Iranian Journal of Pharmaceutical Research (2008), 7 (1): 53-59
Received: August 2006
Accepted: December 2006

Original Article

Antihyperglycemic, Antihyperlipidemic and Antioxidant Effect of *Phyllanthus rheedii* on Streptozotocin Induced Diabetic Rats

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Abstract

The aim of this study was to investigate the effects of ethanolic extract of the whole plant of *Phyllanthus rheedii* wight P.rheedii as antihyperglycemic, antihyperlipidemic and antioxidant effect in streptozotocin (STZ) induced diabetic rats. Male Wistar rats were administered *P.rheedii* (250 mg/kg) orally for 21 days and blood glucose level was measured weekly. At the end of 21 days, the serum lipid metabolites such as total cholesterol, triglycerides, high density lipoproteins (HDL) and protein metabolites such as total protein, albumin, globulin and albumin:globulin ratio (A:G) enzyme level viz serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) were determined. In order to determine antioxidant activity of extract, liver tissues were homogenized in ice cold saline buffer and the assay of lipid peroxides (LPO), superoxide dismutase (SOD) and catalase (CAT) were performed in control, STZ and extract treated rats. All these effects were compared with glibenclamide as a reference antidiabetic drug. Oral administration of *P.rheedii* for 21 days resulted in a significant reduction in blood glucose level, lipid metabolism and enzymes level and significant improvement in LPO, SOD and catalase in liver tissues of STZ induced diabetic rats when compared with untreated diabetic rats. The protein metabolites were significantly altered near to normal. The effects produced by the extract were comparable to that of glibenclamide.

In conclusion The *P.rheedii* showed significant antihyperglycemic, antihyperlipidemic and antioxidants effect in STZ induced diabetic rats.

Keywords: *Phyllanthus rheedii*; Antihyperglycemic; Antioxidant; Antihyperlipidemic; Streptozotocin.

Introduction

Diabetes mellitus is a syndrome, initially characterized by loss of glucose homeostasis resulting from defects in insulin secretion, insulin action both resulting impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (1). Experimental diabetes in animals has provided considerable insight into the physiological and biochemical derangement of the diabetic state. Many of

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these derangements have been characterized in hyperglycemic animals. Significant changes in structure and lipid metabolism occur in diabetes (2). In these cases the structural changes are clearly oxidative in nature and are associated with development of vascular disease in diabetes (3). In diabetic rats, increased lipid peroxidation was also associated with hyperlipidemia (4). Liver, an insulin dependent tissue that plays a vital role in glucose and lipid homeostasis, and it is severely affected during diabetes (5). Liver and kidney participates in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol, phospholipids and triglycerides. During diabetes, a profound alteration in the concentration and composition of lipids occurs. Despite the great strides that have been made in the understanding and management of diabetes, the disease and disease related complications are increasing unabated (6). In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease (7). Many traditional plant treatments for diabetes are used through out the world. Plant drugs (8) and herbal formulations (9-11) are frequently considered to be less toxic and free from side effects than synthetic one (12). Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important (13). The attributed antihyperglycemic effects of these plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or decrease in the intestinal absorption of glucose (12). Hence treatment with herbal drugs has an effect on protecting β -cells and smoothing out fluctuation in glucose levels (14, 15). In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes, but most of the plants have been found to contain substances like glycosides, alkaloids, terpenoids, flavonoids etc., that are frequently implicated as having antidiabetic effects (16).

Phyllanthus rheedii (Family: Euphorbiaceae), a slender branching erect herb, the calyx-lobes usually white margined, found through out in India. It is used as an oriental folk medicine in diabetes mellitus (17). The present study deals with antidiabetic effect of alcohol extract of the whole plant of *Phyllanthus rheedii* (*P.rheedii*) on streptozotocin induced diabetic rats and also we examine antioxidant potential, lipid profile, protein metabolite and liver enzymes level changes in STZ induced diabetic rats. The effect produced by this drug on different parameters was compared with those of glibenclamide, a reference drug (18,19).

Experimental

Matereals

Streptozotocin (STZ) was purchased from Sigma Chemicals Co (St. Louis, MO, USA). Thiobarbituric acid was purchased from E-Merck, India. All other chemicals used were of analytical grade.

Methods

Plant material and preparation of extract

P. rheedii were collected from the local area of Salem, India in the month of October 2004 and were authenticated by the Botanist, Botanical Survey of India, Coimbatore, India. A voucher specimen has been stored and maintained in our laboratory (ET-30). The plants dried in shade and powdered. The powder was extracted with ethanol (95 % V/V) using Soxhlet apparatus. The extract was dried under reduced pressure and stored in a desiccator. The yield of extract was 3.5 % w/w.

Animals

Swiss albino mice (20-25 g) and Male Wistar rats (150-200 g) were purchased from Perundurai Medical College, Perundurai, Tamilnadu and housed in polypropylene cages at room temperature (22±2 °C) with proper ventilation. Prior to the experiments, mice and rats were fed with standard diet for 1 week in order to adapt to laboratory conditions. They were fasted over night but allowed free access to water before the experiment. The study was conducted after obtaining clearance from Institutional animal ethical committee (Ph.Chem/3/2005).

Acute toxicity studies (LD_{50})

The oral acute toxicity study of the extract was carried out in Swiss albino mice. This

Table 1. Effect of on *P. rheedii* blood glucose levels in STZ induced diabetic rats. (n=6, data have been analysed by one way ANOVA followed by Tukey multiple comparison analysis).

Treatment	Dose (mg/kg)	Blood glucose (mg%)				
		0 day	After STZ induction	1st week (after treatment)	2nd week	3rd week
Control	-	85.60±1.20	84.50±2.46	86.50±1.25	86.52±4.30	80.70±5.50
Diabetic control	-	94.23±1.60	460.63±4.90[a]	462.33±3.20[a]	390.00±4.14[a]	420.00±4.76[a]
P.rheedii	250	91.56±1.50	456.25±3.70[a]	266.00±5.60[a,b,§,*]	226.00±3.10[a,b,§,*]	160.00±6.42[a,b,§]
Glibenclamide	500 µg	84.26±1.70	450.85±40.00[a]	165.00±2.10[a,b,§,]	170.00±2.52[a,b,§]	156.00±2.10[a,b,§]

[a]P<0.001 vs control; [b]P<0.001 vs diabetic control

[§]P<0.001 vs after STZ induction in the corresponding group.

*P<0.001 vs glibenclamide.

method was carried out in twelve animals, four animals per treatment group and widely different dose ranges, 1, 2 and 3 g/kg, respectively and observed 24 h.

Experimental induction of diabetes in rats

Rats were made diabetic with an intraperitoneal injection of Streptozotocin (STZ 60 mg/kg body weight) dissolved in citrate buffer (0.1M, pH 4.5). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose level 48 h after the injection of STZ. Rats with blood glucose level above 250 mg/dl were considered to be diabetic and were used in this experiment.

Experimental design

After the induction of diabetes the rats were divided into four groups of six animals each.

Group I-Control rats received the vehicle solution (2% gum acacia).

Group II-Diabetic control received the vehicle solution (2% gum acacia).

Group III-Diabetic rats given ethanol extract of *Phyllanthus rheedii* (250 mg/kg, p.o.).

Group IV-Diabetic rats treated with glibenclamide (500 µg/kg, p.o).

The vehicle and drugs were administered orally by an intragastric tube daily for 21 days.

Blood glucose levels were estimated at 1st, 2nd and 3rd week after the administration of test samples. The effect of each test sample on body weight was also monitored at the same days. On the 3rd week, all animals were sacrificed by cervical decapitation. Blood was collected and serum separated out. The liver were immediately removed and suspended in ice-cold saline.

Biochemical estimation

Blood glucose was determined by the O-toludine method (20). Serum was analysed for the following biochemical parameters: serum glutamate oxaloacetate transaminase (SGOT) (21), serum glutamate pyruvate transaminase (SGPT) (21), alkaline phosphatase (22), total protein (23), albumin (24), albumin-globulin (A: G) ratio (25), cholesterol, high density lipoprotein (HDL) and triglyceride (26). A 10% homogenate of the tissue was used for the analysis of lipid peroxidation (LPO) (27), superoxide dismutase (SOD) (28) and catalase (CAT) (29).

Statistical analysis

The values were expressed as mean±SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison test. P value<0.05 were considered as significant.

Results

From the acute toxicity study the LD_{50} value for the alcoholic extract of *Phyllanthus rheedii* was found to be 250 mg/kg. The injection of STZ produced permanent diabetes within 48 h, the animals became progressively hyperglycemic, hyperlipidemic and hypoalbuminemic (Table 1-3). They become restless and irritable. Severe thirst and lack of appetite were noticed. STZ induced animals showed serum albumin level and consequently A:G ratio showed considerable decline (Table 3). Higher levels of SGOT, SGPT, ALP and hypercholesterolemia were encountered (Table 2). There was significant elevation of LPO and decrease SOD and catalase levels in

Table 2. Effect of *P. rheedii* on lipid metabolites and enzymes in STZ induced diabetic rats.(n=6, data have been analysed by one way ANOVA followed by Tukey multiple comparison analysis).

Treatment	Dose (mg/kg)	Cholesterol (mg%)	HDL (mg%)	Triglyceride (mg%)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Control	-	130.50±0.70	20.15±1.24	80.35±1.40	177.90±3.40	109.50±2.60	307.50±7.75
Diabetic control	-	249.40±1.10[a]	26.56±1.40[b]	175.40±2.24[a]	226.10±2.90[a]	152.40±2.10[a]	580.80±8.70[a]
P.rheedii	250	147.20±1.40[d]	19.00±1.120[d]	88.60±1.90[c]	210.30±2.50[e]	123.00±2.80[c]	422.00±9.40[c]
Glibenclamide	500 µg	138.50±1.60[c]	19.00±1.12[d]	105.60±1.20[c]	187.30±2.90[c]	116.00±1.10[c]	326.00±5.60[c]

[a]P<0.001; [b]P<0.01 vs Control; [c]P<0.001; [d]P<0.01; [e]P<0.05 vs Diabetic control

liver tissue of diabetic control animals when compared to the corresponding control group (Table 4).

The administration of *P.rheedii* at a dose of 250 mg/kg showed significant (P<0.001) antihyperglycemic effect was evident from the 1st week onwards; the decrease in blood glucose was significant on the 3rd week in the group treated with *P.rheedii*. All the protein parameters viz., total protein, albumin, globulin and A: G ratio did not show any deviation from normal range in *P.rheedii* treated rats (Table 3). Lipid parameters viz cholesterol, HDL and triglycerides showed improvement after 21 days treatment compared to control. Serum enzymes namely SGOT, SGPT and ALP showed considerable improvement after treatment with *P.rheedii* in comparison to control. The tissue antioxidant levels such as SOD, LPO and catalase were significantly (P<0.001) altered near to normal after treatment with *P.rheedii*. Administration of *P.rheedii* and glibenclamide tends to bring the values to near normal. The effect of *P.rheedii* was prominent when compared with glibenclamide.

Discussion

Streptozotocin is toxic to β cells and has been widely used to induce diabetes in animals (30). Diabetes is associated with profound alterations in the plasma lipids and lipoprotein profile and with increased risk of coronary heart disease (31). The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins (32). Many herbs and plant products have been shown to have hypolipidemic properties (33). In the present study the feeding of *P.rheedii* to STZ induced diabetic rats mimics insulin in its effect as observed by the lowering of lipids in addition to the antidiabetic activity. *P.rheedii* also posses lipid lowering properties in diabetic animals.

An increase in serum cholesterol, HDL and triglyceride levels were observed in STZ induced diabetic rats but in *P.rheedii* treated STZ induced rats there is a reduction of cholesterol, HDL and triglyceride levels (Table 2). These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics (34).

There is an increase in transaminase activities in the liver and serum of diabetic animals. The increased levels of transaminases, which are active in the absence of insulin because of

Table 3. Effect of *P. rheedii* on treatment on protein metabolites in STZ diabetic rats. (n=6, data have been analysed by one way ANOVA followed by Tukey multiple comparison analysis).

Treatment	Dose (mg/kg)	Total protein (mg%)	Albumin (mg%)	Globulin (mg%)	A: G ratio
Control	-	6.67±0.10	3.14±0.12	2.76±0.08	1.20±0.02
Diabetic control	-	5.49±0.24[b]	2.12±0.10	2.36±0.50	0.93±0.04[a]
P.rheedii	250	5.90±0.30	3.05±0.14	2.85±0.02	1.17±0.06[c]
Glibenclamide	500 µg	6.05±0.42	3.10±0.70[c]	2.80±0.04	1.10±0.05

[a]P<0.001; [b]P<0.01 vs normal; [c]P<0.001 vs Diabetic control

Table 4. Antioxidant effect of *P.rheedii* on STZ induced diabetic rats.(n=6, data have been analysed by one way ANOVA followed by Tukey multiple comparison analysis).

Treatment	Dose (mg/kg)	LPO[1]	SOD[2]	Catalase[3]
Control	-	72.00±1.46	2.60±0.01	56.00±1.24
Diabetic control	-	114.00±2.62[a]	1.02±0.06[a]	32.00±1.00[a]
PR	250	88.00±1.20[b]	1.92±0.02[b]	48.00±1.60[b]
Glibenclamide	500 µg	82.00±1.25[b]	2.12±0.02[b]	49.00±1.60[b]

1 - µ mole of MDA/min/mg protein
2 - Unit/min/mg protein
3 - mole of H_2O_2 consumed/min/mg protein
[a]P<0.001 vs control; [b]P<0.001; [c]P<0.01 vs Diabetic control

increased availability of amine acids in diabetes, are responsible for the increased glucogenesis and ketogenesis observed in diabetes (35). There is an improvement noticed in the levels of SGOT, SGPT and ALP are as a consequence of improvement in the carbohydrate, fat and protein metabolism due to the therapy of alcoholic extract of *P.rheedii*. The restoration of SGOT, SGPT and ALP to their normal levels may be due to the presence of flavonoids in the alcoholic *P.rheedii* extract, which are reported to be hepatoprotective agents (36, 37).

Among the parameters of protein metabolism, the present study showed a slight decline in total proteins, sharp fall in serum albumin, globulin and A: G ratio in uncontrolled diabetic rats. This is in agreement with hypoalbuminemia observed in diabetics (38). On the other hand, in the extract treated diabetic rats protein metabolism never deviated from normal range. Hypoalbuminemia is a common problem in diabetic animals and is generally attributed in the presence of nephropathy. An overall reduction in serum total protein in diabetic animals and consequents albumin and A:G ratios were observed in the present study. This corroborates earlier reports (39). The reversal of these changes by alcoholic *P.rheedii* extract therapy proved that insulin deficiency had been grossly corrected.

Another important factor determining the level and composition of serum and tissue lipids is LPO associated with cellular membrane studies have reported an increase in hepatic, MDA concentration in STZ induced diabetic rats when compared with the normal rats as also evidenced in the present study. In diabetes, hypoinsulinaemia increases the activity of the enzyme fatty acyl coenzyme A oxidase, which initiates β oxidation of fatty acids, resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane function by decreasing membrane bound enzymes and receptors. Its products (lipid radicals and lipid peroxides) are harmful to the cells in the body and associated with atherosclerosis, brain and kidney damage. The extract showed a significant reduction in LPO in liver tissues as compared to the diabetic control.

The destruction of superoxide radical or H_2O_2 by SOD or CAT would ameliorate STZ toxicity, as would substances able to scavenge of hydroxyl radical (40). The altered balance of antioxidant enzymes caused by decrease in SOD, CAT activities may responsible for the inadequacy of antioxidant defense in combating ROS mediated damage. The decreased activities of CAT and SOD may response to increased production of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation (41). A reduced activity of SOD and catalase in liver has been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides (42). *P.rheedii* extract treated rats showed decreased lipid peroxidation, which is associated with increased activity of SOD and CAT. This means that the extract can reduce reactive oxygen free radicals and improve the activities of the hepatic anti-oxidant enzymes.

Conclusion

The *P.rheedii* leaf extract is beneficial in controlling the blood glucose level, improves

the lipid metabolism and prevents diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats. This could be useful for prevention or early treatment of diabetic disorders. Further studies are in progress to isolate, identify and characterize the active principles.

References

(1) Scheen JA. Drug treatment of non- insulin dependent diabetes mellitus in the 1990s. Achievements and future development. *Drug* (1997) 54: 355-368

(2) Sochar M, Baquer NZ and Mclean P. Glucose under utilization in diabetes. Comparative studies on the changes in the activities of enzymes of glucose metabolism in rat kidney and liver. *Mol. Physiol.* (1985) 7: 5-68

(3) Baynes JW and Thrope SR. Role of oxidative stress in diabetic complications. *Diabetes* (1999) 48: 1-4

(4) Morel DW and Chisolm GM. Antioxidant treatment of diabetic rats inhibits lipoprotein oxidation and cytotoxicity. *J. Lipid Res.* (1989) 30: 1827-1834

(5) Seifter S and England S. Energy metabolism. In: Arias I, Papper M and Schacter D. (eds.) *The Liver: Biology and Pathology*. Raven Press, New York (1982) 219-249

(6) Tiwari AK and Madhusudana RJ. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci.* (2002) 83: 30-38

(7) Bhattaram VA, Graefe U, Kohlert C, Veit M and Derendorf H. Pharmacokinetics and bioavailability of herbal medicinal products. *Phytomed.* (2002) 9: 1-36

(8) Bailey CJ and Day C. Traditional treatments for diabetes. *Diabetes Care* (1989) 12: 553-564

(9) Mitra SK, Gopumadhavan S, Muralidhar TS, Anturlikar SD and Sujatha MB. Effect of a herbomineral preparation D-400 in streptozotocin induced diabetic rats. *J. Ethnopharmacol.* (1996) 54: 41-46

(10) Annapurna A, Kanaka Mahalakshmi D and Murali Krishna K. Antidiabetic activity of a polyherbal preparation (tincture of punchparna) in normal and diabetic rats. *Indian J. Exp. Biol.* (2001) 39: 500-502

(11) Bhattacharya SK, Satyan KS and Chakrbarti A. Effect of Trasina, an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. *Indian J. Exp. Biol.* (1997) 35: 297-299

(12) Pari L and Saravanan R. Antidiabetic effect of diasulin, an herbal drug, on blood glucose, plasma insulin and hepatic enzymes of glucose metabolism in hyperglycaemic rats. *Diabetes, Obesity and Metabolism* (2004) 6: 286-292

(13) World Health Organization. *The WHO Expert Committee on Diabetes Mellitus*, Technical Report Series 646. WHO, Geneva (1980)

(14) Jia W, Gao WY and Xiao PG. Antidaibetic drugs of plant origin used in China: Composition, pharmacology and hypoglycemic mechanisms. *Zhongguo Zhong Yao Za Zhi.* (2003) 28: 108-113

(15) Elder C. Ayurveda for diabetes mellitus: a review of the biomedical literature. *Altern. Ther. Health Med.* (2004) 10: 44-50

(16) Loew D and Kaszkin M. Approaching the problem of bioequivalence of herbal medicinal Products. *Phytother. Res.* (2002) 16: 705-711

(17) Rajan S, Sethuraman M and Mukherjee PK. Ethnobiology of the Nilgiri Hills, India. *Phytother*. Res. (2002) 16: 98-116

(18) Babu V, Ganga devi T and Subramonium A. Antidiabetic activity of ethanol extract of *Cassia kleinii* leaf in streptozotocin induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J. Pharmacol.* (2003) 35: 280-296

(19) Augusti KT and Sheela CG. Antiperoxide effect of s-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. *Experientia* (1996) 52: 115-9

(20) Sasaki T, Matsy S and Sonae A. Effect of acetic acid concentration on the colour reaction in the *O*-toludine boric acid method for blood glucose estimation. *Rinshbokagaku* (1972) 1: 346-353

(21) Reitman S and Frankel S. A colorimetric method for determination of serum glucose oxaloaectate and glutamic pyruvate transaminase**.** *Am. J. Clin. Path.* (1957) 28: 53-56

(22) Kind PRN and King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with antipyrin. *J. Clin. Path.* (1954) 7: 322-330

(23) Lowry OH, Rosebrough NJ and Far AL. Protein measurement with Folin - Phenol reagent. *J. Biol. Chem.* (1951) 173: 265-275

(24) Gardwohl RBH. Clinical laboratory methods and diagnosis, 5th ed. Mosby Company, St. Louis (1958) 253

(25) Greenberg DM. Estimation of serum albumin-globulin ratio. *J. Biol. Chem.* (1929) 82: 545

(26) Hawk PB, Oser L and Summerson WH. *Practical Physiology Chemistry.* The Maples Press Co, New York (1954) 126-132

(27) Devasagayam TPA and Tarachand U. Decreased lipid peroxidation in the rat kidney during gestation. *Biochem. Biophys. Res. Commun.* (1987) 56: 836 - 842

(28) Marklund S and Marklund G. Involvement of superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.* (1974) 4: 469-474

(29) Sinha AK. Colorimetric assay of catalase. *Anal. Biochem.* (1972) 4: 389-394

(30) Cooperstin SJ and Watkin D. *Action of Toxic Drugs on Islet Cells. In the Islets of Langerhans.* Academic Press, New York (1981) 387-425

(31) Betterridge J. Lipid disorders in diabetes mellitus. In: Pickup J and Williams G. (eds.) *Textbook of Diabetes*. Blackwell Science, London (2002) 551-553

(32) Brown GB, Xue - Qiao Z, Soccoand DE and Albert

JJ. Lipid lowering and plaque regression. New insights into prevention of plaque disruption and clinical vents in coronary disease. *Circulation* (1993) 8: 1781-1791

(33) Karunanayake EH and Tennekoon KH. Search for novel hypoglycemic agents from medicinal plants. In: Sharma AK. (ed.) *Diabetes Mellitus and its Complication, an Update*. Macmillon India Ltd, New Delhi (1993)

(34) Cho SY, Park JY, Park EM, Choi MS, Lee MY, Jeon SM, Jang MK, Kim MJ and Park YB. Alternation of hepatic antioxidant enzyme activities and lipid profile in streptozotocin-induced diabetic rats by supplementation of dandelion water extract. *Clin. Chim. Acta* (2002) 317: 109-117

(35) Feling P, Marliss E, Ohman J and Cahill JR. Plasma amino acids level in diabetic ketoacidosis. *Diabetes* (1970) 19: 727-730

(36) *Merck Index, An encyclopedia of Chemicals, Drugs and Biologicals*, Merck Co., USA (1996) 8697 and 2320

(37) Mustaq Ahmad M, Shoib A, Tahira M and Anwar H. Hypoglycaemic action of the flavonoid fraction of *Cuminium nigram* seeds. *Phytother. Res.* (2000) 14: 103-106

(38) Porte DJ and Halter JB. In: Williams RH. (ed.) *Textbook of Endocrinology.* WB Saunders Co., Philadelphia (1981) 715

(39) Soon YY and Tan BKH. Evaluation of the hypoglycemic and antioxidant activities of *Morinda officinalis* in streptozotocin- induced diabetic rats. *Singapore Med. J.* (2002) 43: 077-085

(40) Walling C. Fenton's reagent revisited. *J. Am. Chem. Soc.* (1975) 97: 125-129

(41) Lubec B, Hayn M, Denk W and Bauer G. Brain lipid peroxidation and hydroxyl radical attack following the intravenous infusion of hydrogen peroxide in an infant. *Free Rad. Biol. Med.* (1996) 21: 219-223

(42) Searle AJ and Wilsion RL. Gultathion peroxidase effect of super oxide, hydroxyl and bromine free radicals on enzyme activity. *Int. J. Rad. Biol.* (1981) 34: 125-129

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