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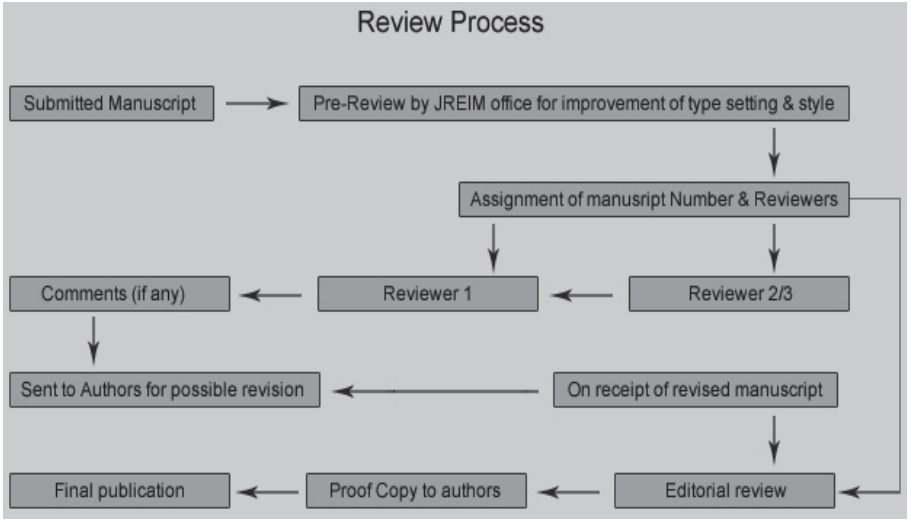

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PROKINETIC EFFECT OF HERBOMINERAL UNANI FORMULATION (*DOLABI*) IN DIABETIC RATS

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Abstract: This study was undertaken to investigate the prokinetic activity of Unani herbomineral formulation (*Dolabi*) in streptozotocin induced diabetic rats and its *in vitro* antioxidant activity. Rat model of diabetes was established by intraperitoneal injection of streptozotocin (55 mg/kg, i.p). Rats were divided into three groups: Normal control, diabetic control and treatment groups. After two weeks of treatment, rats were administered with phenol red meal followed by last dose of *Dolabi* and they were screened for gastric emptying (GE), intestinal transit (IT) and *in vitro* study of distal colonic smooth muscle. *In vitro* antioxidant activity of *Dolabi* was assessed on the basis of radical scavenging activity of the stable Diphenyl-2-picryl-hydrazyl (DPPH) free radical. Percentage of GE and IT was significantly ($P < 0.05$) decreased in diabetic rat as compared to normal control groups. In streptozotocin induced diabetic rats, *Dolabi* significantly ($P < 0.05$) accelerated both GE and IT as compared to diabetic control rats. Significant ($P<0.01$) increase in EC_{50} of ACh in rat distal colon was observed in diabetic rats as compared to normal rats. Where as diabetic rats treated with *Dolabi* showed significant ($P<0.01$) decrease in EC_{50} of ACh in distal colon as compared to diabetic control group. In *in vitro* study, *Dolabi* showed potent radical scavenging activity to stable DPPH-free radical with IC_{50} value of 231.09. *Dolabi* may exert its prokinetic effect by reducing oxidative stress and therefore can be used as drug for treating diabetic patients with gastrointestinal impairments.

Keywords: *Dolabi*, Unani medicine, Herbal medicine, Diabetes, Oxidative stress, Medicinal plants, Diabetic neuropathy.

Introduction

Oxidative stress play an important role in the pathogenesis of chronic complications of diabetes mellitus **(Ziegler and Gries, 1997)** including gastroparesis **(James *et al*., 2008).** The conditions that lead to the over production of the precursors of ROS and/or reduce the efficiency of scavenging system are shown to be responsible for the development of oxidative stress. Hyperglycemia play an important role in generation of reactive oxygen species (ROS) and that lead to chronic diabetic complications including diabetic autonomic neuropathy. Gastroparesis is the most common symptom of diabetic autonomic neuropathy **(Kong *et al*., 1999)** which is reported to cause considerable morbidity in patients. Gastroparesis leads to abnormal gastric motility, characterized by delayed gastric emptying (GE) and intestinal transit (IT) **(Shamaila *et al*., 2009).** The abnormal gastrointestinal motility among diabetic patients seemes to be a clinical manifestation of diabetic autonomic neuropathy **(Punkkinen *et al*., 2008)** and some reports concluded that these gastrointestinal disturbances may be due to the damage of peripheral cholinergic neurons as a result of oxidative stress **(Bijender *et al.*, 2003; De Winter *et al*., 2005).**

Herbomineral formulation *(Dolabi)* is used for treatment of diabetes and its complications in an Unani system of medicine. It contains *Gymnema sylvestre, Eugenia jambolana, Bambusa arundinacea, Rumex vescarricus, Acacia arabica,* oxide of egg shell, oxide of iron rust, zinc oxide **(Table 1).** Some of these ingredients have been reported to possess both anti-diabetic as well as antioxidant activity such

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as *E. jambolana* **(Sagrawat *et al.*, 2006),** *A. arabica* **(Wadood *et al.*, 1989; Sundaram and Mitra, 2007),** zinc oxide **(Robert and Silvestro, 2000)** where as *Gymnema sylvestre* known for its antidiabetic effect **(Gholap and Kar, 2003)**. So far there is no scientific evidence about efficacy of this formulation in preclinical models of impaired gastrointestinal motility and *in vitro* antioxidant activity. The present investigation was under taken to evaluate the effect of *Dolabi* on impaired gastrointestinal motility, colonic smooth muscle response to exogenous acetylcholine (ACh) in streptozotocin (STZ) induced diabetic rats and *in vitro* antioxidant activity.

Materials and Methods

Drug and Chemicals

Dolabi (Hamdard) a herbomineral formulation, purchased from local market. Streptozotocin, phenol red and DPPH were purchased from Sigma (U.S.A.).

Rat model of diabetes

Wistar rats of either sex weighing 180-230g were purchased from Haffkine Bio-Pharma Corporation Ltd., Mumbai (India). All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/Approval/2008-09/05) by the Institutional Animal Ethics Committee. Animals were housed under standard laboratory conditions at controlled temperature 25 ± 1ºC with 50-60% relative humidity in a normal 12 h light and dark cycle with free access to water and standard laboratory feed *ad libitum*.

Overnight fasted rats were injected with streptozotocin (55 mg/kg, i.p.) dissolve in 0.1 M cold citrate buffer (pH 4.45). The blood was withdrawn 48 h later by retro orbital method, serum was separated and fasting serum glucose level was determined using the glucose oxidase -peroxidase method **(Miskiewicz *et al.*, 1973).** Rats with a serum glucose level > 250 mg/dl were considered as diabetic and used for further study. Age matched five non-diabetic rats were used as normal control group and received 0.5ml of 0.1 M cold citrate buffer (pH 4.45).

Study design for gastrointestinal transit and in vitro study on rat distal colon

After persistent hyperglycemia for two weeks, diabetic rats were divided into two groups. Group one was diabetic control, received distilled water (10 ml/kg) and second group served as treatment, received suspension of *Dolabi* in distilled water (140 mg/kg, *p.o)* for next two

Table 1. Contents of Unani herbomineral formulation (*Dolabi*)

Sr. No	Name of content			Quantity
	Unani Name	Botanical Name (Family)	English Name	
1	Aqaqiya	*Acacia arabica* (Fabaceae)	Gum Arabic Tree	166.6 mg
2	Banslochen	*Bambusa arundinaceae* (Bambusaceae)	Thorny bamboo	132.0 mg
3	Tukhm Hammaz	*Rumex vesicarius* (Polygonaceae)	Rosy Dock, Dock Sorrel, Bladder Dock	83.3 mg
4	Gurmar Booti	*Gymnema sylvestre* (Asclepiadaceae)	*Gymnema*	27.7 mg
5	Maghz Jamun Labba Buz	*Syzygium cumini* (Myrtaceae)	Black Plum, Java Plum	27.7 mg
6	Kushta Baiz Murgh	--	Egg gallius domestics (oxide of egg shell)	13.8 mg
7	Kushata Khabsul Hadeed	--	Iron (oxide of iron rust)	13.8 mg
8	Kushta Jast	--	Zinc oxide	41.6 mg
9	Gond Safaid	--	--	41.6 mg
10	Labba Buz	--	--	200.0 mg

weeks. Age matched five non-diabetic rats were used as normal control group and received distilled water (10 ml/kg). Gastrointestinal transit and *in vitro* study on rat distal colon were performed.

Gastric emptying and intestinal transit

After administration of last dose of *Dolabi* to the overnight fasted rats, 1.5 ml of a phenol red meal, consisting of phenol red (0.05%, w/w) in 1.5% methylcellulose, was given through gavage feeding. Twenty minutes later, the rats were sacrificed by cervical dislocation. Their stomachs were clamped with a string above the lower oesophageal sphincter and a string beneath the pylorus to prevent leakage of phenol red. Gastric emptying was determined spectrophotometrically.

The stomach of each rat resected just above the lower oesophageal sphincter and pyloric sphincter. Phenol red remained partly in the lumen of the stomach. The stomach and its contents were put into 5 ml of 0.1 mol/l NaOH. The stomach was minced. The samples containing the total amount of phenol red present in the stomach were further diluted to 25 ml with 0.1 mol/l NaOH and left at room temperature for 1 h. The supernatant (5 ml) was then centrifuged at 800 g for 20 min.

The absorbance was read at a wavelength of 546 nm on a spectrophotometer (Shimadzu, Japan) and the phenol red content in the stomach was calculated. Percentage of gastric emptying of the phenol red was calculated as

[(infusion amount-remains) / infusion amount] × 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 the pyloric sphincter to the ileocecal junction and the distance travelled by the phenol red meal was noted and expressed as percentage of intestinal transit calculated as

[distance traveled by phenol red meal/ total length of small intestine] × 100

In vitro study on rat distal colon

Immediately after cleaning and measuring the length of large intestine, 2 cm distal colon was cut and used for in vitro study. The distal colon was dissected out and mounted under resting tension of 0.5 g in an organ bath containing continuously aerated tyrode's solution. Dose response curves were obtained with ascending doses of ACh (100µg/ml). EC_{50} values were calculated from graph plotted using percent responses against log dose.

In vitro antioxidant activity

In vitro antioxidant activity of *Dolabi* was assessed on the basis of radical scavenging effect of the stable DPPH-free radical. Free radical scavenging activity of different concentration of *Dolabi* and ascorbic acid were measured using DPPH, employing method of Blois **(Blois, 1958).** Solutions of different concentration (50, 100, 150, 200, 250 µg/ml) of *Dolabi* and ascorbic acid were added to 0.01mM, solution of DPPH in methanol. After 30 min, absorbance was measured at 517 nm, using spectrophotometer (Shimadzu, Japan). 0.01mM solution of DPPH in methanol was used as control. All tests were performed in triplicate. IC_{50} value was calculated. The DPPH radical scavenging activity was calculated according to the following equation,

$$\text{DPPH radical scavenging activity (\%)} = A_0 - A_1 / A_0 \times 100$$

Where,

A_0 is the absorbance of DPPH,

A_1 is the absorbance of DPPH solution in presence of the extract.

Statistical analysis

Statistical analysis of data was conducted using one-way ANOVA followed by Dunnett's test. Data were expressed as mean ± SEM, $P<0.05$ was considered statistically significant.

Results

Effect of Dolabi *on delayed gastric emptying in diabetic rats*

Gastric emptying (GE) was significantly ($P < 0.01$) decreased in diabetic rats as compared to normal rats (46.24 ± 3.64 *vs* 57.57 ± 1.96 %,

Figure 1). In STZ induced diabetic rats, *Dolabi* significantly (P<0.05) accelerated gastric emptying (GE) as compared to diabetic control rats (59.05 ± 2.09 *vs* 46.24 ± 3.64 %, **Figure 1**).

Effect of Dolabi on delayed small intestinal transit in diabetic rats

Intestinal transit (IT) was significantly (P < 0.05) decreased in diabetic rats compared to normal rats (45.43 ± 1.94 *vs* 60.93 ± 5.00 %, **Figure 2**). In STZ induced diabetic rats, *Dolabi* significantly (P<0.05) accelerated intestinal transit (IT) as compared to diabetic control rats (64.32 ± 4.16 *vs* 45.43 ± 1.942 %, **Figure 2**).

Effect of Dolabi on EC_{50} of ACh in distal colon of diabetic rats

EC_{50} value of Ach in rat distal colon was significantly (P<0.01) increased in diabetic rats as compared to normal rats (41.52±3.94 *vs* 20.89±3.25 µg, **Figure 3**).

In STZ induced diabetic rats, *Dolabi* show significant (P<0.01) decrease in EC_{50} value of ACh in distal colon as compared to diabetic control rats (14.23±2.59 *vs* 41.52±3.94 µg, **Figure 3**).

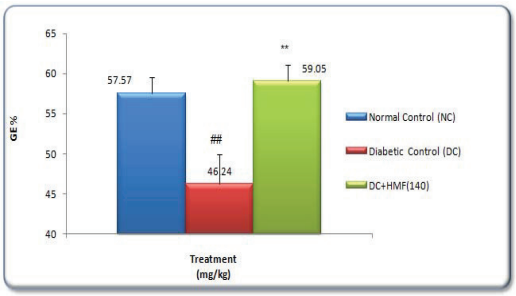
Effect of Dolabi on in vitro antioxidant activity

Dolabi exhibited *in vitro* antioxidant activity with IC_{50} value of 232.11 µg/ml (**Figure 4**).

Discussion

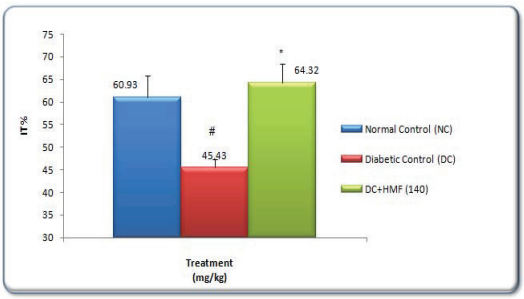
Propulsive motility is termed as peristalsis and it is subserved by a complex pattern of neural reflexes that aim to relax intestinal muscle downstream (descending inhibitory reflex) and

Figure 1. Effect of two weeks repeated dose treatment of *Dolabi* on % of gastric emptying (GE %) in STZ induced diabetic rats



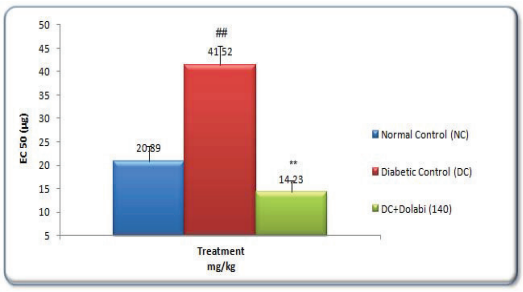
Data represented as mean ± SEM. ## P< 0.01, compared to normal control group; **P< 0.05, compared to diabetic control group (ANOVA followed by Dunnett' test)

Figure 2. Effect of two weeks repeated dose treatment of *Dolabi* on % of intestinal transit (IT %) in STZ induced diabetic rats



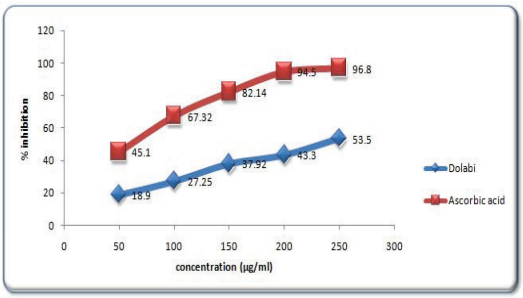
Data represented as mean ± SEM # P< 0.05, compared to normal control group; * P< 0.05, compared to diabetic control group
(significance by one way ANOVA followed by Dunnett' test)

Figure 3. Effect of two weeks repeated dose treatment of *Dolabi* on EC_{50} of ACh in rat distal colon in STZ induced diabetic rats



Data represented as mean ± SEM. ## P< 0.01, compared to normal control group; **P< 0.01, compared to diabetic control group
(significance by one way ANOVA followed by Dunnett' test)

Figure 4. Effect of *Dolabi* on DPPH free radical scavenging activity



Data expressed as mean ± SEM from three observations

contract the muscle upstream (ascending excitatory reflex) of the intestinal bolus. Intestinal transit is controlled by both neural and myogenic mechanisms **(Huizinga *et al.*, 1998).** An increase of the contractile activity of the smooth muscle layers is in general responsible for acceleration of intestinal propulsion. Several mediators and neurotransmitters are responsible for these motor patterns. Acetylcholine is the main excitatory neurotransmitter in the enteric nervous system, whereas NO is the major transmitter of the inhibitory motor neurons **(Waterman and Costa, 1994).** Disorder of autonomic functions **(Punkkinen *et al.*, 2008)** and extrinsic nerve supply to the gut are known to be responsible for the disturbed gastric motility associated with diabetes.

In the present study, STZ induced diabetic rats had mild or moderate gastroparesis which is characterized by slow gastric emptying and intestinal transit as compared with normal controls. Similar delayed gastric emptying, intestinal transit were seen in the STZ diabetic rats which is in agreement with previous studies **(Bijender *et al.*, 2003; Young *et al.*, 2006; El-Salhy, 2002a; Anjaneyulu and Ramarao, 2002; El-Salhy, 2002b).** Thus, rat with STZ induced diabeties could be used as an animal model of diabetic gastroparesis. Disturbed motility of gastrointestinal tract was also reported in the human with diabetes mellitus **(Russo *et al.*, 1997).**

The exact cause of slow gastrointestinal transit in diabetic patients is not known, but several mechanisms have been proposed. Most important among them, is damage of peripheral cholinergic nerve as a result of oxidative stress **(Bijender *et al.*, 2003).** A significant reduction in the contractile response of distal colonic smooth muscle to exogenous Ach was reported in STZ induced diabetic rats.

Treatment with antioxidant vitamin E, significantly increase contractile response of distal colonic smooth muscle to exogenous Ach as well as accelerate small intestinal transit in diabetic rats which confirm role of oxidative stress in damage of peripheral cholinergic neuron associated with diabetic autonomic neuropathy **(Bijender *et al.*, 2003).**

Acute change in blood glucose concentration has also major effect on gastrointestinal motor function in healthy subjects **(Russo *et al.*, 1997)**. In particular, acute hyperglycemia inhibits both the gastrointestinal and ascending components of peristaltic reflex. Poor glycemic control has the potential to cause delayed gastrointestinal transit in diabetic patients **(Jung *et al.*, 2003)**. Therefore drug which control both blood glucose as well as having antioxidant activity will be the best one, for treating diabetic associated gastrointestinal trouble.

To the best of our knowledge, this is the first report on the effect of herbomineral formulation (*Dolabi*) on the gastrointestinal dysmotility in streptozotocin induced diabetic rats and its *in vitro* antioxidant activity using DPPH. *Dolabi* significantly accelerate gastric emptying, intestinal transit and increase contractile response of distal colonic smooth muscle to exogenous ACh in STZ induced diabetic rats. It also exhibit *in vitro* antioxidant activity to DPPH.

Mechanism underlying the action of *Dolabi* on impaired gastric motility may be neuronal dependent and its antioxidant activity may play an important role. Our data strongly reveals that *Dolabi* exert a prokinetic action on gastric emptying and intestinal transit in diabetic rats. The present study also suggests that antioxidant property of *Dolabi* may be responsible for halting progressive changes of chronic diabetes leading to gastric impairment. However, further detailed studies like estimation of endogenous antioxidant enzyme levels are needed.

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