

Effect of Herbal Hypoglycemics on Oxidative Stress and Antioxidant Status in Diabetic Rats

Sunila Vasi¹ and Anoop Austin^{*,2}

¹Department of Chemistry, Arignar Anna College, Aralvaymoli, Nagercoil, India

²Rumi Herbals R&D Centre, 6/15, Ohri Salai, Mugappair East, Chennai 600 037, India

Abstract: Antioxidative potential of four commonly used Indian medicinal plants *Gymnema sylvestre*, *Salacia reticulata*, *Cassia auriculata* and *Eugenia jambolanum* was screened for its antidiabetic activity by streptozotocin induced diabetic rats. Lipid peroxide levels were also measured in normal, diabetic and treated animals. Malondialdehyde (MDA) levels were significantly higher and antioxidant activity was found low in diabetic groups as compared to the control groups, and significant alteration in both the MDA levels and antioxidant activity was also observed when the above herbal hypoglycemic agents were given to diabetic rats. The results confirm that the herbs were not only useful in controlling the lipid peroxide levels but are also helpful in further strengthening the antioxidant potential.

Keywords: Free radicals, diabetes mellitus, antioxidants, herbal antidiabetics, *Gymnema sylvestre*, *Salacia reticulata*, *Cassia auriculata*, *Eugenia jambolanum*.

INTRODUCTION

Diabetes mellitus is a metabolic disorder of the pancreas, which was once considered as a single disease entity diabetes, but now it is described as a heterogeneous group of diseases characterized by a state of chronic hyperglycemia, which causes a number of complications like cardiovascular, renal, neurological and ocular [1]. Diabetes mellitus remains a major health problem and prevention still lies in the realm of future.

Oxidative stress is found to be increased in patients with diabetes mellitus [2]. Evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of Diabetes [3]. Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes [4]. Moreover, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes [5, 6].

Since ages in our Indian civilizations and traditional knowledge many herbal medicines in different oral formulations have been recommended for diabetes and confident claims of cure are on record [7]. It is well known that herbal plants like *Gymnema* (*Gymnema sylvestre*), *Salacia* (*Salacia reticulata*), Tanners cassia (*Cassia auriculata*) and Jamun seed (*Eugenia jambolanum*) not only possess hypoglycemic activity but some of them are used in obesity and cholesterol [8, 9].

With the above considerations the present study was designed to investigate the protective effect of *G. sylvestre*, *S. reticulata*, *C. auriculata* and *E. jambolanum* on plasma lipid peroxide levels and on anti-oxidant enzyme superoxide dismutase (SOD). Moreover, antioxidant molecules, uric

acid and albumin content were measured in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals: Streptozotocin was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Plant Material: *G. sylvestre* Leaves, *S. reticulata* roots, *C. auriculata* flowers and *E. jambolanum* seeds were collected wildy from Coutrallam hills, India. The samples were botanically authenticated. Voucher specimens were deposited for future reference.

PREPARATION OF CRUDE EXTRACT

Preparation of Plant Extract: The ethanolic (50 %) extracts were prepared according to the standard methods [8]. 500 gm of the plant materials were dried, coarsely powdered and then soaked in 2000 ml of 50 % ethanol and subjected for hot percolation method. The solvents were evaporated using rota-vapor at 40-50°C under reduced pressure. A 20% semisolid light greenish material obtained was stored at 0-4° C until used. A known volume of the residual extract is suspended in distilled water and was orally administered to the animals during the experimental period.

ANIMALS

Male Wister albino rats weighing 150-200 gm were used in the present study. All rats were kept at room temperature of 20° C in the animal room of the department. They were maintained on Hindustan Lever food pellets and water *ad libitum*.

48 rats, were selected for the study and divided into 8 groups, each consisting of six animals. Out of 8 groups, seven were made diabetic with a single dose of streptozotocin (65 mg/kg b.w.) by intraperitoneal route [10].

*Address correspondence to this author at the Rumi Herbals R&D Centre, 6/15, Ohri Salai, Mugappair East, Chennai 600 037, India; Tel: 044 26358315; Fax: 044 26180269; E-mail: anoopaustin@gmail.com

Diabetes was confirmed by the determination of fasting blood glucose concentration on the third day post administration of streptozotocin.

Body weight and fasting blood glucose levels of all the rats were determined before the start of the experiment. Rats were divided into the following groups –

- Group 1: Control given only saline [10 ml/kg/once a day, daily].
- Group 2: Streptozotocin induced diabetic given in saline [10 ml/kg/once a day, daily].
- Group 3: Diabetic rats treated with *G. sylvestre* [100 mg/kg/once a day, daily] [11].
- Group 4: Diabetic rats treated with *S. reticulata* [100 mg/kg/once a day, daily] [12].
- Group 5: Diabetic rats treated with *C. auriculata* [200 ml/kg/once a day, daily] [13].
- Group 6: Diabetic rats treated with *E. jambolanum* seeds [50 mg/kg/once a day, daily] [14].
- Group 7: Diabetic rats treated with Insulin [5 units/ kg/once a day, daily] [15].
- Group 8: Diabetic rats treated with Glibenclamide [1000 µg/kg/once a day, daily] [15].

The study drugs and glibenclamide were dissolved in saline and administered to rats by gastric oral tube and Insulin was given subcutaneous. The study period was carried out for 28 days, body weight and fasting blood glucose of the animals were determined on 1, 7, 14, 21 and 28 days. Blood was collected in heparinized vial and in plain vial for hemolysate preparation and for serum separation respectively.

PREPARATION OF HEMOLYSATE

Collected blood was centrifuged for 10-minutes at 3000 rpm. The plasma thus obtained was used for lipid peroxide estimation [16]. Remaining packed RBCs were washed thrice with normal saline to remove the buffy coat. Hemolysis was performed by pipetting out 1 ml of washed red blood suspension in ice cold distilled water. Erythrocyte ghosts were sedimented in a high speed refrigerated centrifuge at 12000 rpm for 40-minutes. The cell content was separated out carefully and used for superoxide dismutase estimation [17].

SERUM SEPARATION

The blood collected in plain vial was kept for 30 minutes and stored at -20° C prior to analyses. Serum from blood after clotting separated out and collected in clean centrifuge tube and again centrifuged for 5 minutes at 3000 rpm. The serum thus obtained was used for albumin and uric acid estimations [18, 19].

SUPEROXIDE DISMUTASE (SOD) ESTIMATION

The estimation of SOD (Sigma St. Louis, USA), a free radical scavenging enzyme, was performed using the photo oxidation method [20] in the hemolysates. Two ml of packed cells were lysed by addition of an equal volume of cold deionized water. Hemoglobin was then precipitated by adding chloroform: ethanol (1.5: 1) mixture. The mixture

was centrifuged at 3000 rpm for 15 min, and the SOD activity was measured in the supernatant.

To 0.88 ml of riboflavin (Acto Pharma, Warangal, AP, India) solution (1.3 X 10⁻⁵ mM in 0.01M potassium phosphate buffer, pH 7.5) 60 µl of O-dianisidine (Sigma St. Louis, USA) solution (10-2 mM in ethanol) was added. To this 1 ml of distilled water was added and kept away from light. Hundred µl of the separated SOD was added and optical density (OD) measured at 460 nm using the spectrophotometer. The cuvette was then transferred to the illumination box (40 Watt white fluorescent tube) for exactly 4 min and the OD was remeasured against blank containing ethanol in place of enzyme. The SOD was estimated from the standard graph plotted using different concentrations of pure bovine SOD.

LIPID PEROXIDES ESTIMATION

MDA, a secondary product of lipid peroxidation, was estimated in the plasma samples utilizing the colorimetric reaction of thiobarbituric acid (TBA) (BDH Fine Chemicals, Mumbai) [21]. It gives an index of the extent of progress of lipid peroxidation. Since the assay estimates the amount of TBA reactive substance e.g. MDA, it is also known as TBARS (thiobarbituric acid reactive substance) test.

To 0.5 ml of the serum, 0.5 ml of 30% trichloroacetic acid (TCA) (BDH Fine Chemicals, Mumbai) and 100 µl of 1% TBA reagent were added. The tubes were covered with aluminum foil and kept in a shaking water bath for 60 min at 80° C. The tubes were then kept in ice-cold water for 10 min followed by centrifugation at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against blank. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3' tetra-ethoxy propane (BDH Fine Chemicals, Mumbai). The results were presented in nanomoles of MDA per ml of serum.

SERUM ALBUMIN ESTIMATION

Serum albumin was analyzed using Spin React Diagnostic Kit (Spain) using a semi autoanalyzer [22].

URIC ACID ESTIMATION

To 4 ml of N/23 sulfuric acid, 0.5 ml of serum is added and mixed. 0.5 ml of 5.6 % sodium tungstate is added, mixed, and the whole centrifuged. 3 ml of the decanted supernatant is placed in a test tube, to which is added 0.2 ml Phosphotungstic acid reaction reagent (PTR), mixed and then 1.0 ml 0.6 N sodium hydroxide. Reading is taken at 15 min. using wave length of 720 on a spectrophotometer. Test blank is prepared freshly using 3 ml of water, 0.2 ml of PTR, 1.0 ml of 0.6 N NaOH. The optical density of blank subtracted by test sample gave the content.

STATISTICAL ANALYSIS

Data are expressed in Mean ± SEM. Student t' test was used to compare the mean difference between the vehicle and the drug treated groups. P<value of 0.05 was considered to be significant.

RESULTS AND OBSERVATIONS

In streptozotocin induced diabetic rats there was a significant increase in fasting blood glucose and a comparative

decrease in body weight and protein concentration. There was a slight increase in body weight and protein and a significant decrease in fasting blood glucose (Table 1) in diabetic rats treated with *G. sylvestre*, *C. auriculata*, *E. jambolanum* and *S. reticulata* and the effects were quite similar to insulin and glibenclamide treated group.

Table 2 shows a statistically significant increase in lipid peroxide levels in streptozotocin induced diabetic rats with respect to normal controls and there was a significant decrease in lipid peroxide levels in diabetic rats treated with herbal drugs. In contrast to this, activity of the enzyme superoxide dismutase was significantly decreased in diabetic rats as compared to that of normal control (Table 2). All the anti-diabetic agents including herbal preparations, insulin and glibenclamide used in the present study significantly elevated the activity of superoxide dismutase when compared with diabetic control.

Albumin, another antioxidant, albumin was also found decreased in diabetic group as compared to normal control (Table 3). Following treatment with herbal preparations, except *E. jambolanum*, significantly increased albumin level. Insulin and glibenclamide also showed significant increase in albumin levels as compared to the control group (Table 3).

Following treatment with *S. reticulata* and *C. auriculata* increased in uric acid levels, whereas *E. jambolanum*, *G. sylvestre* and insulin exhibited non-significant changes (Table 3).

DISCUSSION

The results of the present study showed that extract of all the four plants namely *G. sylvestre*, *S. reticulata*, *C. auriculata* and *E. jambolanum* produced a marked decrease

in blood glucose levels in streptozotocin induced diabetic rats. The hypoglycemic effect of *G. sylvestre*, *S. reticulata*, *C. auriculata* and *E. jambolanum* extract increased gradually and was observed to be maximum at the end of the study period i.e. 4-weeks. Our findings are similar to those reported previously in case of *G. sylvestre* [11], *S. reticulata* [12], *C. auriculata* [13] and *E. jambolanum* [14].

Earlier studies demonstrated aqueous extract of *E. jambolana* seeds lowered blood glucose in diabetic rabbits [23] and produced a marked symptomatic relief in diabetic patients [24]. Alcoholic extract of *G. sylvestre* lowered blood glucose in diabetic animals and stimulates insulin secretion in rabbits [25]. Salacino isolated from *S. reticulata* is a potent inhibitory activity against several alpha-glucosidases, such as maltase, sucrase, and isomaltase, and the inhibitory effects on serum glucose levels in maltose- and sucrose-loaded rats (*in vivo*) were found to be more potent than that of acarbose, a commercial alpha-glucosidase inhibitor [26].

In line with the previous works carried out [13, 14, 27], present study demonstrated elevated plasma lipid peroxide levels in streptozotocin-induced diabetic rats along with a significant decrease in the anti-oxidant enzyme, superoxide dismutase activity. Moreover, we also found reduced levels of serum albumin and uric acid in diabetic rats. Earlier there have been many reports documenting elevated serum lipid peroxide levels and diminished antioxidant status in diabetic subjects [28, 29].

As diabetes and its complications are associated with free radical mediated cellular injury [30] herbal hypoglycemic agents were administered to diabetic rats to assess their anti-oxidant potential. Our results show that *G. sylvestre*, *S. reticulata*, *C. auriculata* and *E. jambolanum* not only have

Table 1. The Effect of Herbal Hypoglycemic Agent on Body Weight and Blood Glucose in Normal and Diabetic Rats

Groups	Change in Body Weight (gm)					Fasting Blood Glucose (mg/dl)				
	0	7	14	21	28	0	7	14	21	28
Control	93.33 ± 1.67	102.67 ± 2.41	105.67 ± 2.20	114.33 ± 2.59	121.17 ± 0.88	81.67 ± 1.80	81.83 ± 2.26	82.00 ± 1.28	81.67 ± 1.68	81.83 ± 2.06
Diabetic control	113.67 ^b ± 3.20	108.33 ± 2.32	102.50 ± 1.66	100.83 ^b ± 1.49	97.83 ^b ± 0.83	237.17 ^b ± 8.95	242.50 ± 8.09	245.50 ^b ± 9.97	248.83 ^b ± 10.19	250.17 ^b ± 11.52
Diabetic rats + <i>Gymnema sylvestre</i>	99.50 ^a ± 2.34	98.5 ± 2.21	99.83 ± 2.29	102.17 ± 2.30	103.50 ± 1.95	247.83 ± 10.38	214.50 ± 7.06	183.83 ^b ± 6.04	144.33 ^b ± 4.99	99.5 ^b ± 5.25
Diabetic rats + <i>Salacia reticulata</i>	102.17 ^a ± 2.47	102.00 ± 2.25	105.17 ± 2.06	108.00 ^a ± 2.01	110.83 ^b ± 2.19	242.00 ± 10.74	179.50 ^b ± 7.45	126.67 ^b ± 4.66	98.83 ^b ± 3.30	81.17 ^b ± 1.43
Diabetic rats + <i>Cassia auriculata</i>	106.50 ± 3.97	105.50 ± 3.79	107.83 ± 4.08	109.00 ± 3.36	110.17 ^b ± 2.66	234.83 ± 11.03	173.83 ^b ± 4.98	123.50 ^b ± 5.54	99.00 ^b ± 1.27	84.67 ^b ± 0.84
Diabetic rats + <i>Eugenia jambolanum</i>	101.83 ± 3.66	101.33 ± 4.05	102.33 ± 4.09	103.50 ± 4.40	104.50 ± 4.22	250.00 ± 10.99	196.17 ^b ± 4.90	139.83 ^b ± 3.23	112.67 ^b ± 3.53	82.50 ^b ± 1.32
Diabetic rats + Insulin	96.83 ^b ± 3.52	96.17 ^a ± 3.47	100.83 ± 3.28	102.50 ± 2.10	106.50 ± 3.25	252.17 ± 10.61	117.67 ^b ± 2.44	93.33 ^b ± 1.21	81.17 ^b ± 1.17	75.33 ^b ± 1.05
Diabetic rats + Glibenclamide	103.00 ± 3.60	102.83 ± 3.52	103.17 ± 3.27	104.17 ± 3.31	104.33 ± 3.24	248.33 ± 10.46	170.33 ^b ± 6.65	128.00 ^b ± 3.18	110.67 ^b ± 2.40	83.67 ^b ± 1.31

Values are expressed as mean ± S.E. (n=6). Group 2 is compared to group 1. Group 3, 4, 5, 6, 7 and 8 are compared to group 2. p-values: ^ap<0.01, ^bp<0.001 vs diabetic control group.

Table 2. Effect of Herbal Hypoglycemic Agents on the Levels Protein Concentration, Lipid Peroxide and Superoxide Dismutase

Groups	Protein Concentration (mg/ml)	MDA (nmol/ml)	SOD (U/mg Protein)
Control	59.17 ± 2.30	2.98 ± 0.078	86.70 ± 1.37
Diabetic control	38.50 ± 1.94 ^a	8.81 ± 0.243 ^a	59.52 ± 1.74 ^a
Diabetic rats + <i>Gymnema sylvestre</i>	42.00 ± 1.23	2.91 ± 0.148 ^a	81.71 ± 1.83 ^a
Diabetic rats + <i>Salacia reticulata</i>	45.33 ± 1.53	3.34 ± 0.152 ^a	83.05 ± 2.91
Diabetic rats + <i>Cassia auriculata</i>	43.83 ± 1.05	3.66 ± 0.091 ^a	78.68 ± 1.82 ^a
Diabetic rats + <i>Eugenia jambolanum</i>	41.33 ± 1.87	2.92 ± 0.228 ^a	68.40 ± 1.21 ^a
Diabetic rats + Insulin	43.17 ± 1.46	3.19 ± 0.162 ^a	127.88 ± 6.96 ^a
Diabetic rats + Glibenclamide	42.17 ± 0.83	3.88 ± 0.128 ^a	101.44 ± 3.70 ^a

Values are expressed as mean ± S.E. (n=6).

^a - p<0.001 vs diabetic control group.

Table 3. Effect of Herbal Hypoglycemic Agents on the Levels of Albumin and Uric Acid

Groups	Albumin (g/dl)	Uric Acid (mg/dl)
Control	2.73 ± 0.055	0.678 ± 0.019
Diabetic control	2.11 ± 0.061 ^a	0.585 ± 0.023 ^a
Diabetic rats + <i>Gymnema sylvestre</i>	2.52 ± 0.054 ^a	0.625 ± 0.037
Diabetic rats + <i>Salacia reticulata</i>	2.69 ± 0.073 ^a	0.670 ± 0.030
Diabetic rats + <i>Cassia auriculata</i>	2.68 ± 0.039 ^a	0.637 ± 0.016
Diabetic rats + <i>Eugenia jambolanum</i>	2.47 ± 0.111	0.615 ± 0.033
Diabetic rats + Insulin	2.73 ± 0.082 ^a	0.543 ± 0.014
Diabetic rats + Glibenclamide	2.65 ± 0.052 ^a	0.533 ± 0.006

Values are expressed as mean ± S.E. (n=6).

^a p<0.001 vs diabetic control group.

hypoglycemic activity but they also significantly reduce the plasma lipid peroxide levels in diabetic rats. Moreover, following the treatment the activity of the anti-oxidant enzyme superoxide dismutase and serum albumin content were also increased. However, the serum uric acid content was not found significantly altered. Uric acid is one of the most abundant chain breaking antioxidants present in human serum and its levels are largely determined by genetic factors, purine intake and renal function [31]. Moreover, excess accumulation of urate in serum and tissues induce gouty pathology, and is by no means beneficial from the medical point of view [30].

SOD and albumin form the primary defense against reactive oxygen metabolites [32]. Such metabolites have been implicated in the damage brought by ionizing radiation, as well as in the effects of several cytostatic compounds [33]. The decreased activity of antioxidant molecules along with elevated lipid peroxide levels in diabetic rats could probably be associated with oxidative stress and/or decreased antioxidant defense potential [34]. The herbal hypoglycemic agents may also act by either directly scavenging the reactive oxygen metabolites, due to the presence of various antioxidant compounds [35], or by increasing the synthesis of anti-oxidant molecules.

Further studies are required not only to assess the status of other antioxidant enzymes and molecules in diabetics

following treatment with herbal hypoglycemic agents, but also to evaluate the levels of metal ions such as copper, zinc, magnesium, manganese and selenium, as altered metabolism of these metals has been reported to occur in both IDDM and NIDDM [27, 36].

REFERENCES

- [1] Anonymous. WHO Expert Committees. In: WHO Technical report series of Diabetes mellitus. 1985; 727.
- [2] Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes* 1991; 40: 405-12.
- [3] Bambolkar S, Sainani GS. Evaluation of oxidative stress in diabetics with or without vascular complications. *J Assoc Phys India* 1995; 43: 10-2.
- [4] Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Antidiabetic agents from medicinal plants. *Curr Med Chem* 2006; 13: 1203-18.
- [5] Wohaieb SA, Godin DV. Alteration in free radical tissue defense mechanism in streptozotocin-induced diabetes in rat. *Diabetes* 1987; 36: 1014-8.
- [6] Asayama K, Kayashibe H, Dobashi K, Niitsu T, Miyao A, Kato K. Antioxidant enzyme status and lipid peroxidation in various tissues of diabetic and starved rats. *Diabetes Res* 1989; 12: 85-91.
- [7] Aslam M, Jafri MA, Javed K, Surendra S. Plant drug with hypoglycemic activity Glimpses in plant Research. Today and Tomorrow's Printers and Publishers, New Delhi - 110005, India. 1998; Vol 12: 271-99.
- [8] Tiwari AK, Rao M. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr Sci* 2002; 83: 30-8.

- [9] Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. *J Ethnopharmacol* 2002; 81: 81-100.
- [10] Kishino E, Ito T, Fujita K, Kiuchi Y. A mixture of *Salacia reticulata* (Kotala himbutu) aqueous extract and cyclodextrin reduces body weight gain, visceral fat accumulation, and total cholesterol and insulin increases in male Wistar fatty rats. *Nut Res* 2009; 29: 55-63.
- [11] Liu B, Asare-Anane H, Al-Romaiyan A, *et al.* Characterisation of the insulinotropic activity of an aqueous extract of *Gymnema sylvestre* in mouse beta-cells and human islets of Langerhans. *Cell Physiol Biochem* 2009; 23: 125-32.
- [12] Li Y, Huang TH, Yamahara J. *Salacia* root, a unique Ayurvedic medicine, meets multiple targets in diabetes and obesity. *Life Sci* 2008; 82: 1045-9.
- [13] Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T. Indian herbs and herbal drugs used for the treatment of diabetes. *J Clin Biochem Nutr* 2007; 40: 163-73.
- [14] Helmstadter A. *Syzygium cumini* (L.) SKEELS (Myrtaceae) against diabetes-125 years of research. *Pharmazie* 2008; 63: 91-101.
- [15] Kanetkar P, Singhal R, Kamat M. *Gymnema sylvestre*: a memoir. *J Clin Biochem Nutr* 2007; 41: 77-81.
- [16] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissue by thio-barbituric acid reaction. *Ann Biochem* 1979; 95: 357-8.
- [17] McCord JM, Fridovich I. SOD enzyme function for erythrocyte. *J Biol Chem* 1969; 244: 6049-55.
- [18] Rodkey FL. Direct spectrophotometric determination of albumin in human serum. *Clin Chem* 1965; 11: 478-87.
- [19] Eichhorn E, Zalmanwaki S, Rotenburg EA, Fanis B. Uric acid estimation in serum and urine. *J Clin Pathol* 1961; 14: 450-3.
- [20] Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T, Krishna DR. Oxidative stress and antioxidant status in acute organophosphorous insecticide poisoning. *Ind J Pharmacol* 2004; 36: 76-9.
- [21] Placer ZA, Crushman LL, Johnson BC. Estimation of products of lipid peroxidation (Malonylaldehyde) in biochemical systems. *J Biol Chem* 1966; 16: 359.
- [22] Mahdy KA, Ahmed HH, Mannaa F, Abdel-Shaheed A. Clinical benefits of biochemical markers of bone turnover in Egyptian children with chronic liver diseases. *World J Gastroenterol* 2007; 13: 785-90.
- [23] Anonymous. Medicinal plants of India. Satyavati GV, Raina MK, Sharma M, Eds. Indian Council of Medical Research, New Delhi 1976; Vol.1: p. 147.
- [24] Kohli KR, Singh RH. A clinical trial of Jambu (*Eugenia jambolana*) in non-insulin dependent Diabetes mellitus. *J Res Ayurveda Siddha* 1993; 14: 89-97.
- [25] Chopra RN, Nayar SL, Chopra IC. The Glossary of Indian Medicinal Plants. Publications and Information Directorate, Council of Scientific and Industrial Research, New Delhi 1956; 127.
- [26] Yoshikawa M, Morikawa T, Matsuda H, Tanabe G, Muraoka O. Absolute stereostructure of potent alpha-glucosidase inhibitor, Salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*. *Bioorg Med Chem* 2002; 10: 1547-54.
- [27] Chandra A, Mahdi AA, Singh RK, Mahdi F, Chander R. Effect of Indian Herbal hypoglycemic agents on antioxidant capacity and trace elements content in diabetic rats. *J Med Food* 2008; 11: 506-12.
- [28] Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohishi N, Yagi K. Lipid peroxide level in plasma of diabetic patients. *Biochem Med* 1979; 21: 102-7.
- [29] Oberley LW. Free radicals and diabetes. *Free Rad Biol Med* 1988; 5: 113-24.
- [30] Asayama K, Uchida N, Nakane T, *et al.* Antioxidants in the serum of children with insulin dependent diabetes mellitus. *Free Rad Biol Med* 1993; 15: 597-602.
- [31] Situnayake RD, Thurnham DI, Kootatpeth S, *et al.* Chain breaking antioxidant status in rheumatoid arthritis: Clinical and laboratory correlates. *Ann Rheum Diseases* 1991; 50: 81-6.
- [32] Mahdi AA. Free radicals and other antioxidants. A Text Book of Biochemistry. Singh SP, Ed. 3rd ed. New Dehli: CBS Publishers and Distributors 2002; pp. 545-55.
- [33] Marklund SL, Westman NG, Lundgren E, Ross G. 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* 1982; 42: 1955-61.
- [34] Mahdi AA, Singh R, Singh RK. Role of reactive oxygen species and antioxidants in human disease. An Overview. *Perps. Biol.* In: Rai V, Naik ML, Manoharachary C, Eds. School of Life Sciences. Raipur: World Laser Graphics 1996; pp. 55-70.
- [35] Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. *Indian J Exp Biol* 2002; 40: 765-73.
- [36] Walter RM, Uri-Hare JY, Olin KL, *et al.* Copper, zinc, magnesium status and complications of Diabetes mellitus. *Diabetes Care* 1991; 14: 1050-6.

Received: April 1, 2009

Revised: May 7, 2009

Accepted: May 8, 2009

© Vasi and Austin; Licensee *Bentham Open*.This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.