DOI: 10.5567/pharmacologia.2013.48.52

Prokinetic Effect of Hyponidd, a Herbomineral Formulation in STZ-Induced Diabetic Rats

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ABSTRACT

Diabetes associated gastrointestinal complications have more common as the rate of diabetes has increased. The present study was undertaken to investigate, prokinetic effect of Hyponidd, a herbomineral formulation (HMF) in streptozotocin induced diabetic rats. Diabetes was induced by single intraperitoneal administration of STZ (55 mg kg^{-1}) in rats. The diabetic rats, after receiving two weeks of drug treatment were screened for Gastric Emptying (GE), Intestinal Transit (IT) and *in vitro* study of distal colonic smooth muscle. Two weeks of treatment with HMF (100 and 200 mg kg^{-1} p.o.) in diabetic rats showed significant restoration of impaired gastric emptying and intestinal transit. Furthermore, there was a significant decrease in EC_{50} on rat colon. Therefore, it may be concluded that Hyponidd exert prokinetic effect and has therapeutic potential in diabetic gastroparesis and related gastrointestinal impairments.

Key words: Diabetic gastroparesis, gastric emptying, hyponidd, metformin, intestinal transit

Pharmacologia 4 (1): 48-52, 2013

INTRODUCTION

Impaired gastric motility and Intestinal Transit (IT) frequently occurs in people suffering from diabetes mellitus (Waseem *et al*., 2009). About 50% of patients, with insulin or non-insulin dependent diabetes have delayed gastric emptying. Autonomic nerve dysfunctions and peripheral neuropathy are mainly responsible for diabetes induced gastroparesis. Delayed gastric emptying is usually attributed to vagal damage, occurring as part of a common symptom of diabetic autonomic neuropathy (Kong *et al*., 1999). Further, it causes a decrease in fundic and antral motor activity, a reduction or a lack of the inter-digestive migrating motor complex, gastric dysrhythmias and pylorospasms. Diabetic peripheral neuropathy has been considered as a primary nervous system complication associated with diabetes (Rahul *et al*., 2012). Further, several investigations suggest oxidative stress is responsible for the damage of peripheral cholinergic neurons (Tembhurne and Sakarkar, 2011).

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Hyponidd, a herbomineral formulation contains 11 herbs (*Momordica charantia, Swertia chirata, Melia azadirachta, Pterocarpus marsupium, Tinospora cordifolia, Gymnema sylvestre, Enicostemma littorale, Emblica officinalis, Eugenia jambolana, Cassia auriculata, Curcuma longa)* and 2 minerals (Aspaltham, Yashadbhasma). Some of these ingredients have been reported to possess either anti-diabetic or antioxidant, or both the activities. Therefore, the present investigation was undertaken to evaluate the prokinetic effect of *Hyponidd* on impaired gastrointestinal motility and colonic smooth muscle response to exogenous acetylcholine (ACh) (Table 1).

MATERIAL AND METHODS

Drug and chemicals: Hyponidd (Charak Pharmaceuticals Pvt. Ltd, India) was purchased from local market at Pune, Metformin was obtained as a gift sample (Cipla Pharmaceuticals, India). Streptozotocin (Sigma Aldrich, USA), Diagnostic kits (Biolab, India) and other chemicals used were of analytical grade (Qualigens, India) were purchased from the local suppliers.

Table 1: Contents of herbomineral formulation, Hyponidd

Botanical name	Common name	Qty. (mg)	Activity reported
Momordica charantia	*Karavellaka*	12.0	Antidiabetic (Raman and Lau, 1996)
Purified aspaltham	*Shilajit*	37.5	Antidiabetic (Babu and Prince, 2004)
Yashadbhasma	*Zinc calyx*	37.5	Hypoglycemic (Prasad and Sharma, 1989)
Swertia chirata	*KiratTikta*	15.0	Antioxidant (Scartezzini and Speroni, 2000)
Melia azadirachta	*Neem*	75.0	Antioxidant (Govindachari, 1992)
Pterocarpus marsupium	*Vijaysaar*	75.0	Antidiabetic (Maniscka*m et al*., 1997)
Tinospora cordifolia	*Guduchi*	75.0	Antidiabetic (Stanely and Menon 2003), Antioxidant (Prince and Menon, 1999).
Gymnema sylvestre	*Meshashringi*	112.5	Antidiabetic (Baskaran *et al*., 1990)
Enicostemma littorale	*Mamejavo*	112.5	Antidiabetic (Murali *et al*., 2002)
Emblica officinalis	*Amalaki*	150.0	Antioxidant (Bhattacharya *et al*., 1999)
Eugenia jambolana	*Raja Jambu*	150.0	Antidiabetic (Grover *et al*., 2000)
Cassia auriculata	*Tarwar*	225.0	Antidiabetic (Pari and Latha, 2002), Antioxidant (Rajagopal *et al*., 2003)
Curcuma longa	*Haridra*	300.0	Antioxidant (Ammon and Wahl, 1991)

Animal: Wistar rats of either sex weighing 180-230 g were purchased from Intox Pvt. Ltd, Pune. Animals were housed under standard laboratory conditions at controlled temperature 25 ±1°C with a relative humidity 50-60% ±5% in 12 h light and dark cycle and had free access to water and standard laboratory feed *ad libitum*. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/Approval/2009-10/09) by the Institutional Animal Ethics Committee.

Experimental design: Overnight fasted rats were injected intraperitoneally, with freshly prepared streptozotocin (55 mg kg^{-1}) dissolved in 0.1 M cold citrate buffer (pH 4.45). The 48 h after the STZ-injection, blood was withdrawn by retro orbital method, serum glucose level was determined using GOD/POD method (Miskiewicz *et al*., 1973). Rats with a serum glucose level ≥250 mg dL^{-1} were considered as diabetic and used for further study.

Age matched normal rats were used as a normal control and received distilled water (5 mL kg^{-1}) (Group I). After two weeks of persistent hyperglycemia, diabetic rats were divided into four groups and received treatment for two weeks, diabetic control rats (Group II) were received distilled water (5 mL kg^{-1}) while, Group III and IV animals were received suspension of Hyponidd (100 and 200 mg kg^{-1}, p.o.) prepared in distilled water. Metformin was administered at a dose of 200 mg kg^{-1}, p.o. (Group V). At the end of two weeks of treatment gastric emptying, intestinal transit and *in-vitro* study were performed on rat distal colon.

Blood sampling: Blood was collected from retro-orbital plexus after rats were subject to light anesthesia and serum was extracted for the determination of blood glucose and oxidative biomarkers.

Blood glucose estimation: Serum glucose level was estimated by GOD/POD method (Trinder, 1969).

Gastric emptying and Intestinal transit: After administration of a last dose of Hyponidd, 1.5 mL of phenol red meal consisting of phenol red in 1.5% methylcellulose was given through gavage feeding. After 20 min, rats were sacrificed by cervical dislocation; stomachs were clamped with a string above lower esophageal sphincter and beneath the pylorus, to prevent the leakage of phenol red. The stomach of each rat resected just above the lower esophageal and pyloric sphincter. The stomach and its contents were minced into 5 mL of 0.1 mol L^{-1} of NaOH and was further diluted to 25 mL of 0.1 mol L^{-1} of NaOH and left at room temperature for 1 h. The supernatant (5 mL) was then centrifuged at 800 g for 20 min, absorbance read at 546 nm and phenol red content was calculated:

$$\text{Gastric emptying (\%)} = \frac{\text{Infusion amount-remains}}{\text{Infusion amount}} \times 100$$

The intestinal transit (IT) of phenol red meal was determined by modified Janseen method (Janseen and Jagenerous, 1957). The small intestine was removed from pyloric sphincter to ilioceacal junction and the distance travelled by the phenol red meal was noted and expressed as percentage of intestinal transit:

$$\text{Intestinal transit (\%)} = \frac{\text{Distance traveled by phenol red meal}}{\text{Total length of small intestine}} \times 100$$

Antioxidant activity: Malondialdehyde (MDA) (Uchiyama and Mihara, 1978), superoxide dismutase (SOD) (Kono, 1978), reduced glutathione (GSH) (Sedlak and Lindsay, 1968), catalase (CAT) (Aebi, 1984) and nitric oxide (NO) (Green *et al*., 1982). The protein concentration was estimated bovine serum albumin as the standard (Lowry *et al*., 1951).

***In-vitro* study on rat distal colon:** Immediately after cleaning and measuring the length of large intestine, a piece (1-2 cm) of distal colon was cut and used for *in-vitro* study. It was mounted under resting tension of 0.5 g

organ bath, with continuously aerated tyrode's solution. Dose response curve was obtained with ascending doses of Ach (100 μg mL^{-1}) and EC_{50} value was calculated.

Statistical analysis: All the values are expressed as Mean±SEM. Statistical analysis was performed using ANOVA followed by Dunnett's test. The values were considered statistically significant when $p<0.05$.

RESULTS

Effect of HMF on blood glucose level: STZ administration (55 mg kg^{-1}; i.p.) resulted in significant ($p<0.001$) increase in blood glucose level as compared to normal control rats. Two weeks treatment with HMF (100 and 200 mg kg^{-1}; p.o.) and metformin in diabetic rats significantly reduced serum glucose level as compared to diabetic control rats (Table 2).

Table 2: Effect of HMF on blood glucose level

Treatment (mg kg^{-1}, p.o.)	Serum glucose (mg dL^{-1}) Pretreatment	Post treatment
Normal control (NC)	72.40±2.20	89.00±1.81
Diabetic control (DC)	350.20±11.95	321.20±5.40@
DC + HMF (100)	346.00±11.47	208.60±3.05*
DC + HMF (200)	378.60±9.20	169.60±7.76*
DC + Metformin (200)	362.40±10.55	147.80±4.23*

n = 6, values are expressed as mean±SEM. @p<0.001 compared to normal control group and *p<0.05, compared to diabetic control group. HMF = Herbomineral Formulation

Table 3: Effect of HMF on gastric emptying in STZ-induced diabetic rats

Treatment (mg kg^{-1})	Gastric emptying (%)
Normal Control (NC)	57.15±1.16
Diabetic Control (DC)	40.44±0.57#
DC + HMF (100)	54.10±1.12*
DC + HMF (200)	52.72±0.65*
DC + Metformin (200)	46.96±0.80*

n = 6, values are expressed as mean±SEM. #p<0.001 compared to normal control group and *p<0.001 compared to diabetic control group. HMF = Herbomineral Formulation

Table 4: Effect of HMF (Hyponidd) on intestinal transit in STZ-induced diabetic rats

Treatment (mg kg^{-1})	Intestinal transit (%)
Normal control (NC)	83.38±1.09
Diabetic control (DC)	36.22±1.50#
DC + HMF (100)	56.57±0.91*
DC + HMF (200)	76.74±0.97*
DC + Metformin (200)	63.81±1.33*

n = 6, values are expressed as mean±SEM. #p<0.001 compared to normal control group and *p<0.001 compared to diabetic control group. HMF = Herbomineral Formulation

Effect of HMF on gastric emptying in STZ-induced diabetic rats: A significant delay in % gastric emptying was observed in diabetic control rats (40.44 vs 57.15%, $p<0.001$) when compared with normal control rats. Two weeks of HMF treatment (100 and 200 mg kg^{-1}) in diabetic rats significantly restored gastric emptying time (54.10 and 52.72%; $p<0.001$; respectively) (Table 3).

Effect of HMF on intestinal transit in STZ-induced diabetic rats: Intestinal transit in diabetic rats was significantly delayed (36.22 vs 83.38%, $p<0.001$) as compared to normal control rats. HMF treatment (100 and 200 mg kg^{-1}) for two weeks in diabetic rats observed significant restoration of intestinal transit (56.57 and 76.74 vs 36.22%; $p<0.001$; respectively) (Table 4).

Antioxidant activity: A significant increase in MDA and NO, whereas decrease in SOD, GSH and CAT were observed in diabetic control rats as compared normal control rats. Administration of HMF (200 mg kg^{-1}) and metformin showed significant decrease in MDA and NO levels whereas increase in SOD, GSH and CAT levels. Treatment with HMF at a dose of 100 mg kg^{-1} could not show any significant activity (Table 5).

***In-vitro* study on rat distal colon:** EC_{50} (33.30±1.04 vs 15.26± 0.41, $p<0.001$) was significantly increase in diabetic rats as compared to normal rats. A significant decrease in EC_{50} (18.04±0.46 and 11.96±0.96 vs 33.30±1.04, respectively) was observed in diabetic rats with two weeks of HMF treatment (Fig. 1).

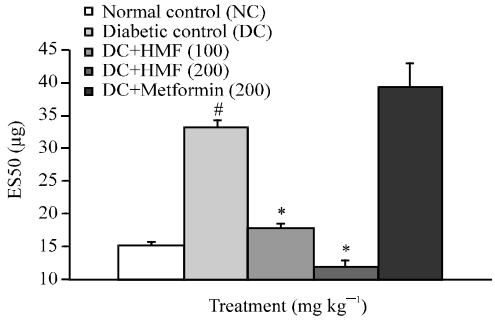

Fig. 1: *In-vitro* study on rat distal colon, n = 6, values are expressed as Mean±SEM. #p<0.001 compared to normal control group and *p<0.001 compared to diabetic control group. (EC50 = Effective Concentration 50)

Table 5: Effect of repeated dose treatment of HMF on oxidative stress markers in plasma

Treatment (mg kg^{-1}, p.o.)	MDA (nmol mg^{-1} of protein)	Nitric oxide (ng mg^{-1} of protein)	SOD (μg mg^{-1} of protein)	GSH (ng mg^{-1} of protein)	Catalase (μg mg^{-1} of protein)
Normal control (NC)	5.33±0.14	167.30±3.92	71.02±2.47	15.87±1.20	35.85±1.79
Diabetic control (DC)	11.28±0.63@	234.80±2.5@	37.82± 1.80@	8.00±0.42@	26.75±1.52@
DC + HMF (100)	9.06±0.26*	212.60±5.27	49.72± 1.27*	12.24±0.63*	28.72±1.63
DC + HMF(200)	7.29±0.24*	145.00±5.50*	65.77± 1.63*	15.54±0.71*	33.17±1.04*
DC + Metformin (200)	6.96±0.24*	132.22±3.31*	72.13± 2.28*	15.48±1.71*	32.76±1.47*

n = 6, values are expressed as mean±SEM. @p<0.001 compared to normal control group and *p<0.05, compared to diabetic control group. HMF = Herbomineral Formulation

DISCUSSION

Delayed gastrointestinal transit, is a well-known diabetic complication leads to vomiting, emaciation and unpredictable changes in blood glucose level (Talley *et al*., 2001; Horowitz *et al*., 2002). Acute hyperglycemia inhibits gastrointestinal peristaltic reflex (Chang *et al*., 1996; Jung *et al*., 2003). The pathogenesis of slow gastrointestinal transit in diabetes mellitus is not clear. However, several mechanisms have been proposed and autonomic neuropathy has been widely accepted as a culprit amongst them (Horowitz *et al*., 2002). Altered gastric motility is due to the disorder of autonomic functions and damage to extrinsic nerve. Bijender *et al*. (2003) showed reduction in contraction of distal colonic smooth muscle of STZ-induced diabetic rats (Bijender *et al*., 2003). Oxidative stress plays a crucial role in the pathogenesis of chronic complication of diabetes, further it damage the peripheral cholinergic neurons (Ziegler and Gries, 1997).

STZ-induced diabetic rats had mild or moderate gastroparesis which is characterized by slow gastric emptying and intestinal transit as compared with normal controls (Bijender *et al*., 2003; Anjaneyulu and Ramarao, 2002; El-Salhy, 2002a, b). Similar delayed in gastric emptying and intestinal transit was observed in the present study which is in agreement with previous reports. Further, significant reduction in contractile response to exogenous Ach was noted in STZ-induced diabetic rats. In the present study, two weeks of HMF treatment (100 and 200 mg kg^{-1}, p.o.) were significantly restored the gastric emptying and intestinal transit in diabetic rats as well as increases contractile response to exogenous Ach.

Diabetes was found to impair the antioxidant status of blood, presumably through production of excessive ROS. In the present study, significantly increased MDA and NO and decreased SOD, GSH and CAT were observed in diabetic rats. Treatment with HMF at a dose of 200 mg kg^{-1} body weight significantly decreased MDA and NO levels and SOD, GSH and CAT levels were significantly increased as compared diabetic control rats. These antioxidant properties of Hyponidd tablets may play an important role in halting progressive changes of chronic diabetes and thus, impaired gastric motility (Zheng *et al*., 2008).

CONCLUSION

The present study suggests therapeutic potential of Hyponidd in diabetic gastroparesis and related gastrointestinal impairments. However, further detailed investigations are warranted in order to clarify detailed mechanism of action.

ACKNOWLEDGMENTS

The authors wish to thanks Prof. M.N. Navale, Founder President, Sinhgad Technical Education Society, Pune for providing facilities to carry out this work.

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