoglycaemic activity of Acacia suma (Roxb.) barks. International Journal of Chemical and Analytical Science, 1(1),10-13.
46. Edwin, E., Sheeja, E., Dhanabal, S.P., & Suresh, B. (2007). Indian J. of Pharmaceutical Sciences, 69(4), 570-571.
47. Sood, R., (1999). Clinical chemistry, in: Medical laboratory technology, methods and interpretations, 5th ed., Jaypee brothers' medical publishers, India, 173-404.
48. Oduola, T., Adeniyi, F.A.A., Ogunyemi, E.O., Bello, I.S., Idowu, T.O., & Subair, H.G. (2007). J of Medicinal Plants Research, 11, 1-4.
49. Caroll, V.V., Longly, R.W. & Joseph, H.R. (1956). J. Biol. Chem, 220, 583-593.
50. Gabbay, K.H., Hasty, K., Breslow, R., Ellison, C. Bunn, H.E. & Gallop, P.M. (1977). J. Clin. Endocrinol. Metab, 44, 859-864.
51. Palmer, J.P., Lernmark, A. (1997). Pathophysiology of Type1 (insulin-dependent) diabetes. In: Porte Jr D, Sherwin, RS (Eds.), Ellenberg and Rifkin's Diabetes Mellitus. Appleton and Lange, Connecticut, U.S.A. 455-486.
52. Szkudelski, T. (2001). Physiological Research, 50, 536-546.
53. SyGy, A., Cisse, R.B., Nangonierma, M., Sarr, N.A., Mbodj, B. (2005). J Ethnopharamacol, 98, 171-175.
54. Chandola, H. M., Tripathi, S. N., & Udupa, K. N. (1980). Hypoglycaemic response of C tamala in patients of maturity onset (insulin independent) diabetes. *J Res Ayurveda Siddha*, *1*, 275-290.
55. Chatterjea, M. N., & Shinde, R. (2002). Textbook of Medical Biochemistry Jaypee Brothers Medical Publishers.