ANTIOXIDANT ACTIVITY OF *WEDELIA CHINENSIS* IN ALLOXAN INDUCED DIABETIC RATS

R. Senthilkumar[1#], S. Ahmedjohn[2], G. Archunan[3] and N.Manoharan[4]

[1]Department of Neurology, Dongguk University International Hospital, Dongguk University College of Medicine, Gyenggi-do, South Korea 410-773.
[2]Postgraduate Department of Botany, Molecular Genetics Laboratory, Jamal Mohamed College, Tiruchirappalli 620 020.
[3]Department of Animal Science, [4]Department of Marine Science, Bharathidasan University, Tiruchirappalli, 620 024.

Summary

The present study was designed to evaluate the oxidative stress related parameters in alloxan induced diabetic animals. After 30 days treatment with *Wedelia chinensis* Ethanol extract (WcEe) in the diabetic experimental animals, the blood glucose, total protein and total cholesterol were analyzed. The results shown, the *Wedelia chinensis* Ethanol extract have firm antihyperglycaemic activity and other parameters seem to be closer to the normal. Also oxidative damage has been studied by estimating serum and liver LPO levels and these were increased and SOD, CAT and GPx activities were reduced in diabetic rats and after treatment with *Wedelia chinensis* Ethanol extract the LPO levels were ameliorated and antioxidant enzyme activities were enhanced. Non enzymic antioxidants such as reduced glutathione (GSH), and nitric oxide (NO) were diminished in diabetic animals and WcEe treated animals revert to normal. These results suggest that the plant *Wedelia chinensis* Ethanol extract has antihyperglycaemic activity and prevent oxidative stress associated with diabetes mellitus in experimental animals.

Keywords: *Wedelia chinensis*, Antioxidant, Allaxon, Diabetes, Superoxide dismutase, Catalase.

Address for correspondence:
[#]Dr. R. Senthilkumar, Post Doctoral Fellow, Department of Neurology, Dongguk University International Hospital, Ilsadong –gu, Gyenggi –do, South Korea, 410-773.
E-mail- rsenthilkumar75@gmail.com
Telephone: 0082-10-8684-9154,

Introduction

Diabetes mellitus is a metabolic disorder in the endocrine system, currently fourth leading cause of death in mankind along with cancer, cardiovascular complication and cerebrovascular disease. It is found in all parts of the world and is rapidly increasing in most parts of the world. (1). In the year 2025, India is predicted to have the larger number of people with diabetes mellitus in the world. Many pharmaceutical companies consider India as the diabetes capital in the world (2).

Oxidative stress or excessive production of reactive oxygen species is being implicated in many diseases such as cancer, atherosclerosis, ageing, diabetes etc. (3). Oxidative stress in diabetes coexists with a reduction in the anti oxidant status (4), and increase in ROS production (5) and which can increase the deleterious effects of free radicals. Increase oxidative stress has been implicated in the etiology of type–1 diabetes mellitus and of spontaneous or chemically induced diabetes mellitus in experimental animals as well as in the development of complications in all types of diabetes mellitus. Oxygen free radicals activity can initiate the peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alterations in the structure and functions of collagen, basement and other membranes, and play a role in the long-term complications of diabetes mellitus (6).

The management of diabetes mellitus is considering a global problem and successful treatment is yet to be discovered. The modern drugs including insulin and oral hypoglycaemic agents control the blood sugar level as long as their regularly administered and they also produce a number of undesirable effects. Drug with multiple mechanism of protective action including antioxidant properties may be one way formed in minimizing tissue injury in human disease (7). External supplementation through antioxidants is recommended to protect cells of pancreas from the deleterious effects of such oxidative stress condition in diabetes mellitus (8). In general, plants and plant products are exhibits medicinal properties. Several Indian plants are also being used in Ayurvedic and Siddha medicines. The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparation has been found to be effective in the treatment of diseases (9). The ethano botanical information reports about eight hundred medicinal plants that may posses antidiabetic potential (10) and some forecasters reported that the many number of medicinal plant extracts for their potential as antioxidants (11).

Plants of the genus Wedelia afford complex constituents, many of which have been used in traditional folk medicine throughout the world. Phytochemical investigations of various Wedelia species have revealed that many components from this genus express significant biological and pharmacological activities (12). *Wedelia chinensis* furnished the two known flavonoid glycosides, hydrazine derivatives (13), new isoflavone,

new compound (9E,11Z,13E)-8,15- dioxooxadeca-9,11,13-trienoic acid, caffeic acid derivatives, etc., (14). The aqueous extract from *Wedelia chinensis* exhibits strong antipyretic-analgesic activity (15). No detailed study has been carried out on the efficacy of *Wedelia chinensis* in the modulation of oxidative stress associated with diabetes mellitus in experimental animals. Hence, the present study was undertaken to investigate possible hypoglycaemic/antihyperglycaemic and antioxidant effects of ethanolic extract of *Wedelia chinensis* in alloxan induced diabetic experimental animals.

Materials and Methods

Collection of Plant materials

Leaves of *Wedelia chinensis* (Osbeck) Merr, (Compositae, tribe Heliantheae, subtribe Ecliptinae) was procured from the Rohini Herbal Garden, Sengipatt, Thanjavur, Tamil Nadu State, India. The specimen sample was dried under room temperature for 15 days and powdered.

Preparation of Alcoholic extraction

The plant leaves were pulverized and extracted as a whole preparation in a Soxhlet apparatus (30g/300 ml 80% aqueous ethanol). The ethanol extract was concentrated to a dry mass, lyophilized, and stored frozen at 0°C. Ethanol extract dry mass was dissolved with phosphate buffer saline and orally administered to the diabetic experimental animals.

Collection of Blood and preparation of Tissue samples

At the end of the experimental periods, experimental rats were sacrificed. Serum was separated by centrifuging the samples at 1500 rpm for 10 minutes and stored in a refrigerator until analyzed. The serum was analyzed for glucose, total protein, and total cholesterol. Liver is homogenized with homogenization buffer using tissue homogenizer and liver antioxidant enzymes such as superoxide dismutase, catalase, GPx, and non enzyme antioxidants were analyzed.

Chemicals

Alloxan and bovine serum albumin were purchased from Sigma chemical company, St. Louis, USA. All other chemicals used for biochemical analysis are purchased from Ranboxy Research Laboratories, Glaxo Laboratories, Nice Pharmaceuticals Company and Dr. Reddy's Laboratory- India.

Animals

Experimental animals were healthy male Swiss Albino Rats (6-8 weeks old) having weight around (180 g – 230 g) were used for the study. They were maintained in an appropriate laboratory condition. All animals were fed standard pellet diet (Gold Mohor Rat Feed, Hindustan (p) Ltd., Mumbai) and water *ad libitum*.

Induction of the diabetes mellitus
The experimental animals were injected intraparetonially with alloxanmonohydrate (150mg/kg bodyweight) dissolved in normal saline (16). After 5 days, the animals were determined the blood glucose and the results were 240 – 280 mg/100 ml of blood. After induction of diabetes mellitus the animals were used for the experimental study.

Experimental protocol
In the present study, four groups of rats were used. Each group consists of six rats of same weight. The animals were treated for 30 days as follows.

Group I – Normal Control Animals(2.0 ml normal saline only)
Group II– Diabetic Animals (Alloxan monohydrate150mg/kg bodyweight)
Group III –Diabetic Treatment Control (Phosphate Buffered Saline - 2.0 ml)
Group IV –Diabetic Treatment with *Wedelia chinensis* (2 mg of WcEe/kg of body weight)

Estimation of blood glucose
Blood glucose was estimated by o-toluidine method (17).

Determination of cholesterol
Cholesterol in serum was determined by the Zak method (18).

Assay of serum and liver LPO
Lipid peroxidation (LPO) was measured by the method of Yagi *et al.*, (1979) (19) and was expressed as nmol of MDA conjugate formed. LPO assay was done in both serum and tissue, tissue LPO was carried out by the modified method of Viana and coworkers (20)

Assay of antioxidant enzymes and protein
The activities of superoxide dismutase, catalase and GPx in liver homogenate were assayed by the methods of Kakkar *et al.,* 1984 (21), Maehlay and Chance, 1954 (22) and Lawrence and Burk, 1976 (23). Protein content of serum was measured by the method of Lowry *et al.,* 1951 (24).

Assay of Non enzymic antioxidants
The reduced glutathione and nitric oxide were analysed from liver homogenate by the method of Kao *et al.,* 1983 (25) and Green *et al.,* 1982 (26) respectively.

Statistical analysis
The values of the biochemical parameters were used to calculate as mean ± S.E.M. by one-way ANOVA followed by by Tukey's Kramer multiple comparison test using GraphPad Instat software.

Results

Data on various biochemical parameters measured in the serum of control and *Wedelia chinensis* treated diabetic animals in table–1. There was the diabetic animals showed a significant increase in blood sugar level is 388.6 ± 14.8 ($P < 0.001$) after a few days of diabetes induction. After thirty days *WcEe* treatment, showed significantly decreased 99.1 ± 6.2 ($P< 0.001$) in blood glucose of the experimental animals.

Table-1: Effect of WcEe on blood parameters in experimental animals

Groups	**Blood glucose mg/100ml**	**Total protein mg/100ml**	**Total cholesterol mg/100ml**
Diabetic Control Animals	96.1 ± 6.2	7.4 ± 0.4	86.3 ± 4.5
Diabetic Animals	388.6 ± 14.8**	5.2 ± 0.3*	179.6 ± 7.4**
Diabetic Treatment Control Animals	386.2 ± 12.3	5.0 ± 0.5	178.2 ± 6.3
Diabetic Animals Treated with *Wed. chinensis*	99.1 ± 6.2$^{\#\#}$	7.3 ±0.4$^{\#}$	88.6 ± 6.5$^{\#\#}$

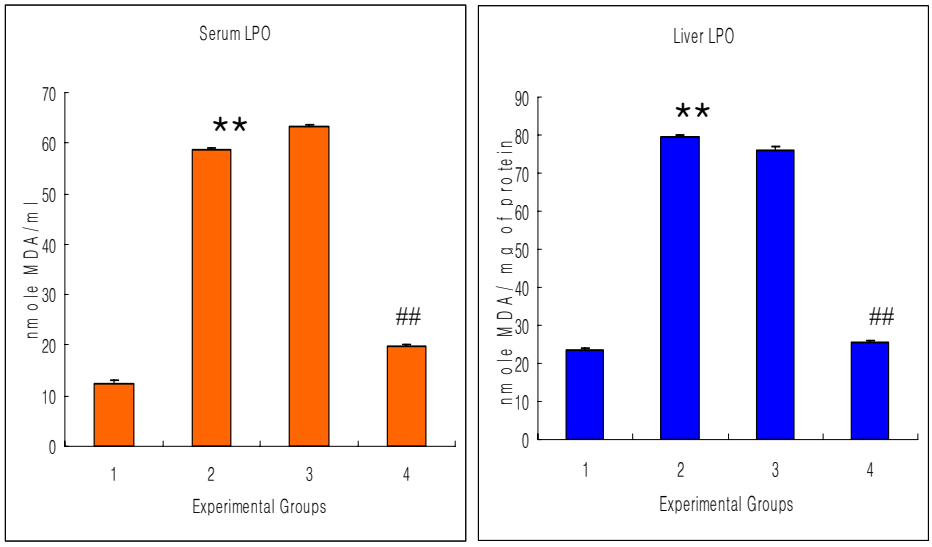
Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software, *P<0.05, **P<0.001, diabetic animals compared with control animals, $^{\#}$P<0.01, $^{\#\#}$P<0.001 diabetic treated animals compared with diabetic animals.

There was a marked decrease in the plasma total protein content of untreated diabetic group 5.2 ± 0.3 ($P < 0.05$) when compared with that of control group animals. After thirty days treatment with *WcEe*, it shows that significantly increased and closed to normal 7.3 ± 0.4 ($P < 0.01$) in the experimental animals. Total cholesterol level in the untreated diabetic group animals were increased significantly 179.6 ± 7.4 ($P<0.001$) when tested against the control group animals. After thirty days treatment with *WcEe*, significantly decreases total cholesterol level 88.6, ± 6.5 ($P < 0.001$) in the experimental animals.

The serum MDA (as an indicator of LPO) level in diabetic rats was significantly higher than their control counterparts. Administration of WcEe to diabetic rats significantly decreased the concentration of MDA in serum values than diabetic group ($p>0.001$). Similar result was also observed in liver homogenate (Figure 1). In the present investigation shows the level of SOD, CAT and GPx in liver of normal rats, diabetic control and treated animals. The liver of the diabetic animals showed reduced level of SOD, CAT and GPx were 3.82 ± 0.8 ($P <0.01$), 55.2 ± 4.4 ($P < 0.01$) and 0.09 ± 0.02 ($P<0.001$) respectively (Table –2).

After thirty days administration with *WcEe* in the diabetic animals SOD, CAT and GPx becomes similar to that of diabetic control 9.5 ± 0.1, 86.2 ± 2.1 and 0.19 ± 0.02 (P<0.001). The effects of WcEe on liver non enzymic antioxidative parameters in alloxan induced diabetes were shown in

Figure 1. Effect of WcEe on serum and liver LPO in experimental animals.



Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals, ##P<0.001 diabetic treated animals compared with diabetic animals.

Figure 2. WcEe treatment (2 mg/kg b.wt.) over a period of 30 days exerted a protective effect on the non enzymic antioxidants namely, reduced glutathione (GSH), and nittic oxide (NO).

Discussion

The diabetogenic effects of alloxan are notoriously variable during the first 48 hrs after administration. Changes in blood glucose levels are often triphasic consisting of initial hyperglycaemia for 24 to 48 hrs followed by more or less permanent hyperglycaemia (27). The alloxan causes massive reduction in insulin release through the destruction of β cells of the islets of langerhans. Hence the secretion may decrease and leads to increase blood glucose. After administration of *WcEe* drug formulation, stimulates insulin secretion from the remnants β cells or and regenerated β cells. Similar effect i.e. insulinogenic activity with treatment of *Trigonella foenum graecum* (28) and *Mormordica charantia* (29).

Table-2: Effect of WcEe on liver antioxidant enzymes in experimental animals

Groups	**SOD in liver** Unit/min/mg protein	**CAT in liver** μmolesH_2O_2 decomposed/ min/mg protein	**GPx in liver** Unit/min/mg of protein
Diabetic control animals	9.3 ± 0.4	86.5 ± 5.3	0.38 ± 0.05
Diabetic animals	3.82 ± 0.8**	55.2±4.4**	0.09±0.02**
Diabetic treatment control animals	3.76 ± 0.7	55.0 ± 3.2	0.10 ± 0.03
Diabetic animals treated with WcEe	9.5 ± 0.1$^{\#\#}$	86.2 ± 2.1$^{\#\#}$	0.19 ± 0.02$^{\#}$

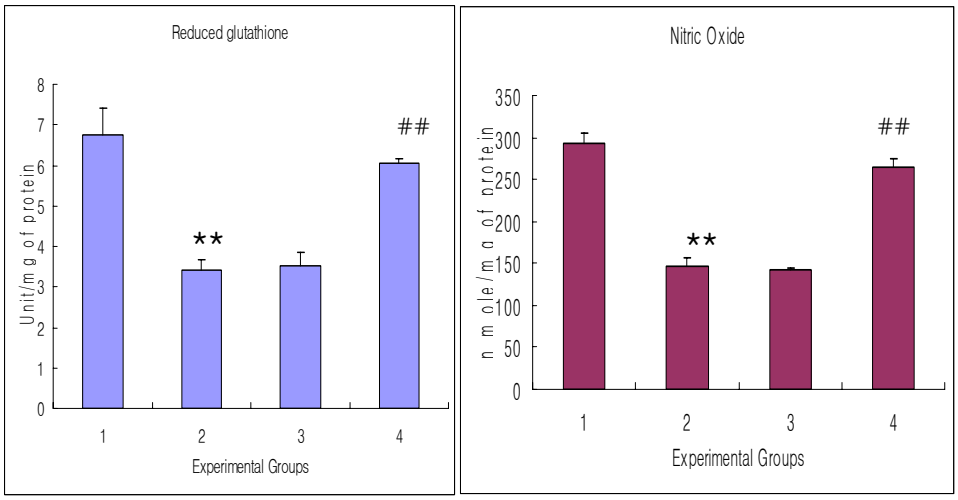
Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals, $^{\#}$P<0.01, $^{\#\#}$P<0.001 diabetic treated animals compared with diabetic animals.

Among these parameters, the total protein is fall in the serum of diabetic rats and this result is very similar as previously showed (30). This is due to hypoalbumiemia , which observed in diabetics (31). Hypoalbuminemia is a common problem of animals and is generally attributed to the presence of diabetic nephropathy. However recent findings indicate that synthesis of protein is also diminished in the diabetic rats (32).

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesteridemia (33). Hypercholestridemia also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose tolerance (34). The observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis (35).

The present data revealed that persistent hyperglycemia through alloxan generated ROS attack produced marked oxidant impact as evidenced by significant increase in LPO levels of diabetic animals than control non-diabetic animals (both serum and tissue). Previous studies done in diabetic patients and diabetic rats reported same phenomenon (36). Elevated levels of MDA were brought down to the normal values by treatment with WcEe.

Figure 2. Effect of WcEe on liver non enzymic antioxidants in experimental animals



Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's Kramer multiple comparison test using GraphPad Instat software **P<0.001, diabetic animals compared with control animals $^{\#\#}$P<0.001 diabetic treated animals compared with diabetic animals.

In our study MDA (as an indicator of LPO) levels in diabetes group were found to be higher than those in control group, indicating increased free radical generation. Treatment of diabetes with the WcEe on subacute studies caused a decrease in MDA levels. This decrease in MDA levels may increase the activity of catalase (CAT) in rats treated with the extract and hence cause inactivation of LPO reactions (37).

Enzymatic antioxidant such as SOD and CAT are considered primary enzymes since they are involved in the direct elimination of ROS (38). SOD is an important defense enzyme and scavenges O2− anion form H2O2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reaction (39).
CAT is a hemoprotein, which catalyzes the reduction of hydrogen peroxides (40). The antioxidant enzymes such as SOD and CAT are known to be inhibited in diabetes mellitus as a result of non-enzymatic glycosylation and oxidation (41). In our study, the activities of SOD and CAT decreased in diabetic rabbits as reported earlier (42) which could be due to inactivation caused by alloxan-generated ROS. The ethanolic extract of *Wedelia chinensis* had reversed the activities of these enzymatic antioxidants, which might be due to decreased oxidative stress as evidenced by decreased LPO.

Alloxan administration decreased reduced glutathione and nitric oxide production in the liver (Figure 2). These findings are in accordance with Sailaja Devi *et al.,* (2000) (43), which reported relatively low liver and blood NO levels in alloxan-induced diabetes. WcEe administration was

increased liver NO production in the diabetic animals. These findings are very similar to the antioxidant enzymes and lipid peroxidation. This finding strengthens our supposition that antidiabetic and antioxidant effects of WcEe.

In conclusion, our observations have clearly demonstrated that the WcEe exerts remarkable antihyperglycaemic activity due to its possible multiple effects involving mechanism. It has also determined that the WcEe extract possessed a capability to inhibit the lipid peroxidation and activate the antioxidant enzymes and non enzymic antioxidants in diabetes conditions.

Acknowledgement

This research in the authors' the partial grant was supported by the TNSCST, Government of Tamilnadu, Chennai, and the authors would like to thank M.I.E.T Institutions and Bharathidasan University, Tiruchirappalli for providing necessary facilities to carryout the research work.

References

(1) Li., WL., Zheng. HC., Bukuru. J., Kimpe., N. De., Natural medicine used in the traditional Chinese medicinal system for therapy of diabetes mellitus. Journal of Ethan pharmacology, 2004; 92:1-21.

(2) Sridhar, GR., Psycosocial and cultural issues in diabetes mellitus. Current science, 2002; 83:791.

(3) Frinkel, T., Holbrook, NJ., Oxidants, oxidative stress and biology of ageing. Nature, 2000; 408: 239-247.

(4) Collier, A, Rumley, A, Rumley AG, et al., Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes, 1990; 41: 909 – 913.

(5) Signorini, AM., Fondelli, C., Renzoni, E., Puccetti, C., Gragnoli, G., Giorgi, G., Antioxidant effect of gliclazide, glibenclamide and metformin in patients with type 2 diabetes mellitus. Current Therapeutic Research, 2002; 63: 411–420.

(6) Baynes, J., Role of oxidative stress in development of complication in diabetes. Diabetes, 1991; 40:405-12.

(7) Barry, H., Antioxidant effects a basis for drug selection. Drugs, 1991; 42: 569.

(8) Naik, GH., Priyadarsini, KI., Satav, JG., Naik, DB., Mohan, H., Antioxidant activity and Phytochemical analysis of the aqueous extract of *Terminalia chebula.* Free Radic. Biol. Med. 2004; 33(S): 547.

(9) Haunda SS., Mundkinajeddu D., Mangal AK., Indian pharmacopia, Government of India, Ministry of Health and Family welfare. Controller of publications; Delhi. 1996.

(10) Alarcon-Aguilara, FJ., Roman Ramos, R., Flores Saenz, IL., Plants medicinals usadas en el control de la diabetes mellitus. Ciencia 1993; 44: 361-363.

(11) Khopde, SM., Priyadarsini, KI., Mohan,H., Gawandi, VB., Satav, J.G., Yakhmi, JV.,Banavalikar, MM., Biyani, MK., Mittal, JP., Characterizing the antioxidant activity of amla (*Phyllanthus emblica*) extract. Curr. Sci., 2001; 81: 185-190.

(12) Li X., Dong M., Liu Y., Shi Q., Kiyota H., Structures and biological properties of the chemical constituents from the genus Wedelia, Chem and Biodiv., 2007; 4: 823-835.

(13) Miles DHV., Chittawong, PA. Hedin, U. Kokpol, Phytochemistry, 1993; 32, 1427.

(14) Farag, S F. Bull. Faculty Pharm., Cairo Univ. 2001; 39:189.

(15) Kuang, L X. Fang, H. Zhou, F. Chin. Tradit. Herbal Drugs, 1997; 28: 370.

(16) Prince, PS., Menon, VP., Pari, L., Hypoglycemic activity of *Syzigium cuminii* seeds; effect on lipid peroxidation in alloxan diabetes rats, Journal of Ethanopharmacology, 1998; 61: 1-7.

(17) Sasaki, T., Matsy, S., Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku 1972; 1: 346-353.
(18) Zak, B., Boyle, AJ., Zlatkis, A., A method for the determination of cholesterol. Journal of Clinical Medicine, 1953; 41: 486-492.

(19) Yagi, K., Ohkawa, H., Ohishi, N., Assay for lipid peroxides in animal tissues by thiobarituric acid reaction. Analytical Biochemistry, 1979; 95: 351–358.

(20) Viana, M., Aruoma, OI., Herrera, E., Bonet, B., Oxidative damage in pregnant diabetic rats and their embryos. Free Radical Biology and Medicine, 2000; 29 (11): 1115–1121.

(21) Kakkar, P., Dos, B., Viswanathan, PN., A modified spectrophotometric assay of superoxide dismutase. Indian journal of Biochemistry and Bio physics, 1984; 21: 130-132.

(22) Maehlay, AC., Chaunce, B., In: Glick, D., Jr (Ed.), Methods of Biochemical analysis. 1st Interscience, New york, 1954; 357.

(23) Lawrence, RA., Burk, RF., Glutathioneperoxidase activity in selenium deficient rat liver. Biochemical and Biophysical Research Communications, 1976; 71: 952–958.

(24) Lowry, OH., Rosenbrough, NJ., Farr, AL., Randall, RL., Protein measurement with Folin-phenol reagent. Journal of Biological chemistry, 1951; 193: 265-275.

(25) Kuo, CH., Maita, K., Sleight, SD., Hook, JB., Lipid peroxidation: a possible mechanism of cephaloridine-induced nephrotoxicity. Toxicology and Applied Pharmacology, 1983; 67: 78–88.

(26) Green, LC.,Wagner, DA., Glogowski, J., Skipper, PL.,Wishnok, JS., Tannenbaum, SR., Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Analytical Biochemistry, 1982; 126: 131–138.

(27) Rerup, CC, Pharmacolo. Rev., 1970; 22: 484.

(28) Khosla, P., Gupta, DD.,Nagpal, RK., Effect of *Trigonella foenum graecum* (Fenugreek) on blood glucose serum lipids in normal and diabetic rats. Indian Journal of Pharmacology, 1995; 39: 173 – 174.

(29) Karunanayake, E H., Welihinda, J., Sirimanne, SR., & Sinnadorai, G, Oral hypoglycaemic activity of some medicinal plants of Sri Lanka. Journal Ethanopharmacology, 1984; 11(2): 223.

(30) Dhanabal, SP., Kokate, CK., Patil MD., Studies on the antidiabetic activity of the roots of *Salacia macrosperma* Wight. Drug lines, 2002; 5: 1 – 4.

(31) Porte, D. Jr & Halter JB., Text book of endocrinology, edited by R. H. Williams (WB. Saunders Co., Phililadelphia), 1981; 715.

(32) Sumana Ghosh, Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. Indian Journal of Experimental biology, 2001; 748 – 759.

(33) Khan, BA., Abraham, A., Leclamma, S., Hypoglycemic action of *Murray Koenigii* (Curry leaf), *Brassica junca* (mustard); mechanism of action. Indian Journal of Biochem Biophys., 1995; 32: 106 – 108.

(34) Gingsberg, HN, Lipoprotein metabolism and its relationship to atherosclerosis. Medicinal and Clinical North America, 1994; 78: 1 – 20.

(35) Bopanna, KN., Kannan, J., Sushma, G., Balaraman, R., Rathod., SP., Antidiabetic and antihyperlipademic effect of neem seed kernel powder on alloxan diabetic rabbits. Indian Journal of Pharmacology, 1997; 29: 162 – 167.

(36) Chang, JM., Kuo, CM., Kuo, HT., Chiu, YW., Chen, HC., Increased glomerular and extracellular malondialdehyde levels in patients and rats with diabetic nephropathy. Journal of Laboratory and Clinical Medicine, 2005; 146: 210–215.

(37) Afshari, AT., Shirpoor, A., Farshid, A., Saadatian, R., Rasmi, Y., Saboory, E., Ilkhanizadeh, B., Allameh, A., The effect of ginger on diabetic nephropathy, plasma antioxidant capacity and lipid peroxidation in rats. Food Chemistry, 2007; 101: 148–153.

(38) Arulselvan, P., Subramanian, SP., Beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic _-cells in experimental diabetes in rats. Chemico-Biological Interactions, 2007; 165: 155–164.

(39) Manonmani, G., Bhavapriya,V., Kalpana, S., Govindasamy, S., Apparanantham, T., Antioxidant activity of *Cassia fistula* (Linn.) flowers in alloxan induced diabetics rat. Journal of Ethnopharmacology, 2005; 97: 39–42.

(40) Punitha, ISR., Shirwaikar, A., Shirwaikar, A., Antidiabetic activity of benzyl tetra isoquinoline alkaloid berberine in streptozotocin–nicotinamide induced type 2 diabetic rats. Diabetologia Croatica, 2005; 34: 117–128.

(41) Al-Azzawie, H., Alhamdani, MSS., Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. Life Sciences, 2006; 78: 1371–1377.

(42) Sepici, A., G¨urb¨uz, I., Cevik, C., Yesilada, E., Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. Journal of Ethnopharmacology, 2004; 93: 311–318.

(43) Sailaja Devi, M.M., Suresh, Y., Das, U.N., Preservation of the antioxidant status in chemically-induced diabetes mellitus by melatonin. Journal of Pineal Research, 2000; 2: 108–115 .