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Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats

P. Subash Babu and P. Stanely Mainzen Prince

Abstract

Hyponidd is a herbomineral formulation composed of the extracts of ten medicinal plants (*Momordica charantia*, *Melia azadirachta*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Gymnema sylvestre*, *Enicostemma littorale*, *Emblica officinalis*, *Eugenia jambolana*, *Cassia auriculata* and *Curcuma longa*). We have investigated hyponidd for its possible antihyperglycaemic and antioxidant effect in diabetic rats. Rats were rendered diabetic by streptozotocin (STZ) (45 mg kg^{-1} body weight). Oral administration of hyponidd (100 mg kg^{-1} and 200 mg kg^{-1}) for 45 days resulted in significant lowered levels of blood glucose and significant increased levels of hepatic glycogen and total haemoglobin. An oral glucose tolerance test was also performed in experimental diabetic rats in which there was a significant improvement in blood glucose tolerance in the rats treated with hyponidd. Hyponidd administration also decreased levels of glycosylated haemoglobin, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and α-tocopherol in diabetic rats. Plasma reduced glutathione and vitamin C were significantly elevated by oral administration of hyponidd. The effect of hyponidd at a dose of 200 mg kg^{-1} was more effective than glibenclamide (600 μg kg^{-1}) in restoring the values to near normal. The results showed that hyponidd exhibits antihyperglycaemic and antioxidant activity in STZ-induced diabetic rats.

Introduction

In traditional practice, medicinal plants are widely used in many countries for the treatment of diabetes mellitus. The antihyperglycaemic effect of several plant extracts and herbal formulations that are used as antidiabetic remedies has been confirmed (Grover et al 2000; Pari & Saravanan 2002). Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Momin 1987). Combined extracts of herbs are used as the drug of choice rather than individual plant extracts. Herbal formulations such as D-400 (Mitra et al 1996) and Trasina (Bhattacharya et al 1997) exhibit antidiabetic effects. Studies have also shown that herbal formulations such as Diamed have antidiabetic and antioxidative effects (Pari et al 2001).

Active oxygen metabolism plays an important role in the normal functioning of the β-cells of the pancreas. Free oxygen radicals and oxidative stress appears to be an important factor in the production of secondary complications in diabetes mellitus (Thornalley et al 1996). Hyperglycaemia generates reactive oxygen species, which in turn cause lipid peroxidation and membrane damage (Hunt et al 1988). Studies carried out in recent years have shown elevated lipid peroxidation in plasma in diabetic rats (Kamalakkannan & Stanely Mainzen Prince 2003). Circulating lipid peroxides were shown to be capable of initiating arteriosclerosis, which is a well known late feature of diabetes mellitus (Hessler et al 1983).

Hyponidd is a herbomineral formulation composed of ten medicinal plants (Table 1). These plants are known to possess antidiabetic and antioxidant properties (Table 2) and have been used in indigenous systems of medicine to treat diabetes mellitus (Baskaran et al 1990; Ammon & Wahl 1991; Raman & Lau 1996; Manickam et al 1997; Bhattacharya et al 1999; Stanely Mainzen Prince & Menon 1999; Grover et al 2000; Scartezzini & Speroni 2000; Murali et al 2002; Pari & Latha 2002). According to Ayurvedic text, a combination

Department of Biochemistry, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India

P. Subash Babu and P. Stanely Mainzen Prince

Correspondence: P. Stanely Mainzen Prince, Department of Biochemistry, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.
E-mail: p_smprince@yahoo.co.in

Table 1 Composition of hyponidd

	Concentration (mg per tablet)
Powder	
Momordica charantia	12
Purified aspaltham	37.5
Yashad bhasma	37.5
Extract	
Swertia chirata	15
Melia azadirachta	75
Pterocarpus marsupium	75
Tinospora cordifolia	75
Gymnema sylvestre	112.5
Enicostemma littorale	112.5
Emblica officinalis	150
Eugenia jambolana	150
Cassia auriculata	225
Curcuma longa	300

of substances is used to enhance the desired action and eliminate unwanted side effects. In view of the above facts, this investigation was undertaken to evaluate the effect of hyponidd on blood glucose, oral glucose tolerance test (OGTT), liver glycogen, total haemoglobin, glycosylated haemoglobin, plasma thiobarbituric acid reactive substances, hydroperoxides, reduced glutathione, vitamin C, vitamin E and ceruloplasmin in streptozotocin-induced diabetic Wistar rats. The effect of hyponidd was compared with that of glibenclamide, a well known hypoglycaemic drug.

Materials and Methods

Drugs

Hyponidd was purchased from a Pharmacy in the Cuddalore District, Tamil Nadu, India (Manufactured by Charak Pharmaceuticals (I) Private Ltd., Haryana, India).

Table 2 Effect of hyponidd constituents

	Phytochemicals	Effect observed
Plant species		
Momordica charantia	Charantin, momordicosides A and B, acylglucosyl sterols, P-insulin, V-insulin, stigmasterol (Raman & Lau 1996)	Antidiabetic (Raman & Lau 1996)
Swertia chirata	Polyoxygenated xanthones, mangiferin, swertinin, swertianin, swerchirin, chiratin, chirataini (Rastogi & Mehrotra 1993)	Antioxidant (Scartezzini & Speroni 2000)
Melia azadirachta	β-carotene, nimbin, azadirachtin, nimbidiol, quercetin, nimbidin and nimbatiktam (Govindachari 1992)	Antioxidant (Govindachari 1992)
Pterocarpus marsupium	Pteroside, pteroisoauroside, marsuposide, vijayosin, sesquiterpene (Maurya et al 2004); pterosupin, pterostilbene, marsupin (Manickam et al 1997)	Antidiabetic (Manickam et al 1997)
Tinospora cordifolia	Tinosporin, isocolumbin, palmatine, tinocordiside (X), tinocordifolioside (XI), cordioside, β-sitosterol (Singh et al 2003)	Antidiabetic (Stanely Mainzen Prince & Menon 2003), antioxidant (Stanely Mainzen Prince & Menon 1999)
Gymnema sylvestre	Gymnemic acids, saponins, stigmasterol, quercitol, betaine, choline, trimethylamine (Kapoor 1990)	Antidiabetic (Baskaran et al 1990)
Enicostemma littorale	Vanillic acid, ferulic acid, p-coumaric acid, apigenin, genkwanin, isovitexin, swertisin, saponarin (Murali et al 2002)	Antidiabetic (Murali et al 2002)
Emblica officinalis	Phyllemblin, gallic acid, ellagic acid, phyllantidine, phyllantine, lupeol, emblicanin A and B (Scartezzini & Speroni 2000)	Antioxidant (Bhattacharya et al 1999)
Eugenia jambolana	Gallic acid, ellagic acid, corilagin, ellagitannins, quercetin (Bhatia & Bajaj 1975)	Antidiabetic (Grover et al 2000)
Cassia auriculata	Flavonoids, anthracene derivatives, dimeric procyanidins, myristyl alcohol, β-D-glucoside, quercetin 3-O-glycoside, rutin (Rao et al 2000)	Antidiabetic (Pari & Latha 2002), antioxidant (Kumar et al 2003)
Curcuma longa	Curcumin, desmethoxy curcumin, bisdemethoxy curcumin, dihydrocurcumin, α- and β-turmerones, eugenol, campesterol, stigmasterol (Rastogi & Mehrotra 1993)	Antioxidant (Ammon & Wahl 1991)
Powders		
Yashad bhasma (Zinc calx)		Used in the treatment of diabetes mellitus in Ayurvedic system
Aspaltham		

Streptozotocin (STZ) was purchased from Sigma Chemical Company Inc. (St Louis, MO). All other biochemicals used in this study were of analytical grade.

Animals

Female Wistar rats, 180–200 g, bred in the Central Animal House, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu, were used. The animals were fed on a standard pellet diet (Hindustan Lever Ltd, Mumbai, India) and water was freely available. All the experimental protocols were approved by the Ethical Committee of Annamalai University.

Experimental induction of diabetes

Diabetes mellitus was induced in rats by a single intraperitoneal injection of freshly prepared STZ ($45\,mg\,kg^{-1}$ body weight) in 0.1 M citrate buffer (pH 4.5) in a volume of $1\,mL\,kg^{-1}$ (Kamalakkannan et al 2003). After two days of STZ administration, blood glucose levels of each rat were determined. Rats with a blood glucose range of $250–300\,mg\,dL^{-1}$ were considered diabetic and included in the study. Blood was collected by sinocular puncture.

Experimental design

In the experiment, a total of 36 rats (12 normal; 24 STZ-diabetic surviving rats) were used. The rats were divided into 6 groups of 6 rats each: Group I, normal untreated rats; Group II, normal rats treated with hyponidd ($200\,mg\,kg^{-1}$) orally in distilled water using an intragastric tube daily for 45 days; Group III, STZ-treated diabetic rats; Group IV & V, STZ-treated diabetic rats administered hyponidd orally ($100\,mg\,kg^{-1}$ and $200\,mg\,kg^{-1}$, respectively) in distilled water using an intragastric tube daily for 45 days; Group VI, STZ-treated diabetic rats given glibenclamide orally ($600\,\mu g\,kg^{-1}$) in distilled water using an intragastric tube daily for 45 days.

After 45 days of treatment, the rats were decapitated after an overnight fast. Blood was collected in heparinized tubes and plasma was separated after centrifugation. Liver tissues were excised immediately and stored in ice-cold containers.

Estimation of blood glucose, hepatic glycogen and plasma insulin

Fasting blood glucose was estimated by the o-toluidine method (Sasaki et al 1972). Hepatic glycogen was estimated by the method of Morales et al (1973). Plasma insulin was performed by the ELISA method using a Boehringer Mannheim kit (Boehringer analyzer ES 300), Mannheim, Germany.

Determination of total haemoglobin and glycosylated haemoglobin

Total haemoglobin was estimated by the cyanomethaemoglobin method (Drabkin & Austin 1932) and glycosylated haemoglobin was estimated by the method of Sudhakar Nayak & Pattabiraman (1981).

Oral glucose tolerance test

An oral glucose tolerance test was performed by the method of Du Vigneaud & Karr (1925). After overnight fasting, a baseline (0 min) blood sample (0.2 mL) was taken from rats in groups I–VI by sinocular puncture. Without delay, a glucose solution ($2\,g\,kg^{-1}$ body weight) was administered by gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. Blood samples from all the groups were collected in potassium oxalate- and sodium fluoride-containing tubes for the estimation of glucose.

Estimation of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP)

Plasma thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP) were determined by the methods of Yagi (1976) and Jiang et al (1992), respectively.

Determination of non-enzymatic antioxidants

Plasma vitamin C and reduced glutathione (GSH) were estimated by the methods of Omaye et al (1979) and Rotruck et al (1973), respectively. Plasma ceruloplasmin and α-tocopherol were determined according to the procedures of Ravin (1961) and Desai (1984), respectively.

Statistical analysis

Statistical analysis was performed using SPSS software package, version 6.0. The values were analysed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) (Duncan 1957). All the results were expressed as mean $\pm$ s.d. for six rats in each group. P values < 0.05 were considered as significant.

Results

Glucose levels measured in blood of normal and experimental rats are given in Table 3. STZ-treated diabetic rats showed significant increased levels of blood glucose as compared with normal rats. Hyponidd treated groups significantly ($P < 0.05$) decreased blood glucose in diabetic rats.

Table 4 shows the changes in body weight, liver glycogen, total haemoglobin and glycosylated haemoglobin of normal and experimental rats. In diabetic rats, the body weight, liver glycogen, total haemoglobin were decreased and that of glycosylated haemoglobin was increased as compared with normal rats ($P < 0.05$). Hyponidd-treated diabetic groups showed significant ($P < 0.05$) increases in body weight, liver glycogen and total haemoglobin and decreased glycosylated haemoglobin compared with untreated diabetic rats.

The oral glucose tolerance level measured in the blood of normal and experimental rats after overnight fasting is shown in Figure 1. In STZ-treated diabetic rats, the peak increase in blood glucose concentration was observed after

Table 3 Effect of hyponidd on blood glucose levels in diabetic rats

Groups	Blood glucose (mg dL^{-1})			
	Day 0	Day 15	Day 30	Day 45
Normal	93.28 ± 7.24	93.33 ± 8.63^{a}	$98.72 \pm 6.94^{a'}$	98.31 ± 6.26^{ab}
Normal rats + hyponidd (200 mg kg^{-1})	101.83 ± 8.74	96.00 ± 6.28^{a}	98.31 ± 6.68^{a}	95.27 ± 5.56^{a}
Diabetic control	324.83 ± 13.86	328.88 ± 15.09^{b}	343.71 ± 17.26^{b}	351.09 ± 21.05^{c}
Diabetic + hyponidd (100 mg kg^{-1})	294.51 ± 16.68	231.18 ± 6.05^{bc}	162.79 ± 6.05^{c}	148.98 ± 8.04^{b}
Diabetic + hyponidd (200 mg kg^{-1})	299.24 ± 14.01	204.10 ± 8.54^{c}	148.60 ± 6.45^{c}	98.51 ± 8.42^{ab}
Diabetic + glibenclamide (600 μg kg^{-1})	289.43 ± 15.34	228.98 ± 8.59^{c}	151.18 ± 7.04^{c}	108.39 ± 7.39^{ab}

Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d) differ significantly at $P < 0.05$ (DMRT).

Table 4 Effect of hyponidd on body weight, glycogen, haemoglobin, glycosylated haemoglobin and plasma insulin in diabetic rats

Groups	Body weight (g)		Glycogen (g/100 g wet tissue)	Haemoglobin (mg dL^{-1})	Glycosylated haemoglobin (mg dL^{-1})	Plasma insulin (μU mL^{-1})
	Initial	Final				
Normal	180.9 ± 7.1	205.7 ± 9.36^{a}	3.71 ± 0.24^{a}	12.78 ± 0.96^{a}	0.44 ± 0.03^{a}	17.2 ± 0.80^{a}
Normal rats + hyponidd (200 mg kg^{-1})	186.5 ± 9.2	221.80 ± 13.61^{b}	3.80 ± 0.31^{a}	14.98 ± 0.69^{b}	0.45 ± 0.02^{a}	18.4 ± 1.29^{a}
Diabetic control	206.4 ± 8.7	168.70 ± 7.91^{b}	1.74 ± 0.09^{b}	6.09 ± 0.38^{c}	0.98 ± 0.05^{b}	8.2 ± 1.20^{b}
Diabetic + hyponidd (100 mg kg^{-1})	178.6 ± 9.3	189.32 ± 8.20^{c}	2.27 ± 0.20^{c}	8.73 ± 0.43^{d}	0.75 ± 0.03^{c}	12.8 ± 1.00^{c}
Diabetic + hyponidd (200 mg kg^{-1})	185.6 ± 6.2	203.80 ± 8.79^{ac}	3.08 ± 0.25^{d}	10.84 ± 0.61^{e}	0.52 ± 0.02^{d}	16.1 ± 0.90^{d}
Diabetic + glibenclamide (600 μg kg^{-1})	181.4 ± 9.2	198.44 ± 8.16^{ac}	2.93 ± 0.20^{d}	9.27 ± 0.54^{d}	0.58 ± 0.02^{e}	14.7 ± 0.81^{d}

Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d,e) differ significantly at $P < 0.05$ (DMRT).

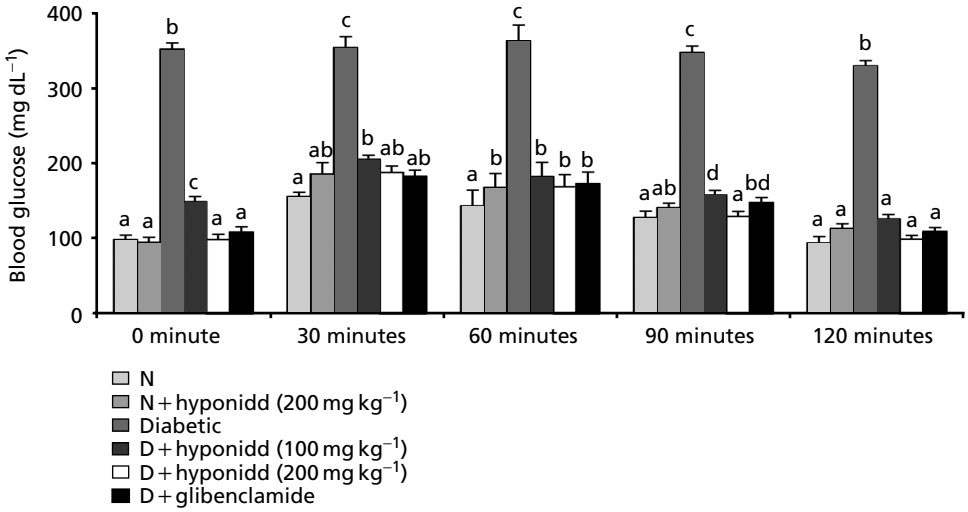

Figure 1 Effect of hyponidd on oral glucose tolerance test (OGTT) in diabetic rats. Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d) differ significantly at $P < 0.05$ (DMRT); N, normal: D, diabetic.

Table 5 Effect of hyponidd on plasma TBARS and hydroperoxides in diabetic rats

Groups	TBARS (nmol mL^{-1})	Hydroperoxides (value $\times 10^{-5}$ mmol dL^{-1})
Normal	2.41 ± 0.12^{ab}	8.91 ± 0.42^{a}
Normal rats + hyponidd (200 mg kg^{-1})	2.64 ± 0.09^{ab}	8.28 ± 0.41^{a}
Diabetic control	4.08 ± 0.18^{c}	16.32 ± 0.67^{b}
Diabetic + hyponidd (100 mg kg^{-1})	3.27 ± 0.12^{c}	12.71 ± 0.89^{c}
Diabetic + hyponidd (200 mg kg^{-1})	2.62 ± 0.09^{a}	9.47 ± 0.43^{a}
Diabetic + glibenclamide (600 μg kg^{-1})	2.81 ± 0.10^{bd}	10.82 ± 0.46^{d}

Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d) differ significantly at $P < 0.05$ (DMRT).

Table 7 Effect of hyponidd on plasma vitamin E and ceruloplasmin in diabetic rats

Groups	Vitamin E (mg dL^{-1})	Ceruloplasmin (mg dL^{-1})
Normal	1.89 ± 0.14^{a}	22.93 ± 1.29^{a}
Normal rats + hyponidd (200 mg kg^{-1})	1.91 ± 0.12^{a}	26.41 ± 1.28^{a}
Diabetic control	3.81 ± 0.22^{b}	36.82 ± 1.82^{b}
Diabetic + hyponidd (100 mg kg^{-1})	2.57 ± 0.18^{c}	29.18 ± 1.44^{c}
Diabetic + hyponidd (200 mg kg^{-1})	2.07 ± 0.96^{ac}	24.71 ± 1.42^{ad}
Diabetic + glibenclamide (600 μg kg^{-1})	2.19 ± 0.03^{ac}	25.22 ± 1.29^{ad}

Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d) differ significantly at $P < 0.05$ (DMRT).

60 min. Even after 120 min, the blood glucose concentration in this group remained high. In normal and hyponidd treated groups, however, a decrease in blood glucose concentration was observed after 60, 90 and 120 min in diabetic rats.

The level of lipid peroxides viz. thiobarbituric acid reactive substances and hydroperoxides in plasma of normal and experimental rats is depicted in Table 5. Diabetic rats showed significantly ($P < 0.05$) increased levels of TBARS and hydroperoxides as compared with normal rats. Hyponidd-treated diabetic rats had significantly ($P < 0.05$) decreased levels of TBARS and hydroperoxides compared with untreated diabetic rats.

Plasma vitamin C and reduced glutathione levels of normal and experimental rats are shown in Table 6. Diabetic rats showed significantly ($P < 0.05$) diminished levels of plasma vitamin C and reduced glutathione as compared with normal rats. Hyponidd-treated groups showed a significant ($P < 0.05$) increase in plasma vitamin C and reduced glutathione compared with untreated diabetic rats.

The levels of plasma ceruloplasmin and α-tocopherol in normal and experimental rats are shown in Table 7. Diabetic rats showed significantly ($P < 0.05$) increased levels of ceruloplasmin and α-tocopherol as compared with normal rats. Hyponidd-treated groups showed a significant ($P < 0.05$) reduction in plasma ceruloplasmin and α-tocopherol compared with untreated diabetic rats.

For all the parameters studied, hyponidd-treated groups at doses of 100 and 200 mg kg^{-1} showed significant effects. The 200 mg kg^{-1} dose had a highly significant effect and brought back all the parameters to near normal levels. The effect of the 200 mg kg^{-1} hyponidd dose was better than glibenclamide. Oral administration of hyponidd (200 mg kg^{-1}) to normal rats had no significant effect.

Table 6 Effect of hyponidd on plasma reduced glutathione (GSH) and vitamin C in diabetic rats

Groups	GSH (mg dL^{-1})	Vitamin C (mg dL^{-1})
Normal	22.83 ± 0.98^{a}	1.62 ± 0.05^{a}
Normal rats + hyponidd (200 mg kg^{-1})	22.08 ± 1.29^{ab}	1.81 ± 0.19^{b}
Diabetic control	15.81 ± 0.65^{c}	0.98 ± 0.06^{c}
Diabetic + hyponidd (100 mg kg^{-1})	19.08 ± 0.83^{d}	1.28 ± 0.03^{d}
Diabetic + hyponidd (200 mg kg^{-1})	21.39 ± 0.97^{be}	1.52 ± 0.05^{ae}
Diabetic + glibenclamide (600 μg kg^{-1})	20.28 ± 0.70^{de}	1.43 ± 0.02^{de}

Each value is mean ± s.d. for 6 rats in each group. Values not sharing a common superscript (a,b,c,d,e) differ significantly at $P < 0.05$ (DMRT).

Discussion

Oral administration of hyponidd, an Ayurvedic herbo-mineral formulation, to streptozotocin-induced diabetic rats showed antihyperglycaemic and antioxidant effects.

Effect of hyponidd on blood glucose, plasma insulin and body weight

Diabetes mellitus causes a disturbance in the uptake of glucose as well as glucose metabolism. The use of a lower dose of STZ (45 mg kg^{-1}) produced an incomplete destruction of pancreatic β-cells even though the rats became permanently diabetic (Aybar et al 2001). After treatment with a low dose of STZ there should be many surviving β-cells, and regeneration is also possible (Gomes et al 1995). The increased levels of blood glucose in STZ-induced diabetic rats was lowered by hyponidd administration. The antihyperglycaemic action of hyponidd results from the potentiation of insulin from existing β-cells of the islets of Langerhans.

This is evident from the significant increase in plasma insulin concentration in STZ-diabetic rats. An increase in insulin secretion may lead to the inhibition of lipid peroxidation due to a decrease in blood glucose levels. As hyponidd comprises many antioxidant phytochemicals, these are also responsible for scavenging reactive oxygen species and inhibiting lipid peroxidation. In this context, Saravanan et al (2002) have also reported lowered levels of blood glucose by administration of Cogent db, an Ayurvedic herbal formulation in experimental diabetic rats.

Decrease in the body weight of diabetic rats is possibly due to dehydration and the catabolism of fats and proteins seen during diabetes mellitus (Hakim et al 1997). Rajkumar et al (1991) have also reported that increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats. Moreover, the increased secretion of insulin due to its anabolic effect may also result in increased synthesis of proteins. Oral administration of hyponidd partially improved the body weight in STZ-diabetic rats. This effect of hyponidd is due to its ability to reduce hyperglycaemia.

Oral administration of hyponidd showed a significant effect on orally administered glucose load in both normal and diabetic rats without inducing the hypoglycaemic state. The glucose load was well tolerated in hyponidd-treated groups. In this context, Diamed, an Ayurvedic herbal formulation, also had a significant effect on the oral glucose tolerance test in experimental diabetic rats (Pari et al 2001).

Effect of hyponidd administration on liver glycogen and total and glycosylated haemoglobin

The liver glycogen content was reduced in STZ-induced diabetic rats. The lack of insulin causes a decrease in the hepatic glycogen content in the diabetic state, which results in inactivation of the glycogen synthase system (Singh et al 2001). Oral administration of hyponidd significantly improved hepatic glycogen levels in STZ-diabetic rats, possibly because of the reactivation of the glycogen synthase system as a result of increased insulin secretion.

The lowered levels of total haemoglobin observed by us in diabetic rats might be due to the increased formation of glycosylated haemoglobin (Pari et al 2001). In diabetes, protein synthesis is decreased in all tissues due to relative insulin deficiency and thus the synthesis of haemoglobin is also suppressed (Chatterjee & Shinde 1994). Increased glycation of protein has been found to be a consequence of diabetic complications. A number of proteins, including haemoglobin, are glycated to a greater degree in diabetes (Alberti & Press 1982). Oral administration of hyponidd decreased the levels of glycosylated haemoglobin. This effect might be due to the lowering of blood glucose levels.

Effect of hyponidd on lipid peroxidation and plasma antioxidants

The increase in plasma TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defence mechanism to prevent the formation of excess free radicals. Increased levels of peroxides observed in plasma are due to the consequences of increased production, and liberation into the circulation, of lipid peroxides due to pathological changes. Oral administration of hyponidd lowered significantly the plasma TBARS and hydroperoxides in STZ-induced diabetic rats.

In diabetic rats, antioxidants in plasma are altered. The plasma protein ceruloplasmin is a powerful free radical scavenger that inhibits lipid peroxidation by binding to copper (Halliwell & Gutteridge 1990). Dormandy (1980) has shown that ceruloplasmin levels increase under conditions leading to the generation of oxygen products, such as the superoxide radical and hydrogen peroxide. The observed rise in plasma ceruloplasmin in diabetic rats might be due to increased lipid peroxides. The increase in ceruloplasmin levels is an indication of increased antioxidant defence to compensate for the loss of other antioxidants.

α-Tocopherol interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals and protects cell structures against damage by lipid hydroperoxides (Kinalski et al 2000). The elevated level of α-tocopherol observed in diabetic rats plays a protective role against increased peroxidation in diabetes mellitus. The increased level of vitamin E may be due to mobilization of vitamin E from adipose tissue to the liver in diabetes (Sukalski et al 1993). The increase may also be due to an alteration in the metabolism or storage of vitamin E by diabetic rats (Jain & Levine 1995).

Glutathione (GSH) is an important inhibitor of free-radical-mediated lipid peroxidation (Meister & Anderson 1983). We have observed a decrease in the level of plasma GSH in diabetic rats. It appears that generation of oxygen radicals by increased levels of glucose causes utilization of GSH and thus lowers GSH levels in the plasma in diabetic animals.

Vitamin C is an excellent plasma hydrophilic antioxidant because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species (Frei et al 1989). It functions as a free radical scavenger of active and stable oxyradicals. The observed decrease in plasma vitamin C might be due to increased utilization as an antioxidant defence against increased reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C (Wefers & Sies 1988). Previous studies have shown that under all types of oxidative stress, ascorbic acid successfully prevents detectable oxidative damage and helps to prevent diseases in which oxidative stress plays a causative or exacerbation role (Zhang & Omaye 2001).

In our study, we observed that the oral administration of hyponidd significantly restored the plasma antioxidants to near normal in STZ-induced diabetic rats. Phytochemical studies of the constituent plants of hyponidd (Table 2) reveal the presence of various alkaloids, flavonoids, sterols and polyphenolic compounds. The following plants in hyponidd exhibit antihyperglycaemic activity: *Momordica charantia* (Raman & Lau 1996), *Pterocarpus marsupium* (Manickam et al 1997), *Tinospora cordifolia* (Stanely Mainzen Prince & Menon 2003), *Gymnema sylvestre*

(Baskaran et al 1990), *Enicostemma littorale* (Murali et al 2002), *Eugenia jambolana* (Grover et al 2000), *Cassia auriculata* (Pari & Latha 2002) and *Emblica officinalis* (Anila & Vijayalakshmi 2000). The antioxidant effect of hyponidd is also due to the presence of *Swertia chirata* (Scartezzini & Speroni 2000), *Melia azadirachta* (Govindachari 1992), *Tinospora cordifolia* (Stanely Mainzen Prince & Menon 1999), *Emblica officinalis* (Bhattacharya et al 1999), *Cassia auriculata* (Kumar et al 2003) and *Curcuma longa* (Ammon & Wahl 1991).

Conclusion

Our findings show that oral administration of hyponidd has antihyperglycaemic and antioxidant effects in streptozotocin-induced diabetic rats. The antioxidant phytochemicals present in the various plant constituents of hyponidd scavenge free radicals and prevent the depletion of endogenous antioxidants. An increase in insulin levels improves the blood glucose levels and thus hyponidd also exhibits antihyperglycaemic activity.

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