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A Comparative Study of Antihyperglycemic Effect of Gymnema Sylvestre and Teneligliptin in Alloxan Induced Diabetic Rats

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ABSTRACT
Diabetes mellitus (Madhumeha) is one of the leading metabolic disorder prevalent in the developing countries which is characterized by high blood sugar level and is associated with macrovascular and microvascular complications. The Indian Ayurveda describes several herbs for the management and treatment of diabetes mellitus among which Gymnema sylvestre (Asclepiadaceae) is revered as a potential antidiabetic herbal drug which has the capability of simultaneously regenerating β-cell and stimulating insulin secretion. Gymnema sylvestre also possesses anti-obesity, anti-hyperlipidemic, anti-inflammatory, and anti-cancerous activities. This review updates the recent developments in the experimental studies conducted on the Gymnema sylvestre as an effective remedy for diabetes mellitus evidenced by both animals and human studies. Moreover, this study also discussed the toxicity of Gymnema sylvestre and future challenges in the roadmap of formulation for prevention and control of diabetes.
Keywords: Gymnema sylvestre leaves, teneligliptin, alloxan

I. INTRODUCTION

Diabetes mellitus is one of the common metabolic disorders with micro-and macro vascular complications that results in significant morbidity and mortality. It is considered as one of the five leading causes of death in the world. In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus there is increasing demand by patients to use natural products with anti diabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agents. medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine.

Teneligliptin

Teneligliptin is a recently developed oral dipeptidyl peptidase 4 inhibitor indicated for the management of type 2 diabetes mellitus (T2DM) in adults along with diet and exercise. Teneligliptin has been recently available in Japan (Teneria), and India (Tenepure; Teneza) at relatively affordable price. This is a positive step toward the management of T2DM in developing countries, where the cost of medicine is out-of-pocket expenditure and is a limiting factor for health care. This review evaluates the efficacy and safety of teneligliptin in the management of T2DM. Teneligliptin has been systematically evaluated in T2DM as monotherapy with diet and exercise and in combination with metformin, glimepiride, pioglitazone, and insulin in short-term (12 weeks) and long-term (52 weeks) studies. These studies have reported a reduction in HbA1c (glycated hemoglobin) of 0.8%–0.9% within 12 weeks of therapy. Two 52-week studies reported sustained improvement in glycemic control with teneligliptin. Teneligliptin has been found to be well tolerated, and the safety profile is similar to other dipeptidyl peptidase 4 inhibitors. Hypoglycemia and constipation are the main adverse events. Teneligliptin can be administered safely to patients with mild, moderate, or severe renal impairment or end-stage renal disease without dose adjustment. Similarly, it can be used in patients with mild-to-moderate hepatic impairment. Teneligliptin is effective and well tolerated and may have an important role in the management of T2DM.

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II. MATERIAL AND METHOD

Experimental animal

ClassificatiON
Kingdom- Animalia
Phylum-Chordata
Sub-phylum- Vertebrata
Class- Mammalia
Order – Rodentia
Genus- Rattus
Species-norvegicus

ANIMAL HOUSING AND FEEDING CONDITION

Male rats (24) weighing 150-200gm of SD strain obtained from animal house, of Pacific College of Pharmacy, Udaipur were used and received human care in compliance with the guideline for the care and use of laboratory Animals. Animals were kept in polypropylene autoclavable (dimension: 43×27×15cms) cage at 24±0ºC. Bedding husk was changed daily. 12 hr day light cycle was maintained in the room with the help of artificial lighting. For feeding, laboratory pellet diet was provided ad lib through a container of appropriate size, water was also provided ad lib by means of water feeding bottles fitted with a nozzle. Experimental Protocols were approved by the Institutional Animals ethics Committee (IAEC) which follows guidelines of committee for the purpose of control and supervision of animals (CPCSEA) which conforms to international norms of Indian national science Academy (INSA). Initially animals were allowed to acclimatize for seven days with free access to water and feed.

MARKING FOR IDENTIFICATION OF ANIMALS: -

Colour coding of fur-coat of animals was done at different sites for giving a unique identity to each rat. Marking (after selection and random grouping) of animals was done by saturated aqueous solution of 1% picric acid.

EXPERIMENTAL PHYTO – EXTRACT (GYMNEMA SYLVESTRE)

GYMNEMA SYLVESTRE PLANT

FINE POWDER OF GYMNEMA SYLVESTRE

GYMNEMA SYLVESTRE EXTRACTION BY USING SOXHLET APPARATUS:-

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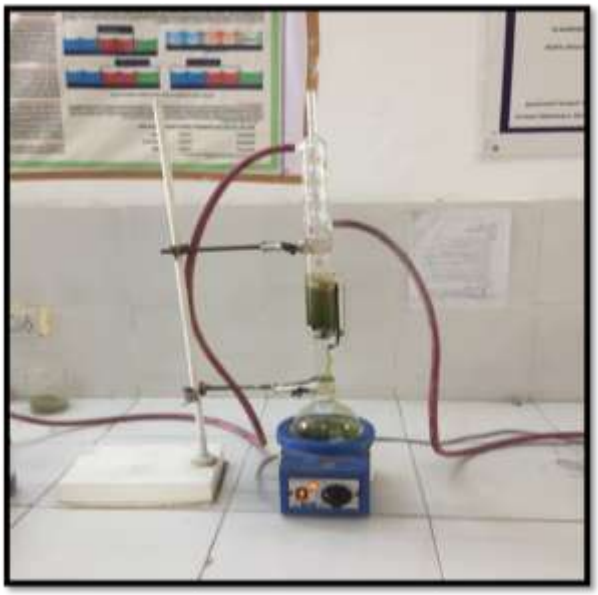

EXTRACTED GYMNEMA SYLVESTRE

SCIENTIFIC CLASSIFICATION:-

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliopsida
Class	Asteridae
Subclass	Gentianales
Order	Gentianales
Family	Asclepiadaceae
Genus	Gymnema
Species	Sylvestre

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PLANT MATERIAL:-

One (1) kilogram leaves were collected from SISARMA BOTANICAL GARDEN, Udaipur. the plant leaves were air dried under shed at 25ºc. And the dried leaves were made in to a fine powder with an auto-mixblender. The powder was kept in deep freezer until the time of use.

PREPARATION OF ETHANOLIC EXTRACT OF GYMNEMA SYLVESTRE:-

One thousand grams of dry fine powder was suspended in 500ml of absolute ethanol (Merck) for 3 days. On the 4th day the leaves material was extracted with ethanol in apparatus for 6 hours and then boiled at 60°C to 65°C for 30 minutes (since boiled decoction of the leaf of this plant has been used as remedy for diabetes). The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40°C yielded 20 percent (20gm).

EXPERIMENTAL INDUCTION OF DIABETES:-

The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg body weight. Blood samples were collected before the administration of alloxan and after 5 days of alloxan administration. Diabetic state was confirmed when the blood sugar level was above 200 mg/dl. The rats with moderate diabetes and hypolipidemia were used for the experiment.

At the end of 0th,7th,14th,& 28th day blood was collected in falcon tubes containing potassium oxylate & sodium fluoride solution for the estimation of glucose and lipid profile.

EXPERIMENTAL DESIGN:-

Healthy animals were selected on the basis of body weights recording and they were randomly divided into four different groups,each group consisting 6 animals(n=6).First group act asnormal control received vehicle(normal saline) orally by oral gavage for 28 days. second group administered alloxan monohydrate (150mg/kg) intraperitoneally (i.p), third group administered standard drug Teneligliptin (0.5mg/kg) after seven days of induction of alloxan monohydrate, orally once a day. And the fourth group administered gymnema leaves extract (100mg/kg), after seven days induction of alloxan monohydrate orally once a day.

• Group I - Normal group (0.9% Normal saline)
• Group II -Diabetic control (DC) group: Alloxan monohydrate (150mg/ kg)
• Group III - Standard drug Teneligliptin (0.5mg/kg) p.o
• GroupIV - gymnema sylvestre extract (400mg/kg) per oral (p.o.)

DOSE SELECTION:-

TENELIGLIPTIN:-

In the present study the dose of Tenegiptin 0.5mg/kg body weight per oral was selected. The daily dose of tenegliptin for albino rats was calculated by extrapolation from the human dose (10mg/day)

Method of Preparation of Teneligliptin Suspension:-

The stock solution was prepared by dissolving 7.5 mg of Teneligliptin in 15 ml of distilled water and administered as a standard drug in a dose of 0.5 mg/kg body weight for the standard group.

Method of preparation of Gymnema sylvestre:-

In the present study the dose of 400 mg/kg was selected. The dose was selected based on the reports in previous study which had antidiabetic and hypolipidemic activity. All doses were administered between 9-9:30am. Method of Preparation of gymnema sylvestre Suspension The stock solution was prepared by dissolving 9 gm of gymnema sylvestre extract in 18 ml of distilled water and administered as a test drug in a dose of 400 mg/kg body weight for the test group.

Collection of blood for estimation of biochemical parameters:-

The blood was collected from the rat tail vein for the estimation of blood sugar by usingglucometer and blood glucose test-strips, supplied by Ascensia Entrust of Bayer Health Care.(Udaipur) For estimation of other biochemical parameters, blood was drawn from the retro-orbitalplexus of the rats (fasted for 12 h), in to sterilize eppendorf tubes. The blood samples wereallowed to coagulate for 30 min at room temperature and then they were centrifuged at 3000rpm for 10 min. The serum used as specimen, should be free from haemolysis and must be seperated from the clot promptly. The resulting upper serum layer was collected in theproperly cleaned, dried, and labeled eppendorf tubes and they were stored at freezer for furtheranalysis of the lipid profiles.

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DRUGS AND CHEMICALS:-

Teneligliptin was purchased from SRL (sisco research laboratories), was dissolved in 0.9% of normal saline. Alloxan was purchased from Merck, India, and the ethanolic extract of gymnema sylvestre was prepared by soxhlet apparatus in chemistry lab. of pacific college of pharmacy, Udaipur and it dissolved in DMSO. Haematoxyline and eosin stains were also purchased from SRL (India).

BIOCHEMICAL ESTIMATIONS:-

ROUTINE PARAMETER:-

According to my experiment i checked Body weight of animals on 0^{th},7^{th},14^{th}&28^{th} day. I also checked feed consumptions & clinical signs of animals accordingly.

EFFECT OF PHYTO EXTRACT ON BLOOD GLUCOSE LEVEL IN RATS:-

Fasting blood glucose was estimated by using a commercial glucometer and test strips (Accucheck Sensor test meter).

LEVEL OF SERUM CONSTITUENTS-UREA, URIC ACID, AND CREATININE IN CONTROL AND EXPERIMENTAL RATS:-

Insulin was measured by using an ultrasensitive Rat Insulin Elisa Kit purchased fromMercodia AB, Sylveniusgatan, Sweden (Cat No. 10-1124-01).Plasma - Insulin levels is expressed as IU/ml.

ESTIMATION OF TOTAL CHOLESTEROL (TC):-

Total cholesterol level was determined by the commercially available reagent kit. It s based on enzymatic method.

ESTIMATION OF HDL-CHOLESTEROL(HDL):-HDL-cholesterol level was also determined by commercially available reagent kit based on phosphotungustate method.

ESTIMATION OF TRIGLYCERIDES:-

Triglycerides level was estimated by using blood sample that a lab analysed.Triglycerides level was estimated by commercially available kit. It is based on enzymatic colorimetric method. This reagent kit was made for in vitro quantitative determination of triglycerides in serum or plasma. Our study was carried out by serum.

PANCREATIC HISTOPATHOLOGY IN FORMALIN FIXED TISSUE OF RATS:-

Pancreatic tissue samples were fixed in buffered 10% formalin overnight. washed next day, then the tissues were dehydrated by ascending grades of ethanol (70% ethanol for first 3 hours followed by 90% ethanol for 3 hours then followed by 100% ethanol for 3 hour)and finally with xylene for 3 hours with continuous shaking through the dehydration process. After that the tissue were dipped in melted paraffin for 4 hours at 60ºC and paraffin blocks were made. A 5µm thick slices of paraffin embedded stomachs were obtained on a poly-L-lysine coated glass slides with the help of Microtome (Leica Model:RM2255). There after the stomach section were stained.

HEMATOXYLIN AND EOSIN (H&E) STAINING:-

Procedure:- Pancreatic section were processed for the H&E staining. The sections were dewaxed with xylene for 5 minutes and this process was repeated thrice, in coupling jars. The section were rehydrated using descending grades of ethanol, 100% 95% and 70% respectively for 2 minutes. Each step was repeated twice. Then the slides were again hydrated with distilled water for 2 minute. After rehydration, the sections were stained with hematoxylin for 1 minute then the slides were washed in running water to remove excess hamatoxyline.After this, the slides were again dehydrated with 95% and 100% ethanol respectively for two minutes .Each, step was repeated twice. Then the slides were processed with three changes of xylene for 5 minutes. Finally, the slides were mounted in DPX solution and allowed to dry at room temperature. The mounted slides were observed under microscope in bright field light. Images of H&E stained sections were acquired at 20x with the help of microscope (leica microscope model DM 5000). The 20x images were quantified by the help of leica Q win V3 software.

III. STATISTICAL ANALYSIS:-

All data are presented as mean ± standard error and analyzed with one-way ANOVA followed by Bonferroni test for multiple group comparisons. Analyses were performedusing Graph Pad Prism Version 5.0 software (GraphPadSoftware Inc., La Jolla, CA). For all comparisons, $P<0.05$ wasconsidered as statistically significant.

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IV. RESULTS:-

The dose of ethanolic extract of Gymnema sylvestre leaves and dose of Teneligliptin were given to the diabetic rats once a day and changes in fating blood glucose ,total cholesterol,HDL-Cholesterol and triglycerides were measured on 0^{th} day,14^{th} and 28^{th} day from the first dose of experiment. An effective reduction in fasting blood glucose (FBG) level was observed on above mentioned time.

Reduction was examined at the dose of given plant extract Gymnema sylvestre (GS) & teneligliptin (0.5mg/kg) was resulted on observed days.

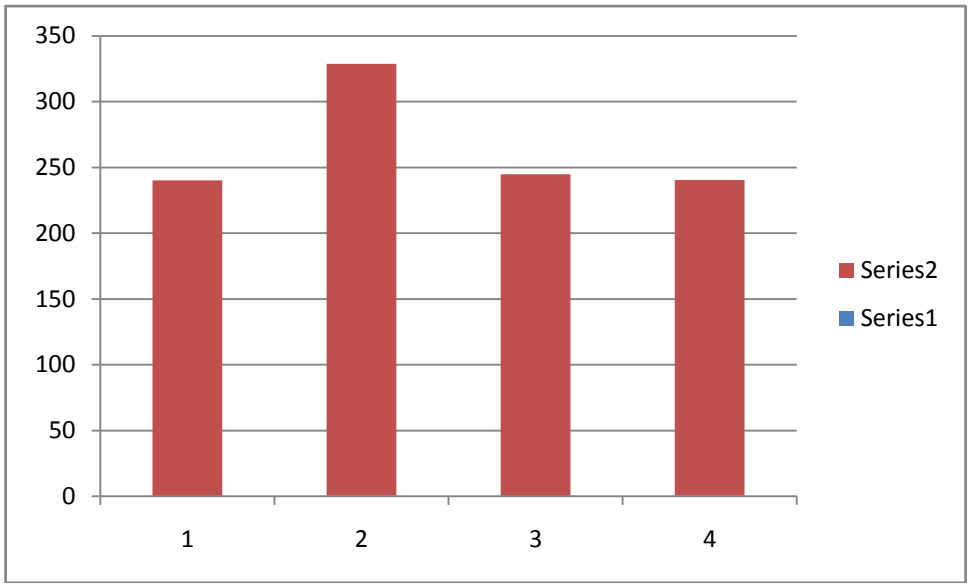
Although a drastic reduction of fasting blood glucose was found to be at 400mg/kg body weight.

Diabetic control showed negligible change whereas maximum percentage reduction of 69% were recorded on 28^{th} day .

The change in the cholesterol, ,HDL-Cholesterol and triglycerides level was measured and observed on potent reduction in serum cholesterol,triglycerides and effective elevation in HDL-Cholesterol level over diabetic control when the rats fed aqueous leaf extract. The level of serum cholesterol was lower in normal rats that were not treated with alloxan and elevation were found in diabetic control .

In respect to HDL- Cholesterol, it showed decrement in normal rats. But maximum elevation of 28^{th} day due to 400mg/kg body weight concentration of aqueous leaf extract kg body weight.However,similar trends of HDL – cholesterol elevation were observed at all doses of treatments with given time periods. Rats fed aqueous leaf extract were showed inhibition in serum triglyceride content. Which was recorded in percentage 50% on 28^{th} day but remarkable reduction of serum triglyceride was noted on feeding at 400mg/kg body weight extract at all days of observations.

BODY WEIGHT OF EXPERIMENTAL ANIMAL:-



A. CONTROL GROUP
B. INDUCED GROUP(Alloxan monohydrate 150mg/kg)
C. STANDARD GROUP (Teneglitine 0.5mg/kg)
D. TEST DRUG (Gymnema sylvestre extract 400mg/kg)

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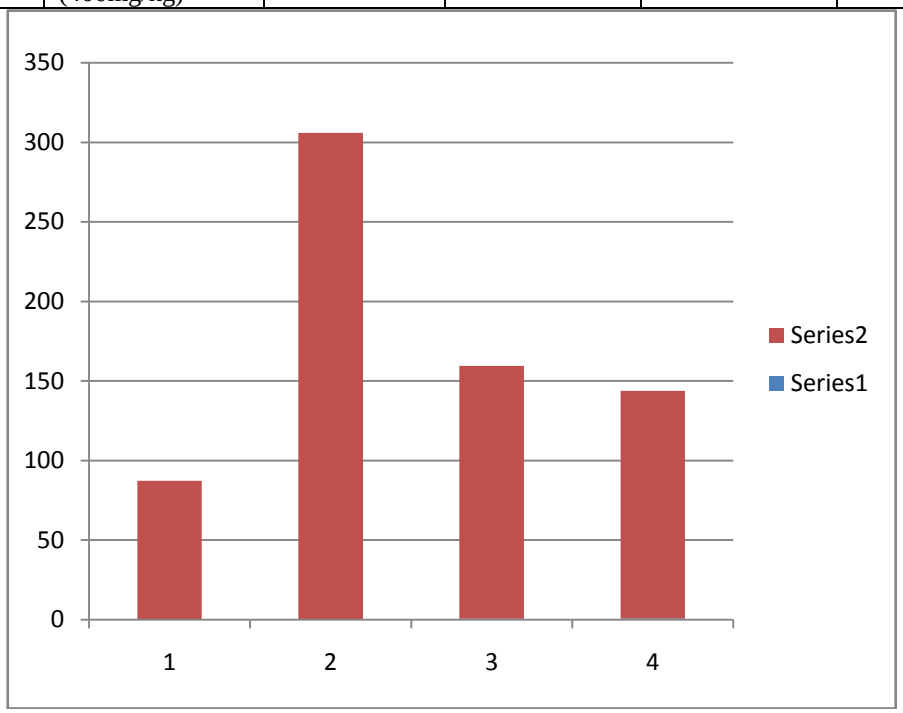
CHANGES IN FASTING BLOOD GLUCOSE LEVELS OF CONTROL AND EXPERIMENTAL ANIMAL:-

Serial no.	GROUP	FASTING BLOOD GLUCOSE(mg/ml)			
		0^{th} day	7^{th} day	14^{th} day	28^{th} day
1.	CONTROL	85±1.6	87.6±2.4	87.3±2.6	89±2.4
2.	DIABETIC (IND	300±3.4	311±2.3	310±2.4	301±2.6
3.	TENELIGLIPTINE	284±2.3	123±2.8	119±3.1	110±2.3
4.	PHYTO EXTRACT (400mg/kg)	289±2.6	98±2.9	95.7±2.8	90±2.5



BLOOD GLUCOSE LEVEL

LEVEL OF SERUM CONSTITUENTS-UREA, URIC ACID, AND CREATININE IN CONTROL AND EXPERIMENTAL RATS:-

Parameters	Urea	Uric Acid	Creatinine
Group	11.58±1.17	0.92±0.12	0.43±0.08
Group	14.32±1.59*	2.00±0.18*	1.38±0.13*
Group	11.96±0.03*	1.22±0.25*	1.72±0.16*
Group	11.07±1.00	0.89±0.09	0.36±0.16

ESTIMATION OF TOTAL CHOLESTEROL LEVEL:-
TOTAL CHOLESTEROL (mg/dl)

Serial no.	Groups	0^{th} day	14^{th} day	28^{th} day
1.	Control	138.6±3.2	136±2.5	134±2.9
2.	Induced grp	265.3±2.1	279.5±2.9	275±3.2
3.	Tenegliptine	261.6±3.3	166±3.2	151±2.5
4.	Phyto-ext.	259±2.9	157.7±2.8	145.2±3.0

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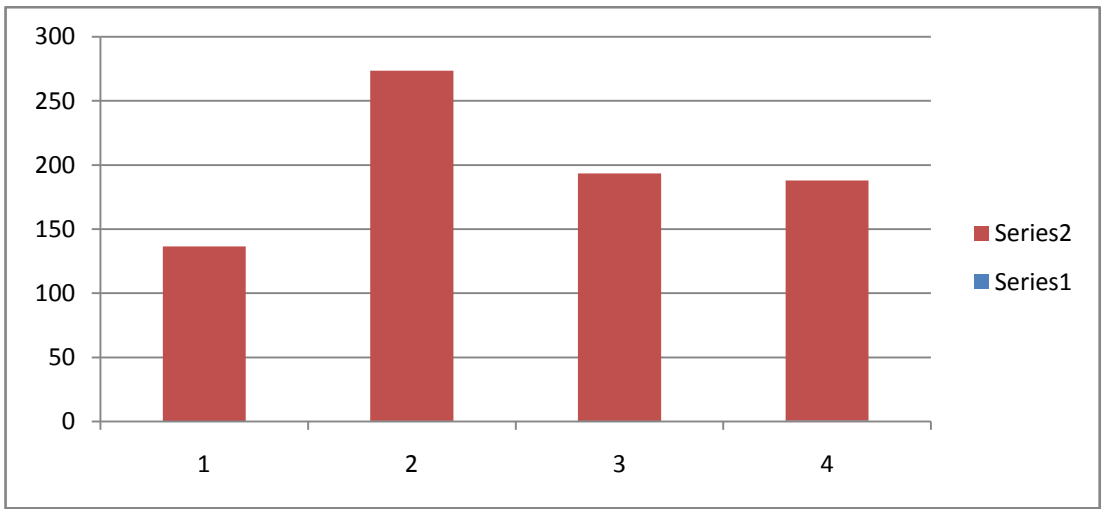
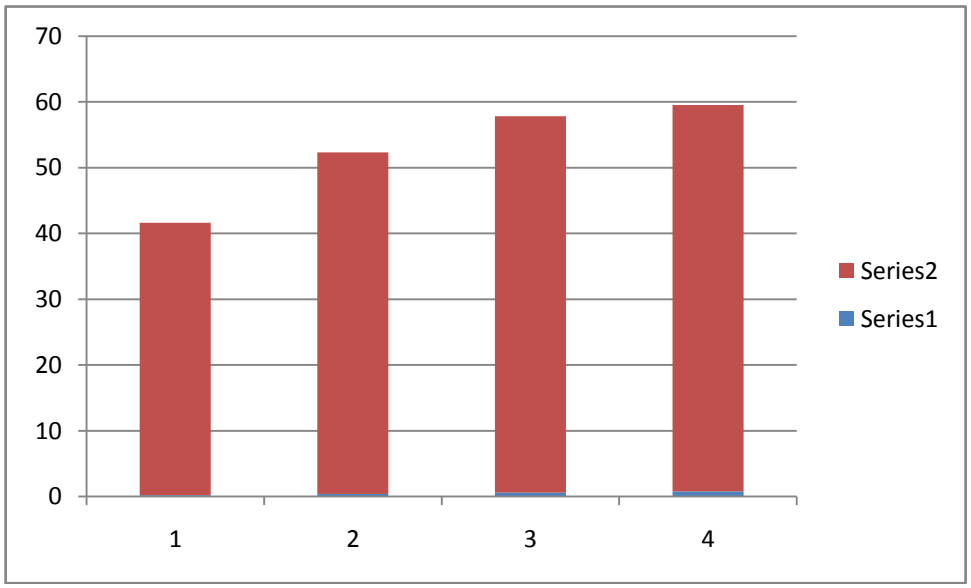


Total cholesterol level

ESTIMATION OF HIGH DENSITY LIPOPROTEIN

Changes in HDL-Cholesterolcontent of control & experimental animals

Serial no.	Group	0^{th} day	14^{th} day	28^{th} day
1.	Control	44.3±1.2	40±2.4	40±2.3
2.	Induced gp	49.6±1.8	51.7±2.7	54.5±2.2
3.	Tenegliptine	51±2.9	59.2±2.6	61.5±1.6
4.	A phyto ext.	49.3±2.3	63±2.9	64.2±1.9



LEVEL OF HIGH DENSITY LIPOPROTEIN

ESTIMATION OF TRIGLYCERIDES (TG):-

Changes in serum triglyceride(TG) content and experimental animals:-

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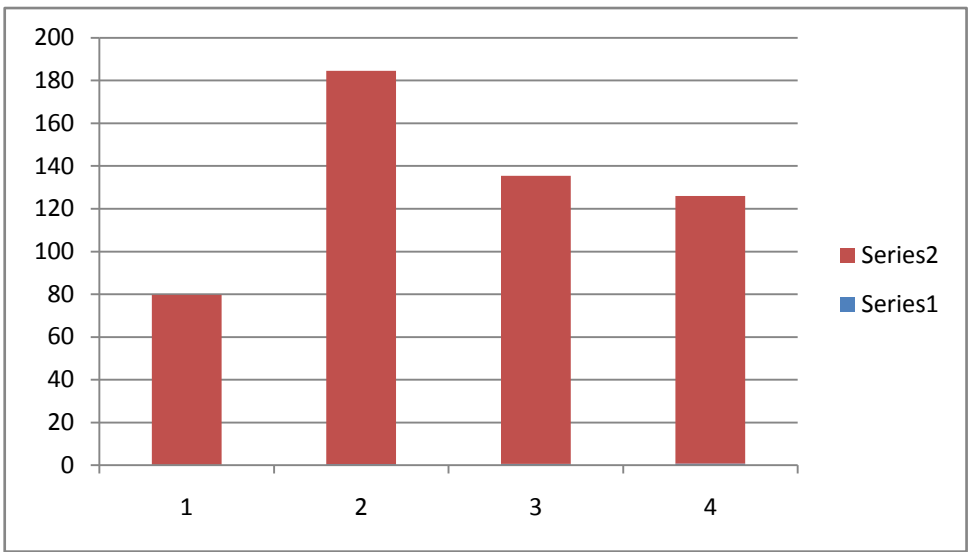
Serum triglyceride (mg/dl)

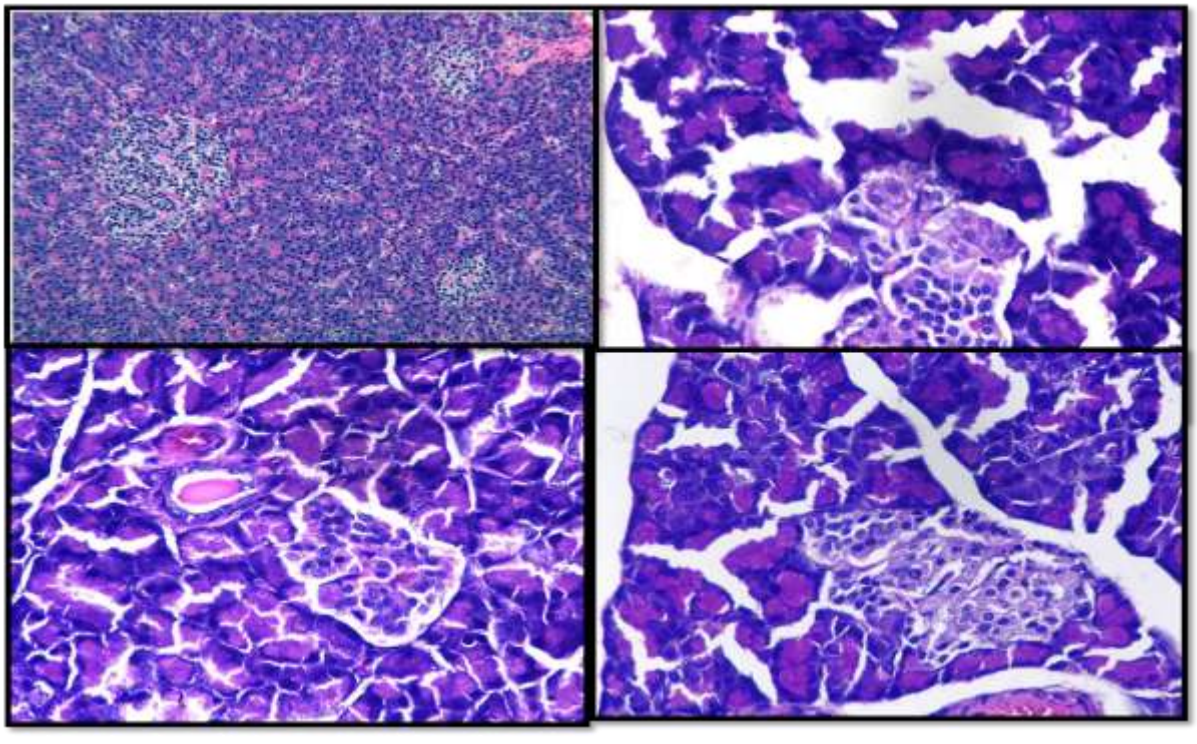
Serial no.	group	0th day	14th day	28th day
1.	Control	80.6±1.6	78±2.7	79.7±2.5
2.	Induced grp	187±2.6	186.7±1.4	179.5±2.5
3.	Tenegliptine	191.3±2.3	107±3.0	106.5±2.2
4.	A Phyto-ext	186±2.9	97.2±2.9	92.5±2.3



LEVEL OF TRIGLYCERIDES IN RATS

HISTOPATHOLOGY OF PANCREATIC TISSUE

A **B**



C **D**

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A. CONTROL GROUP (10x)
B. INDUCED DIABETIC GROUP (10x)
C. STANDARD GROUP (Teneligliptin) (10x)
D. TEST DRUG GROUP (GS phyto extract) (10x)

V. DISCUSSION

Diabetes is a chronic disease caused by inherited or acquired deficiency leading to the lower production of insulin by pancreas or by the ineffectiveness of the insulin produced. This results in increased level of glucose in the blood, damage of many organs of the body systems, blood vessels, nerves, kidney and eyes.Thus our studies provide experimental evidence for the herbal plant Gymnemasylvestre in the prevention and curing of alloxan induced diabetic rats without any side effects.

Diabetes mellitus is cause for global health concern as the disease is rapidly progressing, also the age of onset to younger age groups is alarming. The standard drug therapy has various side-effects and hence need for development of new drugs with better safety profile. Many medicinal plants are used by traditional medicine for treatment of diabetes. Gymnema sylvestre leaves when chewed have property of paralysing the sense of taste for sweet for short period.

The treatment of prediabetic patients is mainly lifestyle modification in the form of weight reduction, exercise and diet control. Lifestyle education at regular health check-up for people with prediabetes lower progression to diabetes by reducing modifiable risk factors. But to follow these lifestyle modifications requires motivation and physicians should assess patient's readiness to work towards change. Studies have shown that people are resistant to lifestyle change.8 Looking at the increasing number of people in prediabetic stage, there is an urgent need to explore different therapeutic options. Since people in India prefer alternative medicine due to their claim of being side effect free, the exploration of vast knowledge of Ayurvedic medicine can help us in understanding their role in various chronic diseases either to prevent further development or to prolong the onset.

Kumar et al, studied the effect of ethanolic extract of Gymnema sylvestre on Alloxan induced diabetic rats. They found that there was significant reduction in blood glucose levels.

Alloxan a β-cytotoxin,induces "chemical diabetes" in a wide variety of animal species by damaging the insulin secreting pancreatic β –cells, resulting in a decrease in endogenous insulin release,which paves the ways for the decreased utilization of glucose by the tissue. In our study ,we have observed that G.Sylvestre decreases fasting blood glucose level in alloxan induced diabetic rats that may be due to decreased level of glucagon and the increased activity of incretin which works to stimulate insulin release and help lower blood sugar. And also the increased activity of enzymes that is responsible for utilization of glucose by insulin dependent pathway or regenerate β-cells in pancreatic islets of like the plant extract.

Teneligliptin also produced significant in blood glucose levels of alloxan diabetic rats,the present findings appear to be in consonance with the earlier suggestion .

In our study,the feeding of G.Sylvestre leaf extract resulted in significantly decreased total cholesterol and serum triglyceride and significantly decreased total cholesteroland serum triglyceride and significantly increased HDL-Cholesterol level; these findings are correlated with the experiment ingestion of G.Sylvestre produced a significant lowering of cholesterol in a hypertension model. Insulin is potent inhibitor of lypolysis since it inhibits the activity of the hormones sensitive lipase in adipose tissueand suppresses the release of triglycerides. The increase in HDL cholesterol levels may be beneficial awing to the negative correlation between HDL-Cholesterol level and cardiovascular diseases.

VI. CONCLUSION

- Ayurveda practice continues today to treat various chronic human diseases and provides positive health benefits to the people and plays significant role in prevention of various diseases. Our investigation demonstrates that ethanolic extract of GS possesses antihyperglycemic activity and so it can be considered as a promising natural remedy in a pre diabetic state to prevent its progression.
- It can also be used as an adjuvant treatment along with the standard allopathic treatment to treat diabetes and hyperlipidaemia. Increase in β cell regeneration activity could be a probable mechanism of action.
- However further long term clinical studies are recommended to define its possible role in diabetes mellitus and hyperlipidemia. Role of GS as a potential hepatoprotective agent also needs further evaluation.

- Diabetic mellitus is a well known clinical entity with various late complications like retinopathy neuropathy etc.
- In our study G.Sylvestre has significant antidiabetic activity so that it can be used as an adjuvant along with allopathic treatment of medicine to treat diabetes as well as to delay the late complication of diabetes.

REFERENCES

[1]. Grijesh Kumar Mall, Pankaj Kishor Mishra, Veeru Prakash. Antidiabetic and Hypolipidemic Activity of Gymnema sylvestre in Alloxan Induced Diabetic Rats. Global J. Biotech. & Biochem. 2009; 4, 37-42.

[2]. Shanmugasundaram, K.R., Panneerselvam, C, Samudram, P, Shanmugasundaram, E.R.B, Enzyme changes and glucose utilization in diabetic rabbits: the effect of Gymnema sylvestre. R.Br. J. Ethnopharmacol. 1983; 7, 205–234

[3]. Bishayee, A. and Chatterjee, M, Hypolipidaemic and anti atherosclerotic effects of oral Gymnema sylvestre R. Br. Leaf extract in albino rats fed on a high fat diet. Phytothera. Res. 1994; 8, 118-120.

[4]. Dateo G.P., Long L. Gymnemic acid, theantisaccharine principle of Gymnema sylvestre. Studies on isolation and heterogenesity of gymnemic acid A1. J. Agric.Food Chem. 1973; 21, 899–903.

[5]. Edward, P.C. Leaf and Febiger Philadelphia,731. Gamble J.S. 1991. Flora of the Presidency of Madras. Reprinted Edition, Vol I – III, Bishen Hooper, D. Isolation and antiviral activity of gymnemic acid. Pharm. J. Trans. 1956; 17, 867-868.

[6]. Kalidass, C., Shanmugasundaram, R. and Mohan, V.R. Pharmacognostic studies of Capparis separia (L.) R.Br. Pharmacognosy Journal. 2009;1, 121 - 125.

[7]. Mohan Lal Kori et al. Antidiabetic Effect of Hydroalcoholic Combined Plant Extract of Portulaca oleracea and Caralluma attenuata in Streptozotocin Induced Diabetic Rats. Indo American Journal of Pharm Research. 2014; 4, 180-201.

[8]. Lpoz-Candales A. Metabolic syndrome X: a comprehensive review of the pathophysiology and recommended therapy. J Med. 2001; 32, 283-300.

[9]. Elizabeth G, Nabel MD. Cardiovascular disease. N Engl J Med. 2003; 349, 60-72.

[10]. Arky RA. Clinical correlates of metabolic derangements of diabetes mellitus in: Kozak GP. (Ed.), Complications of Diabetes mellitus, Saunders WB. Philadelphia. 1982; 16-20.

[11]. Kumar GPS, Arulselvan P, Kumar DS, Subramanian SP. Anti-diabetic activity of fruits of Terminalia chebula on streptozotocin induced diabetic rats. J Health Sc. 2006; 52, 283–291.

[12]. Day C. Traditional plant treatments for diabetes mellitus: pharmaceutical foods. Br J Nutr. 1998; 80: 203–208

[13]. Kapoor LD. CRC Handbook of Ayurvedic Medicinal Plants; CRC, Boca Raton. 1990; 18, 200–201.

[14]. Dixit RS., Pandey HC. Plant used as folk-medicine in Jhansi and Lalitpur sections of Bundelkhand, Uttar Pradesh. Int J Crude Drug Res. 1984; 22: 47–51.

[15]. Gupta SS., Seth CB. Experimental studies on pituitary diabetes II: Comparison of blood sugar level in normal and anterior pituitary extract induced hyperglycemic rats treated with a few Ayurvedic remedies. Indian J Med Res.1962; 50: 708-714.

[16]. Reddy MB., Reddy KR., Reddy MN. A survey of plant crude drugs of Anantapur district, Andhra Pradesh, India. Int J Crude Drug Res.1989; 3, 145–155.

[17]. Kumar NJ, Loganathan P. Hypoglycemic effect of Spinacia oleracea in alloxan induced diabetic rat. Global J Bioech Biochem.2010; 5, 87-91.

[18]. Aziz A.M, EI shafey, Magda M.EI-Ezabi, Moshira M.E Seliem, Hannen H.M Ouda, Doas Ibrahim. Effect of Gymnema Sylvestre R.Br. leaves extract on certain physiological parameters of diabetic rats. Journal of king saud university-science. 2013; 25, 135-141.

[19]. Kalidass, C., Muthukumar, K., Mohan, V.R. and Manickam,V.S. b.Ethnoveterinary medicinal uses of plants from Agasthiamalai Biosphere Reserve (KMTR), Tirunelveli Tamil Nadu, India. My Forest. 2009; 45, 7–14.

[20]. Kapoor, L.D. CRC Handbook of Ayurvedic medicinal plants: Boca Raton, Fl. 1990; 3, 200 - 201.
[21]. Kanetkar, P.; Singhal, R.; Kamat, M. Gymnema sylvestre: A Memoir. J. Clin.Biochem. Nutr. 2007; 41, 77-81.
[22]. Komalavalli, N.; Rao, M.V, In vitro micrpropagation of Gymnema sylvestre:multipurpose medicinal plant. Cell tissue organ culture. Int J Crude Drug Res. 2005; 61, 97-105.
[23]. Kurihara, Y.. Characteristics of antisweet substances, sweet proteins and sweetness inducing protein. Crit. Rev. FoodSci.Nutr. 1992, 32, 231-252.
[24]. Grijesh KM, Pankaj KM and Veeru P. Antidiabetic and Hypolipidemic Activity of Gymnema sylvestre in Alloxan Induced Diabetic Rats. Global Journal of Biotechnology & Biochemistry. 2009; 4, 37- 42.
[25]. Gulab S. Thakur, Rohit S, Bhagwan S. Sanodiya, Mukeshwar P, Prasad GBKS and Prakash SB. Gymnema sylvestre: An Alternative Therapeutic Agent for Management of Diabetes. Journal of Applied Pharmaceutical Science. 2012; 2, 1- 6.
[26]. The Wealth of India. Raw materials, vol. IV. Council of Scientific and Industrial Research; New Delhi: 1956. A Dictionary of Indian Raw materials and Industrial products; pp. 276–277.
[27]. Komalavalli N., Rao M.V. In vitro micropropagation of Gymnema sylvestre: multipurpose medicinal plant. Plant Cell, Tissue and Organ Culture. 2000;61:97–105.
[28]. Dateo G.P., Long L. Gymnemic acid, the antisaccharine principle of Gymnema sylvestre. Studies on isolation and heterogenesity of gymnemic acid A_1. J. Agric. Food Chem. 1973;21:899–903.
[29]. Liu H.M., Kiuchi F., Tsuda Y. Isolation and structure elucidation of Gymnemic acids, antisweet principles of Gymnema sylvestre. Chem. Pharm. Bull. 1992;40:1366–1375.
[30]. Sinsheimer J.E., Manni P.E. Constituents from Gymnema sylvestre leaves. J. Pharm. Sci. 1965;54:1541–1544.
[31]. Sinsheimer J.E., Subbarao G. Constituents from Gymnema sylvestre leaves VIII: Isolation, chemistry and derivatives of gymnemagenin and gymnestrogenin. J. Pharm. Sci. 1971;60:190–193.
[32]. Sinsheimer J.E., Subba R.G., Mc Ilhenny H.M. Constituents from Gymnema sylvestre Leaves V: Isolation and preliminary characterization of Gymnemic acids. J. Pharm. Sci. 1970;59:622–628.
[33]. Yoshikawa K., Amimoto K., Arihara S., Matsuura K. Structure studies of new antisweet constituents from Gymnema sylvestre. Tetr. Lett. 1989;30:1103–1106.
[34]. Yoshikawa K., Nakagawa M., Yamamoto R., Arihara S., Matsuura K. Antisweet natural products V structures of gymnemic acids VIII-XII from Gymnema sylvestreR. Br. Chem. Pharm. Bull. 1992;40:1779–1782.
[35]. Yoshikawa K., Kondo Y., Arihara S., Matsuura K. Antisweet natural products IX structures of gymnemic acids XV-XVIII from Gymnema sylvestreR. Br. Chem. Pharm. Bull. 1993;41:1730–1732.
[36]. Sahu N., Mahato S.B., Sarkar S.K., Poddar G. Triterpenoid Saponins from Gymnema sylvestre. Phytochem. 1996;41:1181–1185.
[37]. Kanetkar P.V., Laddha K.S., Kamat M.Y. Poster presented at the 16th ICFOST meet organized by CFTRI and DFRL. Mysore, India: 2004. Gymnemic acids: A molecular perspective of its action on carbohydrate metabolism.
[38]. Persaud S.J., Al-Majed H., Raman A, Jones P.M. Gymnema sylvestre stimulates insulin release in vitro by increased membrane permeability. J. Endocrinol. 1999;163:207–212.
[39]. Nakamura Y., Tsumura Y., Tonogai Y., Shibata T. Fecal steroid excretion is increased in rats by oral administration of gymnemic acids contained in Gymnema sylvestre leaves. J. Nutr. 1999; 129:1214–1222.
[40]. Agarwal S.K., Singh S.S., Verma S., Lakshmi V., Sharma A. Chemistry and medicinal uses of Gymnema sylvestre (gur-mar) Leaves: A Review. Indian Drugs. 2000;37:354–360.
[41]. Khare A.K., Tondon R.N., Tewari J.P. Hypoglycemic activity of an indigenous drug Gymnema sylvestre in normal and diabetic persons. Ind. J. Physiol. Pharmacol. 1983;27:257–261.

[42]. Maeda M., Iwashita T., Kurihara Y. Studies on taste modifiers II: Purification and structure determination of gymnemic acids, antisweet active principle from Gymnema sylvestre leaves. Tetr. Lett. 1989;30:1547–1550.
[43]. Manni P.E., Sinsheimer J.E. Constituents from Gymnema sylvestre leaves. J. Pharm. Sci. 1965;54:1541–1544.
[44]. Sinsheimer J.E., Subbarao G. Constituents from Gymnema sylvestre leaves VIII: Isolation, chemistry and derivatives of gymnemagenin and gymnestrogenin. J. Pharm. Sci. 1971;60:190–193.
[45]. Dahanukar S.A., Kulkarni R.A., Rege N.N. Pharmacology of medicinal plants and natural products. Indian J. Pharmacol. 2000;32:S81–S118.
[46]. Ramachandran A., Snehalatha C., Satvavani K., Sivasankari S., Vijav V. Type 2 diabetes in Asian-Indian urban children. Diabetes Care. 2003;26:1022–1025.
[47]. Jachak S.M. Herbal drugs as antidiabetic: an overview. CRIPS. 2002;3:9–13.
[48]. Flier J.S. Prevention of obesity reduces the risk of a wide range of health problems. The missing link with obesity? Nature. 2001;409:292–293.
[49]. Claire S. The hormone resistin links obesity to diabetes. Nature. 2001:307.
[50]. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian J Med Res. 2007;125(3):217-30.
[51]. Powers AC, D'Alessio D. Endocrine Pancreas and Pharmacotherapy of Diabetes Mellitus and Hyperglycemia. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th Ed. China: The McGraw-Hill Companies, Inc.; 2011;1237-1274.
[52]. Krishna RB, Reddy SRR, Javangula H, Swapna D, Reddy KJ. Isolation and Characterization of Gymnemic Acid FromGymnema Sylvestre R.BR. in Control of Diabetes. IJLPR. 2012 Jan-Mar;2(1):01-09.
[53]. Palanisamy M, Kanchana G, Murugan R. Antidiabetic efficacy of Ellagic acid in streptozotocin-induced diabetes mellitus in albino wistar rats. Asian J Pharm Clin Res. 2011;4(3):124-8.
[54]. Reddy MN, Kalyani P, Nagalakshmi K, Kaiser J. Therapeutic plants and their antiobesity properties. Global J Res Med Plant and Indigen Med. 2013;2(9):648-55.
[55]. Brunisholz KD, Joy EA, Hashibe M, Gren LH, Savitz LA, Hamilton S, et al. Stepping Back to Move Forward: Evaluating the Effectiveness of a Diabetes Prevention Program Within a Large Integrated Healthcare Delivery System. J Healthc Qual. 2017;39(5):278-93.
[56]. Okada R, Tsushita K, Wakai K, Ishizaka Y, Kato K, Wada T, et al. Lower risk of progression from prediabetes to diabetes with health checkup with lifestyle education: Japan Ningen Dock study. Nutr Metab Cardiovasc Dis. 2017;27(8):679-87.
[57]. Kumar V, Bhandari U, Tripathi CD, Khanna G. Protective effect of gymnema sylvestre ethanol extract on high fat diet-induced obese diabetic wistar rats. Indian J Pharm Sci. 2014;76(4):315-22.
[58]. Kumar V, Bhandari U, Tripathi CD, Khanna G. Evaluation of antidiabetic and cardioprotective effect of Gymnema sylvestre extract in murine model. Indian J Pharmacol. 2012;44(5):607-13.
[59]. Daisy P, Eliza J, Farook KA. A novel dihydroxy gymnemic triacetate isolated from Gymnema sylvestre possessing normoglycemic and hypolipidemic activity on STZ-induced diabetic rats. J Ethnopharmacol. 2009;126:339-44.
[60]. Shanmugasundaram KR, Panneerselvam C, Samudram P, Shanmugasundaram ERB. Enzyme changes and glucose utilisation in diabetic rabbits: the effect of Gymnema sylvestre, R. Br. J Ethnopharmacol. 1983;7:205-34.
[61]. Popkin BM, Horton S, Kim S, Mahal A. and Shiga J. (2001). Trends in diet, nutritional status and diet-related non-communicable diseases in China and India.The economic costs of the nutrition transition. Nutr Rev. 59:379-390.
[62]. Lowery O.H, Roseburg N.J, Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin's phenol reagent.J.Biol.Chem.193: 265-275.
[63]. Babu V, Gangadevi T. and Subramaniam A (2000). Antihyperglycemic activity of

cassia kleiniileaf extract in glucose fed normal rats and alloxan induced diabetic rats. Indian J.Pharmacol.34: 409-415.

[64]. Lowery O.H, Roseburg N.J, Farr A.L. and Randall R.J. (1951). Protein measurement with the Folin's phenol reagent.J.Biol.Chem.193: 265-275.