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## Restoration of Altered Carbohydrate and Lipid Metabolism by Hyponidd, a Herbomineral Formulation in Streptozotocin-Induced Diabetic Rats

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**Abstract:** The purpose of this study was to evaluate the influence of Hyponidd, a herbomineral formulation and its effect on key enzymes of glucose metabolism and lipid metabolism in STZ-induced diabetic rats. Female Wistar rats with a body weight of 180-200 g were used in this study. The rats were divided into seven groups of six rats each after the induction of STZ-diabetes: normal rats; normal rats given Hyponidd (200 mg kg<sup>-1</sup> b. wt.); diabetic control; diabetic rats given Hyponidd (50 mg kg<sup>-1</sup> b. wt.); diabetic rats given Hyponidd (100 mg kg<sup>-1</sup> b. wt.); diabetic rats given Hyponidd (200 mg kg<sup>-1</sup> b. wt.); diabetic rats given glibenclamide (600 µg kg<sup>-1</sup> b. wt.). After 45 days of treatment, fasting blood glucose, plasma insulin, serum and tissue carbohydrate metabolizing enzymes and lipid profiles were determined in normal and streptozotocin induced-diabetic rats. Oral administration of Hyponidd for 45 days resulted in significant (p<0.05) reduction in blood glucose, serum and tissue glucose-6-phosphatase, fructose-1, 6-bis phosphatase, total cholesterol, triglyceride and free fatty acids level. At the same time there was a significant increase in the levels of plasma insulin, hexokinase and HDL cholesterol in streptozotocin induced-diabetic rats. The effect of Hyponidd was compared with an oral hypoglycaemic agent, glibenclamide. Present study shows, that Hyponidd significantly restored the altered carbohydrate and lipid metabolism by exerting a beneficial action against secondary complications associated with diabetes mellitus.

**Key words:** Medicinal plants, streptozotocin, diabetes, carbohydrate, metabolism, hyponidd

### INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. These metabolic disorders include alterations in the carbohydrate, lipid and protein metabolism associated with the lack of insulin secretion or insulin action (Schoenfelder *et al.*, 2006). The influence of diabetes mellitus on lipid metabolism is well established. The association of hyperglycemia and alteration of lipid levels present a major risk of cardiovascular diseases (Pushparaj *et al.*, 2007). Cardiovascular disorders continue to be a major cause of morbidity and mortality. Mortality from cardiovascular abnormalities, including hypertension, atherosclerosis and congestive heart failure, is almost three times more prevalent in the diabetic populations than in the non-diabetic populations (Ortiz-Andrade *et al.*, 2007). Diabetes mellitus affects the lipid metabolism (Prince *et al.*, 2004). Hyperlipidemia is an important risk factor in the initiation and progression of atherosclerotic lesion. The beneficial effect of lowering elevated serum cholesterol level in the prevention of coronary heart disease is well established (Subash Babu *et al.*, 2007).

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In recent years, there has been renewed interest in the treatment against different diseases using herbal drugs as they are generally non-toxic (Momin, 1987). Many indigenous drugs have been used by practitioners of Ayurvedic system for the treatment of diabetes mellitus in India. The WHO has also recommended the evaluation of the effectiveness of plants in conditions where we lack safe modern drugs (Pari and Sathesh, 2006). A hypoglycemic action from some treatments has been confirmed in animal models and non-insulin-dependent diabetic patients and various hypoglycemic compounds have been identified. A botanical substitute for insulin seems unlikely, but traditional treatments may provide valuable clues for the development of new oral hypoglycemic agents and simple dietary adjuncts (Bailey and Day, 1989). A wide array of plant derived active principles representing numerous chemical compounds has demonstrated activity consistent with their possible use in the treatment of diabetes mellitus. Many kinds of natural products, such as alkaloids, glycosides, polysaccharides, peptidoglycans, flavonoids, tannins, steroids, glycopeptides, terpenoids and inorganic ions have been identified. The introduction of these indigenous herbal compounds in the management of diabetes mellitus will greatly simplify the management and make it less expensive. In traditional Indian medicine, plant formulation and several cases of combined extracts of plants are used as drugs of choice rather than individual active principle or plant. Many herbal formulations such as Trasina (Bhattacharya *et al.*, 1997), Diamed (Pari *et al.*, 2001), Diasulin (Saravanan and Pari, 2005), Cogent db (Saravanan, 2002) and D-400 (Mitra *et al.*, 1996) exhibit antidiabetic effect.

Hyponidd is a herbomineral formulation composed of eleven medicinal plants. The plants used in hyponidd formulation are known to possess antidiabetic, antihyperlipidaemic and antioxidants effects (Table 1) and have been used in indigenous system of medicine to cure diabetes (Subash Babu and Prince, 2004). In view of the above facts, the present study was undertaken to evaluate the effect of

Table 1: Name of plants, composition, their phytochemicals and effects of the constituents of Hyponidd (Subash Babu and Prince, 2004)

Plant species	Concentration (mg Tablet <sup>-1</sup> )	Phytochemicals	Effects observed
<i>Momordica charantia</i>	12	Charantin, momordicosides A and B, acylglucosyl sterols, P-insulin, V-insulin and stigmaterol	Antidiabetic effect
<i>Swertia chirata</i>	15	Poly oxygenated xanthenes, mangiferin, swertinin, swertianin, swerchirin, chiratin and chirataini	Antioxidant effect
<i>Melia azadirachta</i>	75	$\beta$ -carotene, nimbin, azadirachtin, nimbidiol, quercetin, nimbidin and nimbatiktam	Antioxidant effect
<i>Pterocarpus marsupium</i>	75	Pteroside, pteroisoauroside, marsuposide, vijayosin, sesquiterpene; pterosupin, pterostilbene and marsupin	Antidiabetic effect
<i>Tinospora cordifolia</i>	75	Tinosporin, isocolumbin, palmatine, tinocordiside (10), tinocordifolioside (11), cordioside and $\beta$ -sitosterol	Antidiabetic and antihyperlipidemic effect
<i>Gymnema sylvestre</i>	112.5	Gymnemic acids, saponins, stigmaterol, quercitol, betaine, choline and trimethylamine	Antidiabetic effect
<i>Encostemma littorale</i>	112.5	Vanillic acid, ferulic acid, p-coumaric acid, apigenin, genkwanin, isovitexin, swertisin and saponarin	Antidiabetic effect
<i>Emblica officinalis</i>	150	Phyllembin, gallic acid, ellagic acid, phyllantidine, phyllantine, lupeol, emblicanin A and B	Antioxidant effect
<i>Eugenia jambolana</i>	150	Gallic acid, ellagic acid, corilagin, ellagitanmins and quercetin	Antidiabetic effect
<i>Cassia auriculata</i>	225	Flavonoids, anthracene derivatives, dimeric procyanidins, myristyl alcohol, $\beta$ -D-glucoside, quercetin 3-O-glycoside and rutin	Antidiabetic and antioxidant effect
Curcuma longa	300	Curcumin, desmethoxy curcumin, bisdemethoxy curcumin, dihydrocurcumin, $\alpha$ and $\beta$ -turmerones, eugenol, campesterol and stigmaterol	Antioxidant effect
<b>Powders</b>			
Yashad bhasma (Zinc calx)	37.5		Used in the treatment of diabetes mellitus in Ayurvedic system
Aspaltham	37.5		

hyponidd on blood glucose, serum and tissue (liver and kidney) carbohydrate metabolizing enzymes (Hexokinase, Glucose-6-phosphatase and Fructose-1, 6-bisphosphatase) and serum HDL, tissue cholesterol, triglycerides (TGs) and free fatty acids (FFAs). The effect of hyponidd was compared with glibenclamide, a well known hypoglycemic drug. In our earlier study, we have shown that Hyponidd exhibits antihyperglycaemic and antioxidant effect in streptozotocin-induced diabetic rats (Subash Babu and Prince, 2004).

## MATERIALS AND METHODS

### Animals

Female Wistar rats with a body weight of 180-200 g were procured from the central Animal House, Raja Muthiah Medical College and Hospital, Annamalai University in October 2005. The animals were fed on standard pellet diet (Hindustan Lever, India) and water *ad libitum*.

### Test Drug and Chemicals

Hyponidd, a herbomineral formulation was purchased from recognized pharmacy, Cuddalore District, manufactured by Charak Pharmaceuticals (I) Private Ltd., Haryana, India. Streptozotocin was purchased from Sigma chemical company Inc. (St. Louis, MO) USA. All other biochemicals and chemicals used in this experiment are of analytical grade.

### Experimental Induction of Diabetes

The rats were injected intraperitoneally with freshly prepared streptozotocin (45 mg kg<sup>-1</sup> body weight) in 0.1 M citrate buffer (pH-4.5) in a volume of 1 mL kg<sup>-1</sup> b. wt. (Subash Babu and Prince, 2004). After 48 h of streptozotocin administration, blood glucose levels of each rat were determined. Rats with blood glucose range of 250-300 mg/100 mL were considered diabetic and included in the study. Blood was collected from the eyes (venous pool).

### Experimental Design and Drug Administration

In the experiment, a total of 42 rats (12 normal, 30 STZ-diabetic surviving rats) were used. The rats were divided into 7 groups of six each. Hyponidd tablets were powdered, suspended in distilled water and different doses of hyponidd were administered orally using an intragastric tube. Group 1: Normal untreated rats; Group 2: Normal rats treated with Hyponidd orally (200 mg kg<sup>-1</sup> b. wt.) in distilled water using an intragastric tube for 45 days; Group 3: Streptozotocin treated diabetic control rats; Groups 4: STZ treated diabetic rats administered with hyponidd (50 mg kg<sup>-1</sup> b. wt.) orally in distilled water using an intragastric tube for 45 days; Group 5: STZ treated diabetic rats administered with hyponidd (100 mg kg<sup>-1</sup> b. wt.) orally in distilled water using an intragastric tube for 45 days; Group 6: STZ treated diabetic rats administered with Hyponidd (200 mg kg<sup>-1</sup> b. wt.) orally in distilled water using an intragastric tube for 45 days; Group 7: STZ treated diabetic rats given glibenclamide orally (600 µg kg<sup>-1</sup> b.wt.) in distilled water using an intragastric tube for 45 days (Subash Babu and Prince, 2004).

All doses were started after two days of STZ injection. No detectable irritation or restlessness was observed after each drug or vehicle administration. No noticeable adverse effect (i.e., respiratory distress, abnormal locomotion and catalepsy) was observed in any animal after the drug administration. Blood glucose levels were estimated every week to ascertain the diabetes status in different groups of rats. At the end of 45 days, all the rats were killed by decapitation (Pentobarbitone sodium) anesthesia (60 mg kg<sup>-1</sup>). Blood was collected in two different tubes (i.e.,) one with whole blood for serum separation and another with anticoagulant- potassium oxalate and sodium fluoride for plasma insulin assay. Liver and kidney were dissected out, washed in ice-cold saline and weighed.

### Biochemical Analysis

**Estimation of Blood Glucose and Plasma Insulin:** Fasting blood glucose was estimated by the o-toluidine method of Sasaki *et al.* (1972). Plasma insulin was performed by the ELISA method using a Bhoehringer Mannheim Kit (Bhoehringer analyzer ES 300), Mannheim, Germany.

### Assay of Carbohydrate Metabolizing Enzymes

Hexokinase was assayed by the method of Brandstrup *et al.* (1957). The activity of Glucose-6-phosphatase and Fructose-1, 6-bisphosphatase were assayed according to the method of Koide and Oda (1959) and Gancedo and Gancedo (1971) and the inorganic phosphate (Pi) liberated was estimated by the method of Fiske and Subba Row (1925).

### Estimation of Lipid Profiles

Lipids were extracted from the tissues by the method of Folch *et al.* (1957), Serum and tissue total cholesterol (Zak *et al.*, 1953), triglycerides (Rice, 1970), free fatty acids (Falholt *et al.*, 1973) and serum HDL-cholesterol (Growenlock, 1988) were estimated.

### Statistical Analysis

Statistical analysis was performed using SPSS software package, version 6.0. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Duncan, 1957). All the results were expressed as Mean±SD for six rats in each group; p-values <0.05 were considered as significant.

## RESULTS AND DISCUSSION

Figure 1 shows the levels of fasting blood glucose and plasma insulin in normal and STZ-induced diabetic rats. Diabetic rats showed an increase in blood glucose and a decrease in plasma insulin compared to normal rats. Administration of Hyponidd brought back the levels of blood glucose and insulin to near normal. Hyponidd treated groups at doses of 100 and 200 mg kg<sup>-1</sup> b. wt. significantly decreased the blood glucose levels and the lower dose (50 mg kg<sup>-1</sup> b. wt.) did not shown any significant effect in STZ-diabetic rats. Two hundred milligram per kilogram dose showed a highly significant effect and brought back all the parameters to near normal levels. The effect at a dose of 200 mg kg<sup>-1</sup> was better than glibenclamide. Therefore, 100 and 200 mg kg<sup>-1</sup> b. wt. doses were selected for further biochemical studies. Oral administration of Hyponidd (200 mg kg<sup>-1</sup>) to normal rats did not show any deleterious effect.

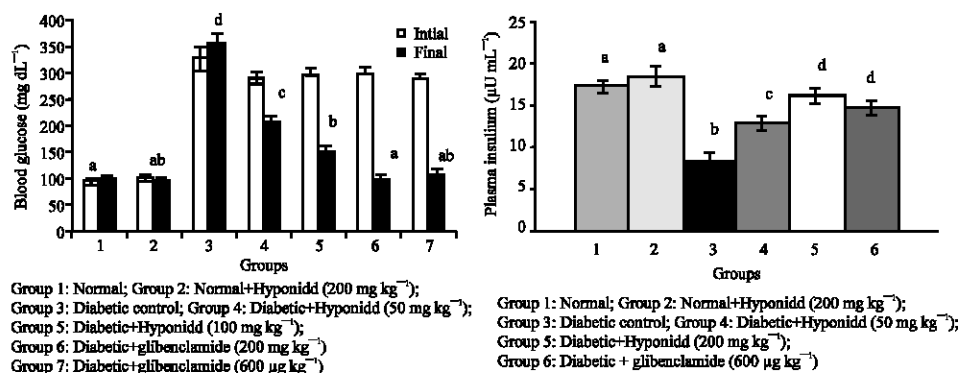
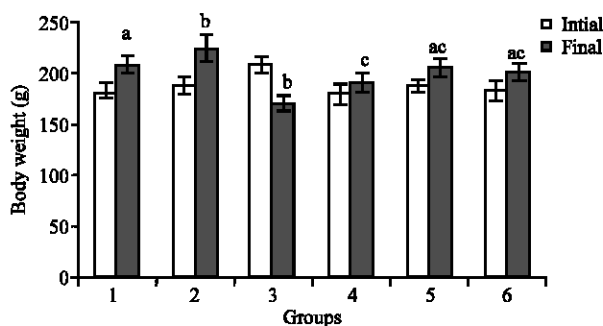


Fig. 1: Effect of hyponidd on blood glucose and plasma insulin levels in normal and diabetic rats, Each value is Mean±SD for 6 rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT)



Group 1: Normal; Group 2: Normal+Hyponidd ( $200 \text{ mg kg}^{-1}$ );  
Group 3: Diabetic control; Group 4: Diabetic+Hyponidd ( $50 \text{ mg kg}^{-1}$ );  
Group 5: Diabetic+Hyponidd ( $200 \text{ mg kg}^{-1}$ );  
Group 6: Diabetic+Glíbenclamide ( $600 \text{ µg kg}^{-1}$ )

Fig. 2: Effect of hyponidd on change of body weight in normal and diabetic rats, Each value is Mean $\pm$ SD for 6 rats in each group. Values not sharing a common superscript differ significantly at  $p < 0.05$  (DMRT)

Table 2: Effect of hyponidd on the activities of serum, hepatic and renal hexokinase, glucose-6-phosphatase and fructose-1, 6-bisphosphatase in normal and diabetic rats

Groups	Normal	Normal+ Hyponidd ( $200 \text{ mg kg}^{-1}$ )	Diabetic control	Diabetic+ Hyponidd ( $50 \text{ mg kg}^{-1}$ )	Diabetic+ Hyponidd ( $200 \text{ mg kg}^{-1}$ )	Diabetic+ glíbenclamide ( $600 \text{ µg kg}^{-1}$ )
<b>Serum</b>						
Hexokinase	0.14 $\pm$ 0.005 <sup>a</sup>	0.17 $\pm$ 0.007 <sup>b</sup>	0.05 $\pm$ 0.003 <sup>c</sup>	0.08 $\pm$ 0.004 <sup>b</sup>	0.12 $\pm$ 0.005 <sup>a</sup>	0.10 $\pm$ 0.004 <sup>a</sup>
Glucose-6-phosphatase	0.24 $\pm$ 0.05 <sup>a</sup>	0.26 $\pm$ 0.02 <sup>ad</sup>	0.48 $\pm$ 0.03 <sup>b</sup>	0.30 $\pm$ 0.02 <sup>c</sup>	0.27 $\pm$ 0.01 <sup>ad</sup>	0.29 $\pm$ 0.02 <sup>d</sup>
Fructose-1,6-bisphosphatase	0.34 $\pm$ 0.02 <sup>a</sup>	0.35 $\pm$ 0.03 <sup>a</sup>	0.71 $\pm$ 0.03 <sup>b</sup>	0.46 $\pm$ 0.02 <sup>c</sup>	0.39 $\pm$ 0.02 <sup>d</sup>	0.42 $\pm$ 0.03 <sup>d</sup>
<b>Liver</b>						
Hexokinase	116.48 $\pm$ 5.26 <sup>a</sup>	122.80 $\pm$ 4.08 <sup>b</sup>	61.81 $\pm$ 2.39 <sup>c</sup>	72.67 $\pm$ 2.64 <sup>d</sup>	96.52 $\pm$ 4.29 <sup>e</sup>	91.30 $\pm$ 4.59 <sup>e</sup>
Glucose-6-phosphatase	15.28 $\pm$ 0.98 <sup>a</sup>	13.75 $\pm$ 0.92 <sup>a</sup>	34.41 $\pm$ 2.34 <sup>b</sup>	23.50 $\pm$ 1.50 <sup>c</sup>	18.03 $\pm$ 1.01 <sup>d</sup>	19.01 $\pm$ 0.71 <sup>d</sup>
Fructose-1,6-bisphosphatase	6.92 $\pm$ 0.41 <sup>a</sup>	7.82 $\pm$ 0.68 <sup>a</sup>	17.36 $\pm$ 0.89 <sup>b</sup>	11.82 $\pm$ 0.71 <sup>c</sup>	8.91 $\pm$ 0.51 <sup>a</sup>	9.28 $\pm$ 0.84 <sup>a</sup>
<b>Kidney</b>						
Hexokinase	98.46 $\pm$ 5.06 <sup>ab</sup>	102.50 $\pm$ 4.08 <sup>a</sup>	62.80 $\pm$ 2.94 <sup>c</sup>	73.52 $\pm$ 3.67 <sup>d</sup>	96.61 $\pm$ 4.50 <sup>ab</sup>	94.32 $\pm$ 4.73 <sup>bc</sup>
Glucose-6-phosphatase	11.53 $\pm$ 0.35 <sup>a</sup>	12.28 $\pm$ 1.29 <sup>a</sup>	24.28 $\pm$ 1.44 <sup>b</sup>	18.03 $\pm$ 0.84 <sup>c</sup>	12.43 $\pm$ 0.47 <sup>a</sup>	14.51 $\pm$ 0.82 <sup>d</sup>
Fructose-1,6-bisphosphatase	10.70 $\pm$ 0.62 <sup>a</sup>	8.58 $\pm$ 0.75 <sup>b</sup>	24.89 $\pm$ 1.82 <sup>c</sup>	18.94 $\pm$ 0.91 <sup>d</sup>	11.08 $\pm$ 0.71 <sup>a</sup>	12.07 $\pm$ 0.48 <sup>a</sup>

Units: Hexokinase: mmoles of glucose phosphorylated/hr/mg protein. Glucose-6-phosphatase:  $\mu\text{mole of Pi liberated/min/mg protein}$ . Fructose-1, 6-bisphosphatase:  $\mu\text{mole of Pi liberated/min/mg protein}$ . Each value is Mean $\pm$ SD for 6 rats in each group. Values not sharing a common superscript differ significantly at  $p < 0.05$  (DMRT)

Changes in the body weight measured in normal and STZ-induced diabetic rats are shown in Fig. 2. In STZ-treated diabetic rats, body weight decreased compared to normal rats. Hyponidd treated groups significantly ( $p < 0.05$ ) increased body weight in diabetic rats.

Table 2 shows the effect of hyponidd on the activities of hexokinase, glucose-6-phosphatase and fructose 1, 6-bisphosphatase in serum, liver and kidney in normal and STZ-induced diabetic rats. The activity of hexokinase decreased while the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase increased in diabetic rats compared to normal rats. Oral administration of Hyponidd ( $200 \text{ mg kg}^{-1}$ ) for 45 days showed a significant effect ( $p < 0.05$ ) and brought back all the activities of enzymes to near normal in diabetic rats.

Table 3 shows the levels of serum and tissue lipids in normal and STZ-induced diabetic rats. There was a significant decrease in the level of serum HDL-cholesterol and a significant increase in the levels of total cholesterol, triglycerides and free fatty acids in diabetic rats compared to normal rats. Administration of Hyponidd brought back the levels of serum lipids significantly ( $p < 0.05$ ) to near normal.

Table 3: Effect of hyponidd on serum HDL-cholesterol, serum and tissue total cholesterol, triglycerides and free fatty acids in normal and diabetic rats

Groups	Normal	Normal+ Hyponidd (200 mg kg <sup>-1</sup> )	Diabetic control	Diabetic+ Hyponidd (50 mg kg <sup>-1</sup> )	Diabetic+ Hyponidd (200 mg kg <sup>-1</sup> )	Diabetic+ glibenclamide (600 µg kg <sup>-1</sup> )
<b>Serum (mg dL<sup>-1</sup>)</b>						
HDL-Cholesterol	58.60±3.80 <sup>a</sup>	56.90±4.00 <sup>a</sup>	31.40±2.90 <sup>b</sup>	47.30±3.50 <sup>c</sup>	55.7±3.60 <sup>d</sup>	51.20±3.40 <sup>d</sup>
Total cholesterol	82.48±3.50 <sup>a</sup>	88.36±4.96 <sup>a</sup>	240.60±12.0 <sup>b</sup>	163.10±6.05 <sup>c</sup>	108.1±3.23 <sup>a</sup>	123.80±5.02 <sup>d</sup>
Triglyceride	12.60±0.60 <sup>a</sup>	14.29±1.29 <sup>a</sup>	36.83±1.82 <sup>b</sup>	26.90±0.83 <sup>c</sup>	11.7±0.62 <sup>ad</sup>	13.89±0.74 <sup>ad</sup>
Free fatty acids	55.80±2.64 <sup>a</sup>	61.26±5.47 <sup>a</sup>	130.60±8.42 <sup>b</sup>	86.70±6.40 <sup>c</sup>	60.9±3.76 <sup>a</sup>	68.29±4.51 <sup>a</sup>
<b>Liver (mg g<sup>-1</sup> wet tissue)</b>						
Total cholesterol	7.64±0.62 <sup>a</sup>	8.91±0.64 <sup>ac</sup>	13.84±0.70 <sup>b</sup>	9.70±0.54 <sup>c</sup>	8.4±0.34 <sup>ac</sup>	8.75±0.32 <sup>ac</sup>
Triglyceride	5.28±0.31 <sup>a</sup>	6.21±0.52 <sup>ab</sup>	12.81±0.72 <sup>c</sup>	8.40±0.51 <sup>d</sup>	6.3±0.43 <sup>ab</sup>	7.10±0.38 <sup>b</sup>
Free fatty acids	7.24±0.59 <sup>a</sup>	8.91±0.64 <sup>a</sup>	21.32±1.50 <sup>b</sup>	13.90±0.93 <sup>c</sup>	9.2±0.62 <sup>a</sup>	10.20±0.58 <sup>a</sup>
<b>Kidney (mg g<sup>-1</sup> wet tissue)</b>						
Total cholesterol	4.11±0.25 <sup>a</sup>	5.28±0.43 <sup>b</sup>	10.24±0.60 <sup>c</sup>	7.43±0.41 <sup>d</sup>	5.3±0.37 <sup>b</sup>	5.72±0.42 <sup>b</sup>
Triglyceride	3.60±0.22 <sup>a</sup>	4.27±0.28 <sup>a</sup>	7.90±0.43 <sup>b</sup>	5.21±0.31 <sup>c</sup>	4.1±0.20 <sup>a</sup>	4.80±0.14 <sup>a</sup>
Free fatty acids	16.53±0.57 <sup>a</sup>	18.22±0.81 <sup>a</sup>	32.53±1.82 <sup>b</sup>	22.30±1.06 <sup>c</sup>	18.9±0.81 <sup>a</sup>	19.30±0.63 <sup>a</sup>

Each value is Mean±SD for 6 rats in each group, Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

The present study revealed the antidiabetogenic effect of hyponidd, a herbomineral formulation, on streptozotocin-induced diabetic rats. Streptozotocin injection resulted in diabetes mellitus, which may be due to the destruction of  $\beta$ -cells of islets of langerhans as reported by others (Kavalali *et al.*, 2002). After injection with a low dose of STZ (45 mg kg<sup>-1</sup>), there should be many surviving  $\beta$ -cells (Gomes *et al.*, 2001). The increased levels of blood glucose in STZ-induced diabetic rats were lowered by Hyponidd administration. The antihyperglycaemic action of Hyponidd results from the potentiation of insulin from existing  $\beta$ -cells of the islets of langerhans or its release from bound insulin (Subash Babu and Prince, 2004).

Glycolysis and gluconeogenesis are the two prime complementary events balancing the glucose load in our body. Hexokinase, glucose-6-phosphatase and Fructose-1, 6-bisphosphatase are the key enzymes of glucose metabolism. They are markedly altered to produce hyperglycemia, which leads to the pathogenesis of diabetes complications (Prasad *et al.*, 2005). The present study was carried out to determine the effect of Hyponidd on the key enzymes involved in carbohydrate metabolism in STZ-diabetic rats. Diabetes mellitus is characterized by partial or total deficiency of insulin resulting in the derangement of carbohydrate metabolism and a decrease in enzymatic activity of hexokinase and increase in the activities of gluconeogenic enzymes.

Hexokinase is the first regulatory enzyme of glycolytic pathway. The activity of this enzyme is decreased in STZ-induced diabetic rats. Oral administration of hyponidd increases the activity of hexokinase. The increased activity of hexokinase can cause increased glycolysis and increased utilization of glucose for energy production (Prince *et al.*, 1997). The lowered levels of blood glucose after Hyponidd treatment in diabetic rats might be due to increased glycolysis, i.e., increased hexokinase activity. The activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase (gluconeogenic enzymes) were elevated in serum, liver and kidney of STZ-induced diabetic rats (Grover *et al.*, 2000). These enzymes were activated in insulin deficiency but under normal state, insulin function as a suppressor of gluconeogenic enzymes (Baquer *et al.*, 1988). Glucose-6-phosphatase plays an important role in glucose homeostasis in liver and kidney (Berg *et al.*, 2001). Administration of Hyponidd was found to restore the activities of these enzymes to near normal levels in STZ-induced diabetic rats. The increased fructose 1, 6-bisphosphatase activity might be due to the change in the allosteric effectors of the enzymes namely Fructose-2, 6-bisphosphate, ATP, AMP and citrate. In diabetic state, there is more lipolysis and lipogenesis, which results in the formation of more AMP and lower utilization of citrate for lipogenesis leading to higher energy state in the cell. Higher concentration of ATP is more favorable for Fructose-1, 6-bisphosphatase activation (Baquer *et al.*, 1988). Administration of Cogent db an Ayurvedic herbal formulation also has been reported to increase

the activity of hexokinase and decrease the activities of both Glucose-6-phosphatase and Fructose-1,6-bisphosphatase in experimental rats (Saravanan *et al.*, 2002).

Lipids play a vital role in the pathogenesis of diabetes mellitus. The most common lipid abnormalities in diabetes are hypertriglyceridaemia and hypercholesterolemia (Mitra *et al.*, 1996). In this study, we have noticed elevated levels of serum lipids such as cholesterol, triglycerides and free fatty acids in diabetic rats. The levels of increased serum lipids in diabetes represent a risk factor for coronary heart disease (Shirwaikar *et al.*, 2005). Under normal circumstances, insulin activates lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzyme thereby causing hypertriglyceridaemia. In insulin deficient diabetes, the concentration of serum free fatty acids get elevated as a result of free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification-triglyceride lipolysis cycle is displaced in favor of lipolysis (Pari and Latha, 2002; Shirwaikar *et al.*, 2004). Administration of Hyponidd lowers serum lipids in diabetic rats.

We have observed a decrease in serum HDL in diabetic rats. HDL is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissues into the liver and thereby acts as a protective factor against coronary heart disease (Pari and Saravanan, 2002). The level of HDL cholesterol which increased after Hyponidd administration might be due to the increase in the activity of lecithin: cholesterol acyl transferase (LCAT), which may contribute to the regulation of blood lipids (Patil *et al.*, 2004).

We have also observed an increase in the levels of tissue (liver and kidney) cholesterol, triglycerides and free fatty acids in STZ-induced diabetic rats. These observations are in line with other reports (Krishnakumar *et al.*, 2000). Hyperlipidaemia is a recognized consequence of diabetes mellitus (Sharma *et al.*, 1996), demonstrated by the elevated levels of tissue cholesterol, triglycerides and free fatty acids (Saravanan *et al.*, 2002). Administration of Hyponidd normalized the tissue lipid levels in diabetic rats. Diabetes-induced hyperlipidaemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of glucose (Krishnakumar *et al.*, 2000). The regression of the diabetic state on Hyponidd administration increases the utilization of glucose, thereby depressing the mobilization of fat.

In the present study, Hyponidd, a herbomineral formulation significantly restored the altered carbohydrate metabolizing enzymes and lipid profiles in STZ-induced diabetic rats. Studies have shown that the constituents of Hyponidd such as *Momordica charantia* (Ahmed *et al.*, 2001), *Pterocarpus marsupium* (Jahromi and Ray, 1993), *Tinospora cordifolia* (Prince and Menon, 2003), *Gynemema sylvestra* (Baskaran, 1990), *Embllica officinalis* (Mathur *et al.*, 1996) and *Eugenia jambolana* (Sharma *et al.*, 2003) possess antidiabetic and antihyperlipidaemic effect in diabetic animals. On the basis of above results, it could be concluded that Hyponidd, a combination of eleven herbal plants exert a significant antidiabetic and antihyperlipidemic effect. This could be due to different types of active principles, each with a single or a diverse range of biological activities, which serves as a good adjuvant in the present armamentarium of antidiabetic drug.

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