SCREENING AND ASSESSMENT OF ANTI-DIABETIC AND REACTIVE OXYGEN SCAVENGING (ROS) EFFECTS OF HERBS IN STREPTOZOTACIN INDUCED MICE.

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Summary

Diabetes is associated with the generation of reactive oxygen (ROS) and oxidative damage to various tissues. Although the mechanisms underlying the development of complication in diabetes remain unclear, much attention has been focused on the role of oxidative stress in this disease. In our on going search for therapeutic approach to the treatment of diabetes .We screened 8 extracts from tradional medical herbs for antioxidant course of this investigation, aqueous extracts of Stevia, Gymnema sylvestre, Momordica charantia, Eugenia jambolana, Trigonella foenum graecum, Swertia chirayita karst, Ocimum sanctum, Azadiracta indica exhibits high antioxidant activates and cell-protective effect. All herbs traditionally been used as medicine for treatment of inflammatory-related disease and recent studies have been reported that their extracts possess antioxidant, anti-inflammatory and anti-diabetic effects. These herbs contain large number of active compounds, such as phenolic acid, flavonoids, triterpenoid saponins, hydroxycitric acid, tannins, urosolic acid, gymnemic acid, glycosides and slevioside, which have known ROS scavenging effects.

Introduction

Diabetes mellitus is a group of metabolic disorders in the endocrine system. The disease is found in all parts of the world and is rapidly increasing. People suffering from diabetes are not able to produce or properly use insulin in the body, so they have a high content of blood glucose. Searching for hypoglycemic agents with origin from domestic herbals was considered as a useful way to find novel therapy of the disease [2].

World Health Organization has recommended that medicinal plant research warrant attention. Plants have been the major source of drugs in Indian system of medicine and other ancient systems in the world. Earliest description of curative properties of medicinal plants was found in Rig Veda (2500- 1800 BC) [14].

Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs. Information on medicinal plants in India has been systematically organized. Medicinal plants have the advantage of having little or no side effects.

Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world. Till today metformin is the only ethical drug approved for the treatment of NIDDM patients, which is derived from a medicinal plant *Galega officialis* and historically used for treatment of diabetes in medieval Europe. There are many anti-diabetic plants, which might provide useful sources for the development of drugs, in the treatment of diabetes mellitus [1]. The literature on medicinal plants with hypoglycemic activity is vast. As many of these plants were used for many centuries and some times as regular constituents of the diet, it is assumed that they do not have many side effects. However chronic consumption of large amounts of traditional remedies must always be taken with caution as toxicity studies have not been conducted for most of plants .Before the advent of insulin injection and other pharmaceutical preparations, healers relied heavily upon herbs to treat diabetes. Although numerous herbs are reported to possess some degree of antdiabetic activity [24], a significant amount of research, as well as traditional usage, suggests that Gurmar leaf (*Gymmnema Sylvetris*), Neem *(Azadiracta indica),* Jamun (*Eugenia jambolana*), Tulsi (*Ocimum sanctum*), Stevia (*Sweeten*), Kalmagh (*Swertia chirayita* karst), Methi (*Trigonella foenum-graeccum*) may be among the best in terms of efficacy and safety. These, as well as several other valuable herbs such as garlic acid ginseng represent safe, useful adjuncts to conventional therapeutic approaches to diabetes management. Also, it is possible that the insulin and glucose normalizing effect of some of these herbs may benefit the non-diabetic with insulin resistance[13].

Streptozotacin (STZ) produces oxygen radicals in the body by causing pancreatic injury and this then cause blood sugar increase [10]. This fore in present study we investigated the effects of aqueous extracts of selected herbs on blood glucoses levels and on lipid per oxidative in normal and STZ induced diabetic mice [20].

Material and Methods

All leaves and seeds were collected from Agriculture University, Jabalpur. All the plants were identified taxonomically by department of Botany, Agriculture University, Jabalpur. All leaves and seeds washed 3 times with fresh water, last wash give distilled water and air dried in shade at room temperature.

Preparation of herbs extracts

The Air dried leaves and seeds were grounded into fine powder by an electrical mixture. The powdered were kept in air tight containers in deep freezer maintained at 4 °C unite the time of further use. The leaves and seeds extracts was prepared by dissolving a known amount of powder (5 gm.) in (10 ml.) distilled water using a magnetic stirrer and keep it for 24 hours. It was then filtered (*watman filter*) and an aqueous suspension was collect in fresh test-tube, which in the form customarily, used in folk medicine, was prepared to facilitate easy handing. The drug solutions were prepared freshly each time and administered intragastrically. The doges schedule for the drug was once a day. [23]

Animals

Male Balb/can.n (I.B.) mices weighing 25-30 gms were purchased from National Institute of Nutrition (NIN) Jamai Osmania, Hyderabad for present study. They were acclimatized to animal house in plastic cage under controlled condition (12h light/ 12h dark cycle, 50% humidity, at 22±1°C.) Animals were allowed free access to drinking water and diet (Hindustan Lever food pellets) during the experiment. All Animal experiments were performed in accordance with the NIH guidelines. The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social justices and Empowerment, Government of India and Institutional Animal Ethics Committee guidelines.

Induction of diabetes in mice

STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents [19, 22]. After overnight fasting (deprived of food for 16 hours had been allowed free access to water), diabetes was induced in mice by intraperitoneal injection of STZ (CALBIOCHEM-Cat #572201, Lot#B69776) dissolved in 0.1M Sodium Citrate buffer pH 4.5-5 at a dose of 55mg/kg body weight [12]. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the mice with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 150 mg/dl) were considered as diabetic mice and used for the further experiments. The change in the body weight was observed throughout the treatment period in the experimental animals. [15]

Experimental Set-up

66 mice, induced for study, were divided into 11 groups, each consisting of six animals. Out of 11 groups nine were made diabetic with a single dose of STZ by intraperitoneal route [13, 35]. Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of STZ [5, 29]. Body weight and fasting blood glucose levels of all the mice were determine before the start of the experiment. Mice were divided into the following groups-

Group 1	Control given only saline				N
Group 2	STZ induced diabetic given in saline				S
Group 3	Diabetic mice treated with *Stevia* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+S
Group 4	Diabetic mice treated with *G. sylvestre* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+GS
Group 5	Diabetic mice treated with *M. charantia* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+MC
Group 6	Diabetic mice treated with *E. jambolana* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+EJ
Group 7	Diabetic mice treated with *T. foenum graecum* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+TFG
Group 8	Diabetic mice treated with *S. chirayita* karst once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+SCK
Group 9	Diabetic mice treated with *O. sanctum* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+OS
Group 10	Diabetic mice treated with *A. indica* once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+AI
Group 11	Diabetic mice treated with Mix Herbs once a day, daily	100 µl/2 Mice	200 µl/2 Mice	300 µl/2 Mice	S+Mix

Table .01: N- Normal, S- STZ-induced, ST- *Stevia,* GS- *G. sylvestre,* MC- *M. charantia,* EJ- *E. jambolana,* TFG- *T. foenum graecum,*SCK- *S. chirayita* karst, OS- *O. sanctum,* AI- *A. indica,* Mix- Mix all of herb

Estimations

After 6- days, 30 days and 60 days of treatment the body weight, fasting blood glucose, HDL, LDL, Albumin and Uric Acid of the animal were again determined [29]. Blood was collected in plain vial to puncher optical nerve with help of fine capillary tubes [14]. Serum/plasma was separated by centrifugation at 3000 rpm for 10 minutes and estimated all the biochemical parameter by kit methods [34, 8] .

Blood Sugar- Enzymatic, GOD-POD, endpoint, auto-analyzer, single reagent chemistry. (Trinder P and Teitz N.W.by autospan kit method)

HDL Cholesterol- Enzymatic, CHOD-POD, endpoint, auto-analyzer (Aspen Ltd. kit method)

LDL- Kinetic Procedure, auto-analyzer (TECODIGNOSTICS U.S.A., kit method)

Albumin- Enzymatic, BCG, endpoint, auto-analyzer (Aspen Ltd. kit method)

Uric Acid- Enzymatic, TBTHBA, endpoint, auto-analyzer (Aspen Ltd. kit method)

Statistical Analysis

All the groups data were statistically evaluated. Hypothesis testing methods included one way analysis of variance (ANOVA) following by least significant difference (LSD) test.[7,27] P values of less than 0.05 were considered to indicate statically significance. All the results were expressed as mean ±S.D. for six animals in each group. [15]

Results

After intra peritoneal injection of STZ induced diabetic mice with 55mg/kg body weight blood glucose levels were increase after 6h. By the oral route of *stevia* (S.) at the dose of 300 µl for 60 days, blood glucose reduce 166.43±8.12 to 79.55±0.91 and comparative increase ($P<0.001$) in body weight up to 29.11±0.27. These effects are quite similar to that obtained by normal mice. (Fig.1) Elevated HDL levels in STZ induced diabetic mice alone with a significant decrease in the LDL levels. More over we also found increase level of serum albumin and uric acid in diabetic mice.

After intra peritoneal injection of STZ induced diabetic mice with Gymnema sylvestre (GS) at the oral dose of 200 µl blood glucose were decrease at 10 to 12days. While increasing the oral dose to 300 µl, the blood glucose was reducing in 6-7days after administration. Elevated HDL or LDL levels were decrease significant. (Fig.1). Moreover we also found increased level of serum albumin and uric acid in diabetic mice. After 6 to 8 days following treatment of mice with, Azadiracta indica

(A.I.) at the dose of 300 µl orally, blood glucose levels decrease compared to the control group. Similar effects were obtained when using stevia (S.), Gymnema sylvestre (G.S.) 300 µl administered orally (Fig.1). After administration orally, extracts of Momordica charantia (M.C.), Ocimum sanctum (O.C.), Eugenia jambolana (E.J.), Trigonella foenum graecum (T.F.G.), Swertia chirayita karst (S.C.K.) and Mix, the blood glucose levels of mice did not differ from control.

But administered orally with Momordica charantia (M.C.) (200 µl and 300 µl), blood glucose of the mice reduced after 20 and 30 days but no effects were found in uric acid and albumin (Fig.1).

After oral administration of Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.) (300 µl orally) there was decrease in blood glucose of the mice. At 6th day After intra peritoneal injection of STZ induced diabetic mice, the blood glucose levels of the control group was 166.43 ± 8.12 , and the blood glucose levels of the groups given Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.) were 161.27 ± 111.15, 162.91 ± 10.15 and 160.45 ± 11.7 respectively. ($P< 0.05$ for all compared to control group). While giving the same dose orally till 30days, the blood glucose of the control group was still high (142.91 ± 15.19) but the blood glucose levels of the group given Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.) were 81.16 ± 1.25, 81.01 ± 1.13 and 82.77 ± 0.79 respectively ($p< 0.001$, $p< 0.01$ and $p< 0.05$ respectively compared to the control group).Till 60 days after continue administrating orally, Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.)still had a hypoglycemic effect, the blood glucose levels of the mice being 79.55 ± 0.91, 80.25± 0.32 and 80.12 ± 32.42 respectively, as compared to 100.32 ± 00.99 in control group ($p< 0.05$ for both) (Fig.1). During screening, we found three herbs (Stevia, Gymnema sylvestre, Azadiracta indica) exert shows hypoglycemic effect in STZ induced mice. Those herbs decreased blood glucose levels, HDL, LDL levels and simultaneously increase body weight when administered orally. Furthermore we also got the fruitful result in Serum albumin and uric acid, when administered Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.) 300 µl orally as compared to all other herbs. Earlier there have been many report documenting elevated HDL and LDL status in diabetic subjects [1, 4 and 17] . Further studies required to know the active components and their molecular interactions, which will help to analyze therapeutic efficacy of the products and also to standerdise the product [11]. Efforts are now being made to investigate mechanism and metabolism of action of some of these plants and human which have been reported to occur in both IDDM and NIDDM [1].

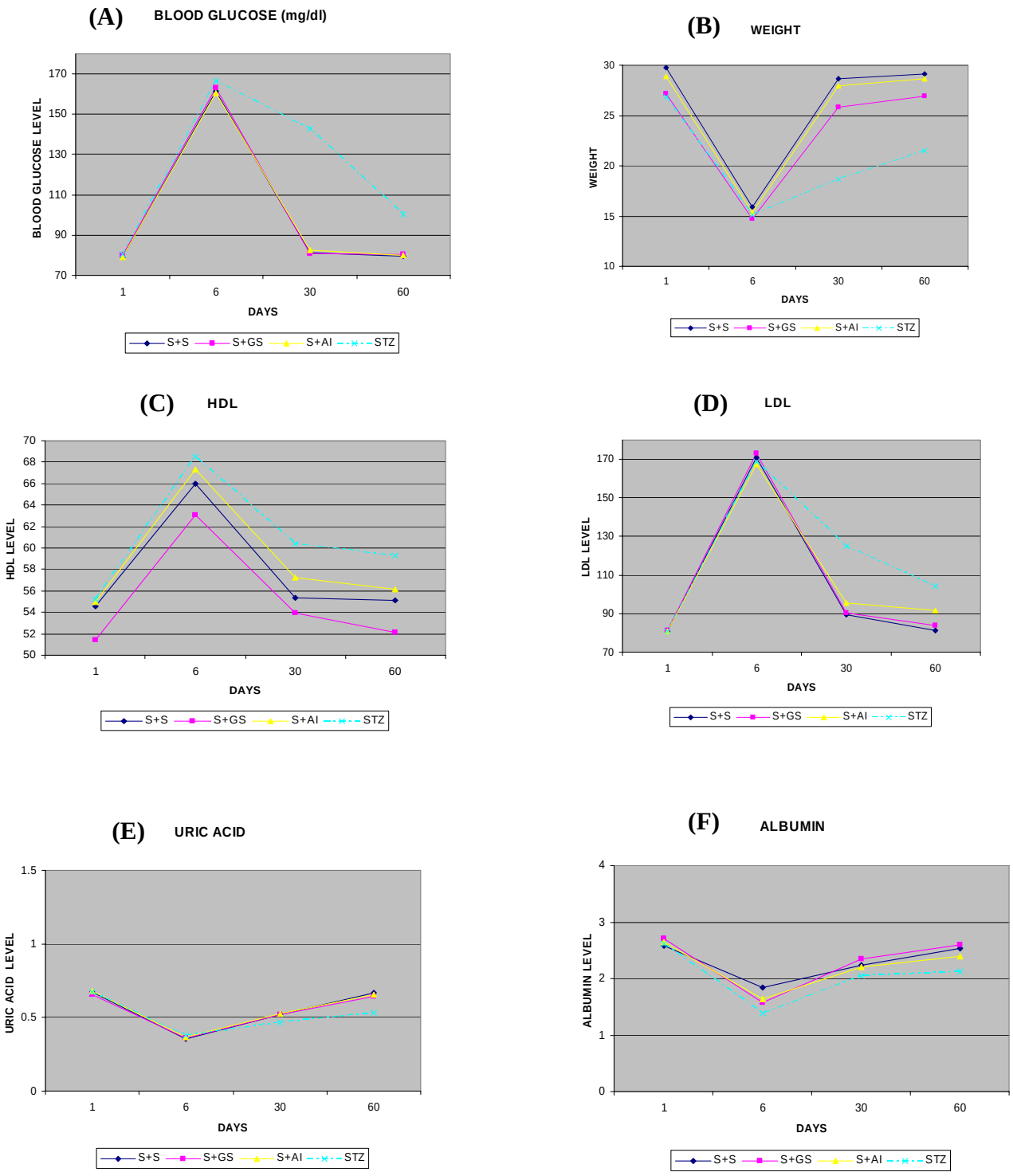

Figure 1: Biochemical estimation for the screening of the Diabetic effect of three herbs (*Stevia, Gymnema sylvestre, Azadiracta indica*) and one normal (STZ induce) in BALB/CAN.N (I.B.) mice

Discussion

Many plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world. Out of these, only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been evaluated in experimental diabetes in animals. [11] The result of the present study showed that extract of all the eight plants namely *Stevia, Gymnema sylvestre, Momordica charantia, Eugenia jambolana, Trigonella foenum graecum, Swertia chirayita karst*, *Ocimum sanctum, Azadiracta indica* and one mix produces a marked decrease in blood glucose levels in normal as well as STZ induced diabetic mice. [1] The hypoglycemic effects of *Stevia, Gymnema sylvestre, Momordica charantia, Eugenia jambolana, Trigonella foenum graecum, Swertia chirayita* karst, *Ocimum sanctum, Azadiracta indica* and one mix extract increased gradually and was observed to be maximum at the end of the study period [1] i,e. 60 days. Our finding are similar to those reported precisely in case of *Stevia* [13], *Gymnema sylvestre,(Shanmugasundaram ERB, et al 1990,) Momordica charantia* [20,30] *, Eugenia jambolana, Trigonella foenum graecum, Swertia chirayita karst* [4], *Ocimum sanctum* [7], *Azadiracta indica.*

The present study was conducted to evaluated the beneficial effect of there varies of concentration of all eight and one mix herbs in STZ induced mice. Elevated HDL levels in STZ induced diabetic mice alone with a significant decrease in the LDL levels. More over we also found reduce level of serum albumin and uric acid in diabetic mice. Earlier there have been many report documenting elevated HDL and LDL status in diabetic subjects [1,4 and 17]. Diabetes and its complication are associated with free radicals mediated cellular injury [2] herbal hypoglycemic agents were administered to diabetic mice to assess their anti-oxidant potential [1]. Our result show that (*Stevia, Gymnema sylvestre, Azadiracta indica*) exert shows hypoglycemic effect in STZ induced mice. Those herbs decreased blood glucose levels, HDL, LDL levels and simultaneously increase body weight when administered orally. Furthermore we also got the fruitful result in Serum albumin and uric acid, when administered Gymnema sylvestre (G.S.), Azadiracta indica (A.I.) and Momordica charantia (M.C.) 300 µl orally as compared to all other herbs.

Moreover, excess accumulation of urate in serum and tissues induce gouty pathology, and are by no means beneficial from the medical point of view [1,2] . Albumin forms the primary defense against reactive oxygen metabolites [23]. Such metabolites have been implicated in the damage brought about by ionizing radiation, as well as in the effects of several cytostatic compounds [24].

The decreased activity of LDL levels in diabetic mice could probably be associated with oxidative stress and/or decreased antioxidant defense potential [23]. The reversal in their content following treatment may be due to decreased oxidative load. The herbal hypoglycemic agents may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds [16], or by increasing the synthesis of anti-oxidant molecules. [1] Thus many different plants have been used individually or in formulations for treatment of diabetes and its complications. [32] One of the major problems with this herbal formulation is that the active ingrediactions are not well defined. Further studies required to know the active components and their molecular interaction. Which will help to analyze therapeutic efficacy of the products and also to standerdise the product [11]. Efforts are now being made to investigate mechanism and metabolism of action of some of these plants and human which have been reported to occur in both IDDM and NIDDM [1].

References

1. Abbas Ali Mahdi, Anu Chandra, Raj Kumar Singh, Sanjeev Shukla, L.C. Mishra and Sohali Ahmad (2003) Effect of herbs hypoglycemic agents on oxidative stress and antioxidant status in Diabetic rats. Indian J. Clinical Biochemistry, 18 (2) 8-15
2. Asayama, K., Uchida, N., Nakane, T.,hayashibe, H., Dobashi, K., Amemiya, S.,Kato, K. and Nakazawa, S. (1993)Antioxidants in the serum of children withinsulin dependent diabetes mellitus. Free Radic.Biol.Med. 15, 597-602.
3. Alvares M, Bazzone RB, Godoy GL, Cury R, Botion LM (1981). Hypoglycemic effect of *Stevia rebaudiana* Bertoni *I Brazilian Seminar on Stevia rebaudiana* 25-26.
4. Bailey CJ, Day C, Turner SL, Leatherdale BA (1985). Cerasee, a traditional treatment for diabetes. Studies in normal and streptozotocin duabetic mice. *Diabetes Res 2*: 81-84
5. Bopanna, K.N., Kannan, J., Sushma, G., Balaraman, R. and Rathod, S.P. (1997) Antidiabetic and anti-hyperlipaemic effects of neem seed kernel powder on alloxan diabetic.
6. Bordia A, et al (1997). Effect of ginger (Zingiber officinale Rosc.) and Fenugreek (*Trigonella foenum graecum L.*) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prost Leuko EFA. 56, 379-384.
7. Chattopadhyay S. Ramanathan M, Das, J. Bhattacharya SK.(1997). Animals modelsin experimental diabetes mellitus. Indian J Exp Bioo ,35:1141.
8. Chandilia, H.B., Talwalkar, B.S. and Rajha, B.S. (1988). Lipid profiles in Diabetes mellitus. J. Dia. Assoc.India 21,155.
9. Chattopadhyay S, Ramanathan M, Das J, Bhattacharya SK.(1997) Animal models in experimental diabetes mellitus. Indian J Exp Bio, 35: 1141.
10. Chang Hwa Jung et al (2006), Antihyperglycemic activity of herbs extracts on streptozotocin-induced diabetic rats, Biosci, Biotechnology, Biochem, 70 (10), 2556-2559.
11. Chen V, Ianuzzo CD. Dosage effect of streptozotocin on rat tissue enzyme activities and glycogen concentration. Can J Physiol Pharmacol (1982). 60: 1251
12. Chattopadhyay, (1993). R.R. Chattopadhyay, Hypoglycaemic effect of *Ocimum sanctum* leaf extract in normal and streptozotocin diabetic rats. *Indian Journal of Experimental Biology* **31** , pp. 891–893.
13. Das, Siddartha, Samai, Khitish Chandram and Tripath, Bibhuti Bhashnt (1992). Factors influencing plasma lipids and liprrotein cheolesterol in indian NIDDM. J. Dia. Assoc. Ind. 32 (2).

14. Dhar, M.L., Dhar, M.M., Dhawan, B.N., Mehrotra, B.N. and Ray, C. (1968). Screening of Indian plants for biological activity (part-I). Ind.J.Exp.Biol. 6, 232-247.
15. Dhanasekar Sathishsekar and Sorimuthu Subramanian, (2005) Antioxidant properties of Momordica Charantia (bitter gourd) seeds on strptozotocin induced diabetic rats. Asia Pac J Clin Nutr 14 (2): 153-158.
16. Gupta, S.K., Prakash, J. and Srivastava, S. (2002) Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Ind.J.Exp.Biol. 40, 765-773.
17. Halim Eshrat M.All Hussain (2002), Hypogycmic, Hpolipidmic and antioxidant properties of combination of *Curcumin* from *curcumin Longa*, Linn, and partially partially purified product from *Abroma Augusta* Linn. In streptozotocin induce Diabetes. Indian J. Clinical Biochemistry, 17 (2) 33-43.
18. Jacob RA. The integrated antioxidant system Nutr Res 1995; 15: 755.
19. Junod A, Lambert AE, Staufacher W, Renold AE. (1969)Diabetogenic action of streptozotocin: relationship of dose to metabolic respose. J Clin Invest 48:2129.
20. Karunanayake EH, et al (1990). Effects of Momordica charantia fruit juice on streptozotocin-induce diabetes in rats. J. Ethnopharmacol 30,199-204.
21. K. Ravi, D.S. Sekar, S.C. Subramanian,(2004) Hypoglycemic activity of inorganic constituents in *Eugenia jambolana* seeds on streptozotoc in induced diabetic rats. *Bio trace Elem Res*, **99 (1- 3)**: 145-55.
22. Ledoux SP, Woodley SE, Patton NJ, Wilson LG. (1966) Mechanism of nitrosourea induced β-cell damage alterations in DNA. Diabetes 35:866.
23. Mahdi, A.A. (2002) Free radicals and other antioxidants. A text Book of Biochemistry by S.P. Singh, 3rd edn. CBS Publishers and Distributors, New Delhi. 545-555.
24. Marklund, S.L., Westman, N.G., Lundgren, E. and Ross, G. (1982) Copper and zinc containing superoxide dismutase, manganese containing superoxide dismutase, catalase and glutathione peroxidase in normal and neoplastic human cell lines and normal human tissues. Cancer Res. 42, 1955-1961.
25. Mahdi, A.A., Singh, R. and Singh, R.K. (1996) Role of reactive oxygen species and antioxidants in human disease: A overview Persp. Biol. (Rai, V., Naik, M.L. Manoharacharry, C. Eds.) School of Life Sciences, World Laser Graphics, Raipur, 55- 70.
26. Mohamed, A.K., Bierhanu, A., Schiekofer, S.,Tritschler, H., Ziegler, H., and Nawroth, P.P.,(1999)The role of oxidative strees and NF (B) activation in late diabetic complication Biofactors, 10, 175-179.
27. Rajaseekaran S. Sivagnanam K, Ravi K and Subramanian S (2004). Hypoglycemic effects of Aloe Vera gel on streptozotocin –induce diabetic rats. J Med Food 7:61.
28. Shanmugasundaram ERB, et al. (1990). Possible regeneration of the islet of lengerhans in streptozotocin-diabetic rats given gylvestre leaf extracts. J Ethnopharamacol, 30, 265-279.
29. Shih, K.C., Kwak, C.F. and Hwa, C.M. (1997). Acipimox attenuates hyperglyceredemia in Dislipidemic Non-insulin dependent diabetes mellites patients without perturbation of insulin sensitivity and glycemic control. Diabetic. Res. Clin pract. 36 (2), 113-119.
30. Svoboda GH, Gorman M, Root MA (1964). Alkaloids of *Vinca rosea* (*Catharanthus roseus*). 28. A preliminary report on hypoglycemic activity. *Lloydia 27*: 361-363.
31. Situnayake, R.D., Thurnham, D.I., Kootathep, S., Chirico, S., Lunec, J., Davis, M. and McConkey, B. (1991) Chain breaking antioxidant status in rheumatoid arthritis: Clinical and laboratory correlates – Ann. Rheum. Dis. 50, 81-86.
32. Tomlinson KC, Gardiner SM, Hebden RA and Bennett T. Functional consequences of streptozotocin-induced diabetes mellitus, with particular reference to the cardiovascular system. Pharmacol Rev. (1992) 44: 103.
33. Yue, K.K.M., Chung, W.S., Lenung, A. W. N. and Cheng, C.H.K.,(2003) Redox change precedue the occurrence of oxidative strees in eyes and arota, but not in kidneys of diabetic rats life Sci., 73, 2557.
34. Yogi, K et al. (1999). Lipid peroxide and human diseases. Chemistry and Physics of Lipid 45, 337-351.
35. Xu,X.,Qi, X., Wang, W., and Chen, G., (2005).Separation and determination of flavonoides in Agrimonia pilosa Ledeb. By capillary electrophoresis with electrochemical detection. J Sep. Sci., 28, 647-652.