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Role of Diasulin, an Herbal Formulation on Antioxidant Status in Chemical Induced Diabetes

Leelavinothan Pari and Ramalingam Saravanan
Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, 608 002, India

Abstract: In the present study the effect of ethanolic extract of the Diasulin was examined for its antioxidant effect in the liver and kidney of alloxan diabetic rats. Oral administration of Diasulin (200 mg kg^{-1}) for 30 days resulted in a significant reduction in thiobarbituric acid reactive substances and hydroperoxides. The Diasulin also caused a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in liver and kidney of alloxan diabetic rats. The results show the antioxidant effect of Diasulin. The effect of Diasulin at 200 mg kg^{-1} was more effective than glibenclamide, a reference drug.

Key words: Diasulin, alloxan, antioxidants, herbal formulation, lipid peroxidation

INTRODUCTION

Diabetes mellitus is a syndrome, initially characterized by a loss of glucose homeostasis. Free radicals and oxidative stress may act as a common pathway to diabetes itself as well as to its later complication. The attack on the cell membrane by free radicals lead to lipid peroxidation. Lipid peroxidation in cell is controlled by a various cellular defense mechanisms consisting of enzymatic and non-enzymatic scavengers systems[1] .The increased oxidative stress in diabetes includes the autoxidation of glucose and non-enzymatic glygation and also changes in the antioxidant defense systems. Therefore the ineffective scavenging of free radicals plays a crucial role in determining tissue injury. Many traditional plant treatments for diabetes are used throughout the world. Plant drugs[2] and herbal formulation[3-5] are frequently considered to be less toxic and more free from side effects than synthetic one. Based on the WHO recommendations hypoglycaemic agents of plant origin used in traditional medicine are important[6]. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than individual. Various herbal formulations such as Diamed[7] cogent db[8] and hyponidd[9] are well known for their antidiabetic effects. Diasulin, a poly herbal drug composed of ten medicinal plants. In our previous study, we have evaluated the antidiabetic, antihyperlipidemic and antiperoxidative effect of Diasulin in alloxan-induced diabetes[10,11]. The present investigation was carried out to study the antioxidant effect of Diasulin in addition to its antidiabetic and antiperoxidative effect in rats. The effects produced by this drug on different parameters were compared with glibenclamide, a reference drug.

MATERIALS AND METHODS

Animals: Male Wistar rats of body wt. 180-200 g were obtained from central Animal House, Raja Muthiah Medical College, Annamalai University. The animals were fed on standard pellet diet (Hindustan Lever, Mumbai, India) and water *ad libitum*. The rats used in the present study were maintained in accordance with guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderbad, India and the study approved by the ethical committee (Vide No. 88, 2002).

Test drug and chemicals: Ethanolic extract of ten medicinal plants, which are involved in preparation of Diasulin was a gift from herbal remedies, pondicherry, india.

The residual extract were mixed and named as Diasulin was prepared (Table 1) on the basis of an ayurvedic antidiabetic formulation proposed by Pandey *et al*.[12]. Five hundred grams of each plant (chopped into small pieces) was extracted individually were, soaked overnight in 1.5 L of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvents were evaporated in a rotavapor at 40-50°C under reduced pressure and lyophilized. Alloxan England. Enzyme Linked Immuno Sorbant Assay (ELISA) kit for insulin assay

Corresponding Author: Leelavinothan Pari, Department of Biochemistry, Faculty of Science, Annamalai University, Annamalai Nagar, Tamil Nadu, 608 002, India Tel: +914144230139 Fax: +914144238080

Table 1: Diasulin (Composition and concentration)

Botanical name	Common name	Family	Part used	Concentration (mg dL^{-1})
Cassia auriculata	Tanner's cassia	Ceasalpinaceae	Flower	40
Coccinia indica	Little gourd	Cucurbitaceae	Fruit	40
Curcuma longa	Turmeric	Zingiberaceae	Rhizome	40
Emblica officinalis	Indian gooseberry	Euphorbiaceae	Fruit	20
Gymnema sylvestre	Ram's horu	Asclepiadaceae	Leaves	20
Momordica charantia	Bitter gourd	Cucurbitaceae	Fruit	30
Scoparia dulcis	Sweet broom weed	Scrophulariaceae	Whole plant	40
Syzigium cumini	Jamun	Myrtaceae	Seed	20
Tinospora cardifolia	Gulancha tinospora	Menispermaceae	Root	20
Trigonella foenum graecum	Fenugreek	Fabaceae	Seed	50

was purchased from Boehringer Mannheim, Germany. All other biochemicals used in this experiment were purchased from Sigma Chemical monohydrate was purchased from BDH Chemicals, Poole, Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade.

Drug administration: Diasulin was suspended in distilled water and adminisitered orally through intragastric tube at the following doses of 50, 100 and 200 mg kg^{-1} of diabetic rats.

Experimental induction: The rats were injected intraperitoneally with alloxan monohydrate dissolved in sterile normal saline at a dose of 150 mg kg^{-1}[13]. After 2 weeks, rats with moderate diabetes having glycosuria (indicated by Benedict's qualitative test) and hyperglycaemia (i.e., with a blood glucose of 200-260 mg dL^{-1}) were used for the experiment.

Experimental design: In the experiment, a total of 42 rats (30 diabetic surviving rats, 12 normal rats) were used. The rats were divided into seven groups of six rats each after the induction of alloxan diabetes. Group 1: Normal untreated rats. Group 2: Normal rats given aqueous solution of Diasulin (200 mg kg^{-1} body weight) daily using an intragastric tube for 30 days. Group 3: Diabetic control rats. Group 4: Diabetic rats given aqueous solution of Diasulin (50 mg kg^{-1} body weight) daily using an intragastric tube for 30 days. Group 5: Diabetic rats given aqueous solution of Diasulin (100 mg kg^{-1} body weight) daily using an intragastric tube for 30 days. Group 6: Diabetic rats given aqueous solution of Diasulin (200 mg kg^{-1} body weight) daily using an intragastric tube for 30 days. Group 7: Diabetic rats given aqueous solution of glibenclamide (600 μg kg^{-1} body weight) daily using an intragastric tube for 30 days. At the end of 30 days, all the rats were killed by decapitation under pentobarbitone sodium (60 mg kg^{-1}) anesthesia. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and plasma was separated for the assay of insulin. The liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

Analytical methods: Fasting blood glucose was estimated by O-toluidine method[14]. Plasma insulin was estimated by using enzyme linked immunosorbent assay (ELISA) kit (Boehringer Mannheim, Germany). Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Fraga *et al.*[5]. Hydroperoxide was determined by the method of Jiang *et al.*[16]. Glutathione (GSH) was estimated by the method of Ellman[17]. The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar *et al.*[18]. Catalase by the method of Sinha[19]. The activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed according to the method of Rotruck *et al.*[20] and Habig *et al.*[21], respectively. Protein content in tissue homogenate was measured by the method of Lowry *et al.*[22]

Statistical analysis: The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan Multiple Range Test (DMRT). Values were considered statistically significant when $p<0.05$ Duncan[23].

RESULTS AND DISCUSSION

Effect of Diasulin on blood glucose and plasma insulin: Table 2 shows the level of blood glucose and plasma insulin in normal and experimental animals. There was a significant elevation in blood glucose level, whereas plasma insulin levels decreased significantly in alloxan diabetic rats, compared with normal rats. Administration of Diasulin and glibenclamide tends to bring blood glucose and plasma insulin towards normal. The effect of Diasulin at 200 mg kg^{-1} was significantly better than 50 and 100 mg kg^{-1} therefore the higher dose was used for further biochemical studies. The administration of Diasulin and glibenclamide to normal rats showed a significant effect in lowering blood glucose and increasing plasma insulin.

Table 2: Changes in blood glucose and plasma insulin levels of normal and experimental animals

Groups	Fasting blood glucose (mg dL^{-1})	Plasma insulin ($\mu U\ mL^{-1}$)
Normal	81.58±2.40^a	11.18± 0.74^a
Normal+Diasulin (200 mg kg^{-1})	78.33±4.70^a	12.68±0.94^b
Diabetic control	265.00± 4.40^b	3.55±0.38^c
Diabetic + Diasulin (50 mg kg^{-1})	210.80±14.26^c	5.61±0.48^d
Diabetic + Diasulin (100 mg kg^{-1})	158.33±11.00^d	6.03±0.41^d
Diabetic + Diasulin (200 mg kg^{-1})	104.16±6.70^e	7.05±0.64^e
Diabetic + Glibenclamide (600 µg kg^{-1})	111.60±9.86^e	6.32±0.55^{de}

Values are given as mean ± SD for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMR).
Duncan procedure, Range for the level 2.89, 3.03, 3.13, 3.20, 3.25.
Diabetic control was compared with normal, † $p<0.001$.
Experimental groups were compared with diabetic control, * $p<0.001$.
+ + +, > 2% sugar, ++; -2% sugar; +, -1%

Table 3: Changes in levels of TBARS and hydroperoxides in liver and kidney of normal and experimental animals

	TBARS (mM per 100 g tissue)		Hydroperoxide (mM per 100g tissue)	
Groups	Liver	Kidney	Liver	Kidney
Normal	0.73±0.066^a	0.845±0.07^a	71.42±4.61^a	53.56±4.61^a
Normal + Diasulin (200 mg kg^{-1})	0.72±0.079^a	0.785±0.065^a	69.63±6.09^a	52.40±3.90^a
Diabetic control	1.77±0.06^b	1.565±0.105^b	102.37±6.41^b	75.35±4.46^b
Diabetic + Diasulin (200 mg kg^{-1})	0.88±0.053^c	0.970±0.072^c	78.56±5.83^c	61.89±6.07^c
Diabetic + Glibenclamide (600 µg kg^{-1})	0.93±0.088^c	1.00±0.086^c	80.90±5.32^c	63.16±6.30^c

Values are given as mean ± S.D for 6 rats in each group, Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT), Duncan procedure, Range for the level 2.95, 3.09, 3.20

Effect of Diasulin on TBARS and hyderoperoxide: Table 3 shows the concentration of TBARS and hydroperoxides in tissues of normal and experimental animals. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes when compared to the corresponding control group. Administration of Diasulin and glibenclamide tends to bring the values to near normal.

Effect of Diasulin on GSH: Table 4 shows the content of GSH in tissue of normal and experimental groups. There was significant decrease in the concentration of GSH in tissues during diabetes when compared with the corresponding control groups. Administration of Diasulin and glibenclamide tends to bring the value to near normal.

Effect of Diasulin on SOD, CAT, GPx and GST in liver and kidney: Table 5 and 6 show the activities of SOD, CAT, GPx and GST in liver and kidney of normal and experimental groups. There was a significant reduction in the activities of SOD, CAT, GPx and GST in tissues like liver and kidney during diabetes. Administration of Diasulin and glibenclamide tends to bring the values to near normal. The effect was more prominent when compared with glibenclamide.

Table 4: Changes in levels of reduced glutathione in liver and kidney of normal and experimental animals

	Reduced glutathione (mg 100 mg^{-1} tissue)	
Groups	Liver	Kidney
Normal	45.33±3.87^a	33.41±2.10^a
Normal + Diasulin (200 mg kg^{-1})	47.99±2.20^a	36.26±2.53^b
Diabetic control	22.71±2.38^b	21.43±1.57^c
Diabetic + Diasulin (200 mg kg^{-1})	39.45±1.79^c	27.76±1.11^d
Diabetic + Glibenclamide (600 µg kg^{-1})	41.90±2.01^c	25.99±2.83^d

Values are given as mean ± SD for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT). Duncan procedure, Range for the level 2.95, 3.09, 3.20.

DISCUSSION

Oxidative stress has been implicated in the pathogenesis of a wide variety of clinical disorders, resulting usually from deficient antioxidant defences. Alloxan, a β cytotoxin induces chemical diabetes (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting cells of the pancreas. This damages a large number of β-cells resulting in decrease in endogenous insulin release. Alloxan administered rats therefore become hyperglycaemic in a short period of time, followed by a hepatic glucose over production[24].

In this study, we have found that Diasulin, a poly herbal drug decreases blood glucose in alloxan-diabetic rats. The possible mechanism by which Diasulin mediates its antidiabetic action may be by potentiation of pancreatic secretion of insulin from existing β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue. It is evidenced by the significant increase in the level of insulin by Diasulin in diabetic, normal and glibenclamide treated rats (Table 2). In this context, a number of medicinal plant such as *Coccinia indica*[25], *Cassia auriculata*[26], *Curcuma longa*[27],

Table 5: Changes in activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in liver of normal and experimental animals

Groups	Catalase (Units[A] mg^{-1} protein)	Superoxide dismutase (Units[B] mg^{-1} protein)	Glutathione peroxidase (Units[C] mg^{-1} protein)	Glutathione–S–transferase (Units[D] mg^{-1} protein)
Normal	74.41±1.78[a]	11.22±0.95[a]	9.50±0.77[a]	6.76±0.63[a]
Normal+Diasulin (200 mg kg^{-1})	76.50±1.50[a]	13.01±0.89[b]	9.67±0.35[a]	6.99±0.43[a]
Diabetic control	41.90±2.70[b]	4.84±0.49[e]	4.36±0.49[b]	2.99±0.28[b]
Diabetic+Diasulin (200 mg kg^{-1})	64.18±2.88[c]	7.62±0.57[d]	8.13±0.75[c]	6.04±0.53[c]
Diabetic+Glibenclamide(600 µg kg^{-1})	59.93±1.22[d]	7.24±0.58[d]	7.47±0.50[c]	5.20±0.26[c]

Values are given as mean ± SD for 6 rats in each group, Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT), Duncan procedure, Range for the level 2.95, 3.09, 3.20, [A]-µ mole of H_2O_2 consumed/minute, [B]-One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in one minute, [C]-µg of GSH consumed/min, [D]-µ moles of CDNB-GSH conjugate formed/min

Table 6: Changes in activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione–S–transferase (GST) in kidney of normal and experimental animals

Groups	Catalase (Units[A] mg^{-1} protein)	Superoxide dismutase (Units[B] mg^{-1} protein)	Glutathione peroxidase (Units[C] mg^{-1} protein)	Glutathione–S–transferase (Units[D] mg^{-1} protein)
Normal	25.68±1.77[a]	16.31±0.58[a]	9.50±0.77[a]	6.30±0.40[a]
Normal + Diasulin (200 mg kg^{-1})	28.00±1.49[b]	17.93±1.53[a]	9.67±0.35[a]	6.26±0.54[a]
Diabetic control	19.12±2.09[c]	8.07±0.68[b]	4.36±0.49[b]	3.18±0.24[b]
Diabetic + Diasulin (200 mg kg^{-1})	24.22±1.27[a]	14.39±1.20[c]	8.13±0.75[c]	5.57±0.51[c]
Diabetic + Glibenclamide (600 µg kg^{-1})	20.27±1.89[b]	11.78±1.02[c]	7.47±0.50[c]	4.54±0.36[d]

Values are given as mean±SD for 6 rats in each group, Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMR),Duncan procedure, Range for the level 2.95, 3.09, 3.20, [A]-µ mole of H_2O_2 consumed/min, [B]-One unit of activity was taken as the enzyme reaction which gave 50% inhibition of NBT reduction in one min, [C]-µg of GSH consumed/min, [D]-µ moles of CDNB-GSH conjugate formed/min

Gymnema sylvestre[28], *Momordica charantia*[29], *Scoparia dulcis*[30], *Syzigium cumini*[31] and *Tinospora cardifolia*[32] (ingredients of Diasulin) have also been observed to have insulin-stimulatory effects.

A marked increase in the concentration of TBARS and hydroperoxides are observed in liver and kidney of diabetic rats[33,34]. Previous report shows that Diasulin and glibenclamide tends to bring the increased concentration of lipidperoxidation products to near normal level[11].

The activities of SOD, CAT, GSH, GPx and GST, were observed to decrease significantly in diabetic rats. SOD is major defense for aerobic cells in combating the toxic effects of superoxide radicals[35]. Catalase is an enzymatic antioxidant widely distributed in all animal tissues[36]. GPx an enzyme with selenium and GST catalyses the reduction of hydrogen peroxide and hydroperoxides to non-toxic products[37]. The most abundant oxidative free radicals such as superoxide anions and hydroxyl radicals are generated in living cells, which induces lipid peroxidation of cell membrane and damage[38]. The depletion in the activity of GPx and GST may result in the involvement of deleterious oxidative changes due to the accumulation of toxic products[37].

GSH is one of the major defense systems. It protects the body from toxic effect of reactive oxygen species and helps to maintain normal cellular redox potential and alternation of both these processes result in the abnormal GSH levels[39]. We observed decreased GSH contents in liver and kidney of alloxan diabetic rats. The lowered levels of GSH represent increased utilization due to oxidative stress and counteract with the increased formation of lipid peroxides.

Administration of Diasulin and glibenclamide increased the activities of SOD, CAT, GPx, GST and GSH in diabetic, liver and kidney. Antidiabetic and Antioxidant effect of Diasulin may be due to the effect of chemical constituents of different plants, viz, alkaloid and pectins from *Coccinia indica*[40], alkaloids from *Tinospora cordifolia*[32], emlicamin A and B from *Emblica officinalis*[41], trigonelline and scopoltin from *Trigonella foenum graecum*[42], alkaloid-6-methoxybenzoxazolinone and terpenoids such as scoparic acids A, B, C and scopadulcic acid A and B from *scoparia dulcis*[43]. Which may be responsible for scavenging free radicals liberated by alloxan and thus enhance the enzymic and non-enzymic antioxidants in diabetic rats.

The decreased level of blood glucose and increased activity of antioxidant enzymes SOD, CAT, GSH and GPx in liver and kidney of diabetic rats showed the above effect. Thus the results of our study show that Diasulin, a poly herbal drug offered an antioxidant activity, along with antidiabetic, antihyperlipedemic and antiperoxidative effect, which could exert a beneficial action against pathological alteration caused by the presence of superoxide and hydroxyl radicals in alloxan diabetes. This could be due to different types of active principles of Diasulin, each with a single or a diverse range of biological activities. Combined extract of plants are used as the drug choice rather than individual.

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