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Add-on therapy of herbal formulation rich in standardized fenugreek seed extract in type 2 diabetes mellitus patients with insulin therapy: An efficacy and safety study

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ABSTRACT

Objective: To assess the safety and efficacy of herbal formulation rich in standardized fenugreek seed extract (IND-2) add-on therapy in type 2 diabetes mellitus (T2DM) patients who were on insulin treatment in prospective, single arm, open-label, uncontrolled, multicentre trial. **Methods:** T2DM patients (n=30) with aged 18-80 years who were stabilized on insulin treatment with fasting blood sugar (FBS) level between 100-140 mg/dL received IND-2 capsules (700 mg, thrice a day) for 16 weeks. The primary endpoints were an assessment of FBS at week 2, 4, 6, 8, 12 and 16. Secondary end-points include post-prandial blood sugar level, glycosylated Hb (HbA1c), reduction in the dose of insulin and number of hypoglycemic attacks, and improvement in lipid profile at various weeks. Safety and adverse events (AEs) were also assessed during the study. **Results:** Study was completed in twenty T2DM patients, and there was no significant reduction in FBS and post-prandial blood sugar level after add-on therapy of IND-2. However, add-on therapy of IND-2 significantly reduced (P<0.01) the HbA1c values, requirements of insulin and hypoglycemic events as compared with baseline. Total cholesterol, high-density lipoproteins-cholesterol, and low-density lipoprotein-cholesterol levels were significantly increased (P<0.01) after IND-2 add-on therapy. Body weight and safety outcomes did not differ significantly in IND-2 add-on therapy group at week 16. Additionally, add-on therapy of IND-2 did not produce any serious adverse events. **Conclusions:** The results of present investigation suggest that add-on therapy of IND-2 with insulin in T2DM patients improves glycaemic control through a decrease in levels of HbA1c and number of insulin doses needed per day without an increase in body weight and risk of hypoglycemia. Thus, IND-2 may provide a safe and well-tolerated add-on therapy option for the management of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a complex, chronic progressive disease which is most common amongst the various forms of diabetes. It is manifested by hyperglycemia, disturbance in the metabolism of carbohydrate, fat, and protein which could be the result from deficiency secretion of insulin or its actions[1]. It is a long-term metabolic disorder which is associated with multiple comorbidities as well as microvascular and macrovascular

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complications such as retinopathy, nephropathy, neuropathy, cardiomyopathy[2,3]. Numerous epidemiological studies have suggested that obesity, insulin resistance, hyperlipidemia, hypertension, and smoking are the risk factors associated with T2DM[4,5]. The prevalence of T2DM is fast-growing worldwide and now become a major public health problem. The projected prevalence of T2DM is predicted to affect more than 350 million people by 2025 worldwide[6,7]. It also produces significant economic burdens, and in a developing country like India, the cost of illness for diabetic care is estimated at USD 336[8].

Due to a progressive increase in the costs of treatment, the intensive lifestyle modifications have been suggested as a treatment alternative to decrease the risk for diabetes development[9,10]. However, it is difficult to achieve a targeted glycaemic control with lifestyle modification alone. Hence, a strategic escalation of antidiabetic therapy has suggested by the American Diabetes Association and European Association for the Study of Diabetes[1]. Thus, metformin has been recommended by the American Diabetes Association and European Association for the Study of Diabetes as first-line therapy in combination with lifestyle modifications for patients with T2DM[1]. However, metformin may not be able to effectively control glycaemic goals in the patients with higher baseline glycated hemoglobin (HbA1c) levels. Thus, in such patient management of diabetes needs multiple pharmacological treatments[1,11].

Dipeptidyl-peptidase-4 inhibitors and sulfonylureas are another optional pharmacological therapies now available[5,12,13] that increases insulin secretion by acting on pancreatic β-cells[14]. However, these treatment regimens are associated with decreased β-cell function along with concern for weight management. Thus, adequate control of glycemia is difficult over a long period[15]. Thiazolidinediones (such as pioglitazone, rosiglitazone) is another class of oral antihyperglycemic agents that act by regulating glucose and lipid metabolism via modulation of peroxisome proliferator-activated receptor γ[16]. However, clinical evidence showed the association of use of these agents with myocardial events risk and nephrotoxicity[17].

It has been suggested that the failure in the maintenance of the targeted level of glycated hemoglobin (HbA1c) is a major cause for the worsening nature of T2DM[18,19]. Thus, the advanced therapy of insulin has been recommended for improvement in glycaemic control[1,20,21]. It has been showed that initiation of the insulin therapy in the patients with metformin-alone helps to achieve the glycaemic control with HbA1c reduction[22]. However, additional risk such as weight gained and hypoglycemia need to be considered while management of T2DM using insulin[23]. Furthermore, cost of insulin, unavailability of insulin by the oral route, medication compliance and insulin resistance are the major obstacles in insulin therapy for the management of T2DM. The add-on therapy with insulin may provide sustained glycaemic control along as well as a decrease in the frequency of use of insulin[24,25]. Therefore, interest in add-on therapies to insulin is increasing amongst diabetic patients.

Herbal medicine can be considered as an efficacious and safe treatment alternative for the fulfillment of the unmet medical need as an "add-on therapy" along with insulin in the long-term management of T2DM[26-28]. A wide range of phytoconstituents isolated from herbal origin can act by multiple mechanisms including stimulation of insulin production, increase on insulin sensitivity, reduction in HbA1c, modulation of insulin action, reduction of carbohydrate absorption amongst others[28]. The traditional Indian and Chinese medicines have documented an array of such medications for the T2DM treatment with proven clinical safety and efficacy[29]. *Camellia sinences*, *Emblica officinalis*, *Gymnema sylvestre*, *Linum usitatissimum*, *Salacia reticulata*, *Tribulus terrestris*, *etc*. are few amongst them which showed clinical efficacy and safety in the management of T2DM[30-40].

Trigonella foenum-graecum Linn. (Fabaceae) (fenugreek) has ancient traditional uses in the treatment of diabetes. Fenugreek possesses a broad spectrum of pharmacological and therapeutic properties including anti-hyperlipidemic, antidiabetic, anticancer, anti-arthritic, antioxidant, antimicrobial, antinociceptive and anti-inflammatory potential[41-47]. Fenugreek plays a vital role in the prevention of heart, liver, kidney and spleen diseases[48-53]. Several investigators have reported the hypocholesterolemic and anti-diabetic potential of defatted fenugreek seed[43,54]. Studies carried out by various researchers have reported the anti-diabetic potential of fenugreek in an array of animal models[41,42,55] as well as in human subjects[43,54]. These effects are related to the presence of constituents such as alkaloid (Trigonelline) and amino acid (2S,3R,4S, 4-hydroxyisoleucine (4-HI))[56-59]. In this view, the herbal formulation (IND-2) has been developed which is rich in standardized fenugreek seed extract. Previously, the researcher also showed the beneficial effect of herbal formulation rich in standardized fenugreek seed extract in the management of T2DM patients inadequately controlled with a sulphonylurea[60]. However, to our knowledge, add-on therapy of herbal formulation rich in standardized fenugreek seed extract (IND-2) for its the efficacy and safety have not been evaluated in a patient with T2DM who were stabilized on insulin treatment. Hence, present investigation aimed to assess the safety and efficacy of IND-2 add-on therapy in T2DM patients on insulin treatment in prospective, single arm, open-label, uncontrolled, multicentre study.

2. Materials and methods

2.1. Study design and protocol

The study was designed as a prospective, open-label, single arm, uncontrolled, multicentre study to assess the safety and efficacy of herbal formulation rich in standardized fenugreek seed extract (IND-2) (Table 1) 700 mg, thrice a day as add-on therapy in T2DM patients who were stabilized on insulin treatment. All patients received 3 capsules of IND-2 per day, one in the morning (preferably at 7 am), one in the afternoon (preferably at 3 pm), and one at night (preferably at 11 pm). The capsules were administered one hour before of taking the food or two hours after taking the food. The dose of IND-2 was to be adjusted only when the blood sugar level < 80 mg/dL or 20% less than the previous value. Patients who complained about hypoglycemic attacks were discontinued from the study.

The study comprised a screening and enrolment period namely week (-2) (visits 1) and week 0 *i.e.* baseline (visit 2), during which patients continued background medication consisting of a stable dose

of insulin, and a treatment period, during which patients received 700 mg thrice a day as add-on therapy of IND-2 added to background medication for 16 weeks. Total eight study visits occurred *viz*., week 2 (visits 3), week 4 (visit 4), week 6 (visit 5), week 8 (visit 6), week 12 (visit 7) and week 16 (visit 8, *i.e.*, treatment end) (Figure 1).

Table 1

Composition of IND-2.

Sr. no.	Name of the ingredient	Standardised to marker	Quantity
1.	*Trigonella foenum-graecum*	20% 4-HI and 20% Trigonelline	500 mg
2.	*Salacia reticulata*	20% Saponins	10 mg
3.	*Camellia sinences*	50% Polyphenols	15 mg
4.	*Emblica officinalis*	20% Tannins	70 mg
5.	*Gymnema sylvestre*	25% Gymnemic acid	15 mg
6.	*Tribulus terrestris*	20% Saponins	70 mg
7.	*Piper nigrum*	95% Piperine	10 mg
8.	*Linum usitatissimum*	40% Alpha linolenic acid	10 mg

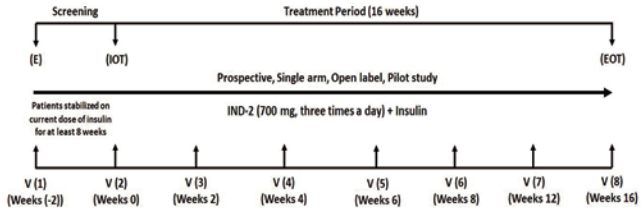

Figure 1. Study design.

E: Enrolment; EOT: End of treatment; IOT: Initiation of treatment; V: Visit.

2.2. Inclusion and exclusion

Eligible patients were: (1) male or female subject aged 18-80 years, (2) T2DM (Non-insulin dependent) patients who were stabilized on insulin treatment, (3) fasting blood sugar (FBS) level between 100 mg/dL and 140 mg/dL including both (100 mg/dL and 140 mg/dL), (4) difference between two blood sugar level readings (Visit 1 and Visit 2) should not be more than 30 mg/dL, (5) written informed consent from the subject, (6) stabilized at the same dose of insulin for a minimum period of 8 weeks, (7) no participation in any other clinical studies during last 60 days.

Exclusion criteria were subjects with conditions such as (1) severe renal insufficiency defined by a creatinine value above 1.5 mg/dL, (2) severe hepatic insufficiency defined by an SGOT (serum glutamic oxaloacetic transaminase) or SGPT (serum glutamic pyruvic transaminase) value equal or higher than the threefold normal values of the respective laboratory safety value, (3) pregnant, willing to get pregnant or nursing women, (4) participation in a clinical trial during the 60 days before the present trial, (5) simultaneous participation in another clinical trial, (6) cardiovascular disorder.

2.3. Withdrawal criteria

The participation in a clinical trial by the subjects may be terminated at any time under the following conditions:

•At the discretion of the investigator: subjects can be withdrawn on the discretion of the investigator at any time during the trial if the possibility of any impairment of the subjects' health cannot be excluded.

•At the subject's request: the subject can withdraw himself from the trial without any reason.

•If an adverse event (AE) occurs: if a subject is withdrawn due to an AE, this should be followed up until it has resulted in a stabilized medical condition (recovered, recovered with residual damage, death).

•Patients who did not get performed their laboratory testing within 7 days from the end of treatment visit

•If patients failed to come within 7 days of the scheduled follow-up visit

•If patients become pregnant during the study

•If the major protocol violation occurred by the patients: like missing two consecutive visits, not taking medication for 1 week, *etc*.

2.4. Compliance with ethics

All patients provided written and informed consent. The study protocol was approved by the institutional review board of each center (The protocol No. IBHM02/2002). The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Guideline for Good Clinical Practice guidelines as defined by the International Conference on Harmonization.

2.5. Compliance with treatment

Patients were encouraged not to skip scheduled medication intake or reduce dosages on their own. Lapses observed during visits were documented on the appropriate page of the Case Report Form. Investigators evaluated treatment compliance by measuring unused medication in the subject medication box. If more than 20% of the total prescribed medication was not consumed by the patient during every 2 weeks, the patient was termed as noncompliant and was excluded from the trial.

2.6. Study endpoints

The effect of IND-2 (700 mg, thrice a day) on FBS compared with baseline (week 0) was the primary efficacy endpoint. Whereas, the effects of IND-2 on post-prandial blood sugar level (PPBS) and glycosylated Hb (HbA1c), to check the feasibility of reduction in the dose of insulin and number of hypoglycemic attacks after addition of IND-2 and to assess improvement in lipid profile [*i.e.*, triglycerides, high-density lipoproteins-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total cholesterol (TC), and LDL-C to HDL-C ratio] as compared with the baseline were the secondary efficacy endpoints. Assessment of FBS, PPBS, HbA1c, insulin dose and hypoglycemic attacks were done on all visits (Week 0, 2, 4, 6, 8, 12 and 16). The lipid profile was assessed on alternate visits (Week 0, 8 and 16).

2.7. Safety parameters

The following safety parameters were investigated to assess the safety of IND-2:

• The clinical safely was evaluated on all visits by vital signs, ECG recordings, and any AEs which may be spontaneously reported and observed directly.

• The following vital signs were considered for a measure of clinical safety variable:

•The temperature of the body (° F)
•Respiratory rate (breath/minute)
•Systolic blood pressure (mmHg)
•Diastolic blood pressure (mmHg)
•Heart rate (beats/minute)

The laboratory safety was evaluated by determining the following parameters at the initiation of treatment (week 0), and after 8 and 16 weeks: Haemoglobin (Hb), white blood cell (WBC): total and differential count, red blood cell (RBC) count, platelet count, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase(ALT), lactic dehydrogenase (LDH), serum proteins, urinalysis, blood urea nitrogen (BUN) and creatinine.

2.8. Blood sampling and measurements

Blood samples were collected after fasting condition of at least 10 h. Then samples of blood were processed and analyzed in one central laboratory. Samples were handled according to laboratory routines, and the laboratory variables were analyzed according to routine assay techniques at the laboratory.

2.9. Statistical analysis

A total of 30 participants were recruited with an assumption of a 20 percent dropout rate. Being a pilot study, no statistical method was applied to decide the sample size. Results are represented as mean ± standard deviation (SD). Mean values of various diabetic markers (FBS and PPBS levels, number of hypoglycemic attacks since the last visit, amount of insulin required) and HbA1c at each visit were compared with baseline. The secondary efficacy parameters, *i.e.*, various lipid markers (TC, LDL-C, HDL-C, triglycerides, the ratio of LDL-C and HDL-C), AEs (haematology and biochemistry) and vital parameters at V6 as well as at V8 were compared with baseline and analyzed using a paired *t*-test. The $P<0.05$ was considered statistically significant. The SPSS version 16.0 software used for statistical analysis.

3. Results

3.1. Patient recruitment, demographics and baseline characteristics

Total 60 subjects were screened, and 30 (male = 16 and female 14) were recruited in the trial. Ten subjects were withdrawn from the trial at various stages. The lost of follow-up [4/10 (40%)], and AE [4/10 (40%)] were the most common reasons for treatment discontinuation. Two subjects discontinued due to lack of compliance and inclusion violation. Thus, 20 subjects were considered for the study and received treatment with IND-2 add-on therapy. Figure 2 depicted the patient recruitment and selection process.

The demographics and baseline characteristics of the subjects were represented in Table 2. At the baseline of the study, mean age was 52 years, HbA1c was 6.19%, BMI was 27.31 kg/m^2, FBS levels was 121.71 mg/dL, and PPBS levels was 193.560 mg/dL.

3.2. Treatment compliance

After each visit, the unused capsules were counted from which the treatment compliance was determined on a study population of 20. If more than 20% of the total prescribed medication was not consumed by the patient during every 2 weeks, the patient was termed as noncompliant and was excluded from the trial. The mean treatment compliance was > 98%, which was deemed acceptable.

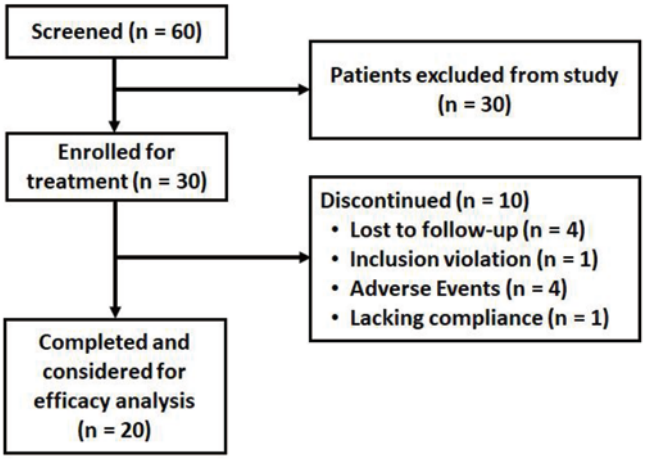

Figure 2. Patient disposition.

Table 2

Demographic data (n = 20).

Parameter	Value (Mean ± SD)
Age (Years)	52.00 ± 6.64
Male (n)	16 (53%)
Female (n)	14 (47%)
Height (cm)	155.03 ± 7.20
Weight (kg)	65.30 ± 5.81
BMI (kg/m^2)	27.31 ± 3.35
FBS (mg/dL)	121.71 ± 13.90
PPBS (mg/dL)	193.56 ± 56.40
HbA1c (%)	6.19 ± 0.64

n: number of patients; BMI: body mass index; FBS: fasting blood sugar; HbA1c: glycated hemoglobin; PPBS: postprandial blood sugar; SD: standard deviation.

3.3. Effects of IND-2 add-on therapy on glucose regulation

There was no statistically significant reduction in FBS and PPBS level at week 2, 4, 6, 8, 12 and 16 after add-on therapy of IND-2 when compared with baseline (week 0) (Figure 3). However, add-on therapy of IND-2 with insulin showed a significant decrease ($P<0.01$) in HbA1c at week 8 compared with the baseline (Figure 4A). Additionally, requirements of insulin (in terms of units consumed per day) at week 2, 6, 8, 12 and 16 were also significantly less ($P<0.001$, $P<0.01$, $P<0.01$, $P<0.01$ and $P<0.01$, respectively) after add-on therapy of IND-2 as compared with from baseline (Figure 4B). Furthermore, reductions in hypoglycemic events after add-on therapy of IND-2 at week 2, 6 and 12 were also significantly reduced ($P<0.01$, $P<0.01$and $P<0.05$, respectively) as compared with baseline (Figure 4C).

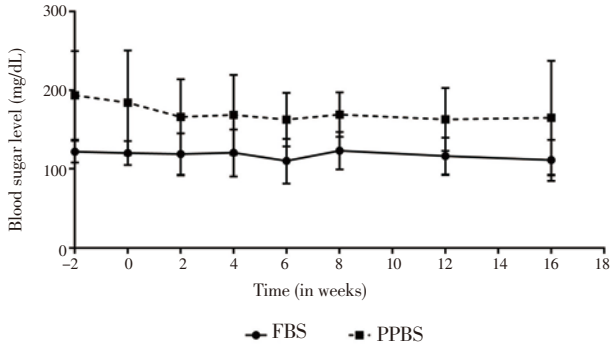

Figure 3. Effect of add-on therapy of IND-2 on FBS and PPBS over 16 weeks. Values are shown as the mean ± standard deviation. Data were analyzed by paired "*t*" test.

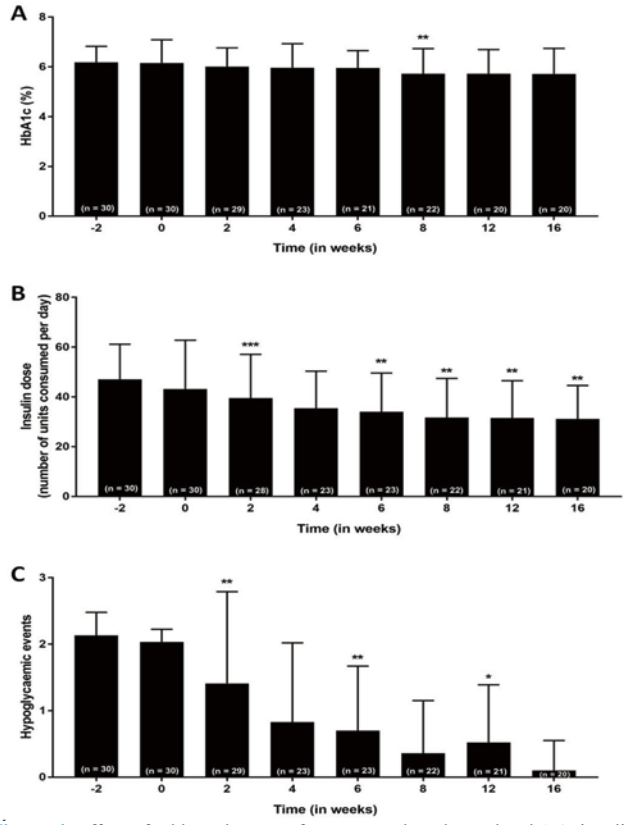

Figure 4. Effect of add-on therapy of IND-2 on the HbA1c level (A), insulin dose (B) and hypoglycemic events (C) over 16 weeks.
Values are shown as the mean ± standard deviation. Data were analyzed by paired "*t*" test, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ as compared with baseline (week 0). HbA1c: Glycated hemoglobin.

3.4. Effects of IND-2 add-on therapy on lipid profile

LDL and TC showed a slight but significant increase ($P<0.01$ and $P<0.05$) at week 8 in the IND-2 add-on treatment group as compared with the baseline. At 16 weeks of treatment, LDL, HDL, and TC were also significantly increased ($P<0.01$) as compared with the baseline. However, triglyceride levels and LDL: HDL ratio did not differ significantly at week 8 and 16 when compared with the baseline (Table 3).

Table 3

Effect of add-on therapy of IND-2 on efficacy variables of lipid profile.

Characteristic	IND-2 + insulin		
	Week 0 (n = 30)	Week 8 (n = 22)	Week 16 (n = 19)
Total cholesterol (mg %)	174.05 ± 45.36	187.14 ± 40.93*	185.63 ± 29.56**
LDL cholesterol (mg %)	111.61 ± 49.18	118.43 ± 51.92**	125.01 ± 44.05**
HDL cholesterol (mg %)$^{\$}$	49.46 ± 9.29	51.91 ± 11.47	60.01 ± 9.79**
Triglyceride (mg %)$^{\$}$	213.51 ± 52.07	217.57 ± 42.19	212.88 ± 44.86
LDL / HDL ratio	2.40 ± 1.29	2.35 ± 1.09	2.17 ± 0.97

Values are presented as the mean ± standard deviation. Data were analyzed by paired "*t*" test, $^{*}P<0.05$ and $^{**}P<0.01$ as compared with baseline (Week 0).
$^{\$}n$ = 20 at week 16.
LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

3.5. Effects of IND-2 on safety outcomes

At week 16, there was no significant difference in body weight, plasma AST, ALT, protein, and creatinine in add-on therapy with IND-2 as compared with baseline (Table 4). However, treatment with IND-2 showed significantly decreased ($P<0.05$) in ALP, bilirubin, and significantly increased ($P<0.05$) in a BUN at week 8 as compared with the baseline but these differences did not continue till week 16 and remain non-significant as compared with the baseline (Table 4).

Table 4

Effect of add-on therapy of IND-2 on safety parameters.

Parameter	Baseline (Week 0) (n = 30)	Week 8 (n = 22)	End of treatment (Week 16) (n = 20)
Body weight (kg)	65.93 ± 14.87	66.09 ± 13.21	66.95 ± 14.87
AST (mg %)	28.82 ± 10.05	29.46 ± 10.35	26.28 ± 9.27
ALT (mg %)	28.60 ± 10.54	29.47 ± 12.64	26.23 ± 12.63
ALP (mg %)	158.28 ± 52.45	127.13 ± 37.79*	151.07 ± 63.08
Bilirubin (mg %)	0.76 ± 0.40	0.61 ± 0.25*	0.59 ± 0.32
Serum proteins (mg %)	7.76 ± 3.10	6.90 ± 3.03	6.92 ± 2.87
Serum creatinine (mg %)	0.93 ± 0.29	0.90 ± 0.26	0.96 ± 0.30
BUN (mg %)	13.14 ± 4.53	16.46 ± 3.73*	15.98 ± 4.28
Hb (mg %)	13.43 ± 4.03	14.21 ± 4.21	13.49 ± 4.34
Haematocrit	43.43 ± 12.27	45.86 ± 10.26	43.70 ± 15.28
MCV (fL/red cell)	79.10 ± 13.46	91.77 ± 10.57**	87.60 ± 10.45
MCHC (g/dL)	32.27 ± 11.83	34.23 ± 12.18	32.00 ± 10.43
RBC (in millions / cmm)	4.78 ± 0.75	4.54 ± 0.83	4.51 ± 0.86
WBC (mg %)	8 954.83 ± 3 080.28	9 934.00 ± 2 667.71	9 006.55 ± 2 914.94
Eosinophils (%)	3.40 ± 1.25	3.32 ± 1.29	3.30 ± 1.38
Neutrophils (%)	62.17 ± 12.65	58.55 ± 13.93	58.35 ± 11.38
Lymphocytes (%)	35.53 ± 9.47	39.86 ± 10.19	39.70 ± 7.18
Monocytes (%)	0.10 ± 0.31	0.09 ± 0.29	0.05 ± 0.22
Platelets (×1 000 / cmm)	271.20 ± 76.25	366.64 ± 99.27**	329.05 ± 75.50
Respiratory rate (breaths per minute)	15.17 ± 3.26	12.91 ± 2.09*	13.35 ± 1.31

Values are presented as the mean ± standard deviation. Data were analyzed by paired "*t*" test, $^{*}P$ <0.05 and $^{**}P<0.01$ as compared with baseline (Week 0).
ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate transaminase; BUN: Blood urea nitrogen; Hb: Haemoglobin; MCV: Mean corpuscular volume; MCHC: Mean corpuscular haemoglobin concentration; RBCs: Red blood cells; WBC: White blood cells.

The levels of Hb, hematocrit, MCHC, RBC, WBC, eosinophils, neutrophils, lymphocytes, and monocytes did not differ significantly in IND-2 add-on therapy at week 8 and week 16 as compared with the baseline. However, add-on therapy of IND-2 for 8 weeks resulted

in a significantly increase (P<0.01) in MCV and platelets levels when compared with baseline, but these changes were discontinued till week 16 and remain non-significant when compared with baseline. Similarly, the respiratory rate was decreased significantly (P<0.05) at week 8 and remained non-significant at week 16 in IND-2 add-on therapy group as compared with the baseline (Table 4).

3.6. AEs

Overall AEs and the most common AEs are summarized in Table 5 and Table 6. Add-on therapy of IND-2 did not produce any serious AEs during the clinical study. Total 99 AEs were documented in the study out of which 5 adverse events were graded as severe which includes a cough (2), dyspnoea (1), diarrhea (1), and gastritis (1). Seventeen events were considered as 'related' to the test drug, and 4 subjects were discontinued from the trial due to adverse events.

Table 5

Summary of adverse events experienced by a number of patients at least once.

Adverse event	No. of patients	% of total
Abdominal pain	3	10.00
Anorexia	1	3.33
Anxiety	1	3.33
Constipation	1	3.33
Cough	2	6.66
Crystals in urine	2	6.66
Diarrhoea	6	20.00
Dry cough	2	6.66
Dyspnoea	1	3.33
ECG abnormal	5	16.66
Epithelial cells 6-8/hpf	1	3.33
Flatulence	1	3.33
Gastritis/Epigastric burning	7	23.33
Glycosuria	1	3.33
Headache	5	16.66
High BP	2	6.66
High BUN	3	10.00
High cholesterol	2	6.66
High eosinophil count	2	6.66
High ESR	8	26.66
High HDL	1	3.33
High MCHC	2	6.66
High MCV	1	3.33
High monocytes	3	10.00
High platelet count	1	3.33
High serum creatinine	3	10.00
High serum proteins	2	6.66
High total bilirubin	1	3.33
High triglycerides	1	3.33
High WBC	1	3.33
Low cholesterol	1	3.33
Low haematocrit	2	6.66
Low lymphocytes	1	3.33
Low plasma albumin	1	3.33
Low RBC	1	3.33
Nausea	3	10.00
Palpitation	1	3.33
Pus cells 4-6 hpf in urine	4	13.33
Restlessness	1	3.33
Sweating	2	6.66
Urine albumin	2	6.66

Taken individually, absolute frequency of adverse events such as increase in ESR, gastritis/epigastric pain and diarrhea were the first three most commonly observed adverse events. In terms of patients experiencing the adverse events, the same adverse events were most commonly observed. Mean values of some laboratory tests were altered after 8 weeks and returned to normal after 16 weeks. There were no deaths, or serious medical event reported in during this clinical study.

Table 6

Absolute frequency of adverse events.

Adverse event	Absolute frequency	Adverse event	Absolute frequency
High ESSR	10	High eosinophil count	2
Gastric / Epigastric burning	8	High S. proteins	2
Diarrhea	6	Anorexia	1
ECG abnormal	6	Anxiety	1
Headache	5	Constipation	1
Pus cells in urine	5	Dyspnoea	1
Nausea	4	Epithelial cells 6-8/hpf	1
Abdominal pain	3	Flatulence	1
High BP	3	Glycosuria	1
High BUN	3	High HDL	1
High monocytes	3	High MCV	1
High S. creatinine	3	High platelet count	1
Low haematocrit	3	High total bilirubin	1
High MCHC	3	High triglycerides	1
Cough	2	High WBC	1
Crystals in urine	2	Low cholesterol	1
Dry cough	2	Low lymphocytes	1
Sweating	2	Low plasma albumin	1
Urine albumin	2	Low RBC	1
High cholesterol	2	Palpitation	1
High eosinophil count	2	Restlessness	1

All adverse events irrespective of causality.

4. Discussion

T2DM is a chronic, complex heterogeneous condition resulted from impairment of insulin secretion from β cells of the pancreas, insulin action, or insulin resistance in the peripheral tissues. In the last few decades, the prevalence of T2DM increases significantly which makes it one of the most critical growing health issues worldwide. Therefore, the guidance of the American Association of Clinical Endocrinologists recommended the various treatment regimen including dipeptidyl peptidase-4 inhibitors (such as sitagliptin), thiazolidinediones, sulfonylureas, and insulin[61,62]. However, these agents are also associated with substantially clinical side effects including a risk of hypoglycemia, increased body weight, lactic acidosis, the risk of myocardial events, *etc*[1]. Hence, many investigators have employed various add-on therapies from the natural origin for the treatment of T2DM[4,63]. In the present investigation, we have also assessed the safety and efficacy of IND-2 add-on therapy in T2DM patients who were on insulin treatment. The outcomes of the present investigation showed that addition of IND-2 (700 mg, thrice a daily) with insulin treatment for 16 weeks significantly improved the amount of insulin required per day along with significant decrease in of the numbers of hypoglycemic attacks without any serious adverse events.

Numerous clinical evidence suggests that control over FBS and PPBS level are essential outcomes in the management of T2DM and reflected as the gold standard for overall glycaemic control[64,65]. However, FBS level determination over time course trend failed to provide the detailed information of diabetes status and is also non-satisfactory[66]. Hence, according to the American Diabetes Association, glycated hemoglobin (HbA1c, with a cut-point ≥6.5%) has been recommended over FBS (≥7.0 mmol/L) for diabetes detection[20]. Furthermore, HbA1c provides an insight about the individual average levels of blood glucose over the last two to three months. Thus, HbA1c is considered as a reliable indicator of chronic glycemia and reflected the risk of various chronic diabetes complications such as retinopathy, neuropathy, nephropathy, *etc*. Hence, currently, HbA1c is widely used as an essential test for chronic management of diabetes. In the present investigation, we have also determined the effect of add-on therapy of IND-2 on HbA1c and result show that it significantly decreased HbA1c levels over 8 weeks. Fenugreek (*Trigonella foenum-graecum*), *E. officinalis*, *G. sylvestre*, *S. reticulata*, and *C. sinensis* were reported to reduce the HbA1clevel in clinical and preclinical studies[30-36,67]. Additionally, the presence of 4-HI in standardized fenugreek seed extract may be responsible for the reduction in HbA1c level in T2DM patients and the findings of the previous investigator also support this notion[56-59].

The management of T2DM many time needs the combination of oral antidiabetic therapies including sulfonylureas, DDP-4 inhibitors. However, these therapies are substantially limited by its side effects[1]. Hence, many T2DM patients eventually moved to insulin therapy for maintaining better glycaemic control. Although insulin is the most effective treatment available for the management of blood glucose levels, it is still also associated with side effects such as weight gain and risk of hypoglycaemia[1,23]. Therefore, in such patients, there is a need for add-on therapy with more viable and safe option. In the present investigation, we have implicated IND-2 as an add-on therapy along with insulin for the management of T2DM. The finding showed that IND-2 with insulin decreased (27% reduction) the number of insulin dose needed per day as compared with baseline. The observed beneficial effects of IND-2 on top of insulin are anticipated from its complementary mechanism of action. This action of IND-2 may depend on the amount of glucose present which caused the secretion of insulin. This mechanism of action of IND-2 is further supported by the decrease in the number of hypoglycemic events over 16 weeks. Whereas, sulfonylurea has a limitation where it caused continuous stimulation of insulin secretion, even with decreasing level of glucose which results in hypoglycemia[1]. Thus, clinically IND-2 can be considered as a potential add-on therapeutic agent with insulin for T2DM management, with a decrease in hypoglycemia risk and a decrease in the number of insulin doses needed per day.

Further, we have also investigated the effect of IND-2 on lipids profile which showed that a moderate but significant increase in total, HDL and LDL cholesterol over 16 weeks. It has been well documented that elevated level of cholesterol associated with risk for CHD (coronary heart disease) and atherosclerosis[68]. However, a recent clinical study also reported that vildagliptin add-on therapy with insulin improves control on glycemia with an increase in the risk of hyperlipidemia[69]. In the present investigation, documented increase in lipid profile might be a secondary effect resulting from decreased HbA1c levels.

According to the ADA/EASD guidelines, the treatment approach for T2DM therapy should be based on patient-centric which should consider various aspects including the need for treatment, preference of patient and values[1]. Although ADA/EASD recommends insulin as effective treatment for the management of T2DM, weight gain is a common side effect associated with it. Thus, weight control during the treatment of diabetes is an important consideration for the T2DM management therapy. In the present study, body weight in add-on therapy of IND-2 with insulin did not differ significantly despite significant improvement in HbA1c which can be considered an advantage of IND-2 along with insulin treatment.

Traditional medicines are considered to be relatively safe option with low side-effects, but their efficacy is found relatively low as compared with Western medicines. Hence, many times the high dose was usually needed to attend the optimal therapeutic efficacy. Thus, their toxicity cannot be neglected in the view of their therapeutic applications[70]. The common adverse event associated with herbal supplementation includes complications related to gastrointestinal tracts such as nausea, vomiting, diarrhea, and constipation[71]. Also, herb-drug interaction related to pharmacokinetics and pharmacodynamics properties needs to be considered during their administration. In the present investigation, add-on therapy of IND-2 with insulin was well tolerated. There was no any serious AEs were reported during the trial. Among the reported AEs, the majority of the events were mild and not related to the IND-2 add-on therapy. However, discontinuation of 4 subjects from the trial due to adverse events is a significant finding. The previous investigator also reported similar kind of cause which lead to treatment discontinuation[72,73]. Increased ESR is reported in a maximum number of patients and tops the list of the number of absolute adverse events. Though, the rise in ESR was reported as not related to drugs, there is a need for further studies with a large number of patient population to confirm the effect of IND-2 on ESR. All the events, which were related to the drug were gastrointestinal tract events. Six significant abnormalities in laboratory parameters were observed which were present during week 8 and not continued till week 16.

Recent year various researchers have investigated the effect of polyherbal formulation on HbA1c levels[74,75]. Administration of polyherbal formulation that contains *Gymnema sylvestre* extract in T2DM for 3 months showed significant improvement in HbA1c levels and antioxidant enzyme activities[74]. Furthermore, a polyherbal formulation containing standardized extracts of *Trigonella foenum-graecum* and *Emblica officinalis* also showed a decrease in HbA1c, FBS and PPBS in the T2DM patient with uncontrolled blood sugar despite a sulfonylurea and metformin stable dose[65]. These studies suggested that *Trigonella foenum-graecum*, *Gymnema sylvestre*, and *Emblica officinalis* were most widely implemented herbs for preparation of polyherbal formulations in the management of diabetes. Additionally, safety and efficacy of *Trigonella foenum-graecum* as an anti-diabetic medication have also been well established clinically[76,77]. Thus, results of current investigation along with findings of earlier researchers both substantially provide the potential of this polyherbal formulation (IND-2) as a safe and well-tolerated add-on treatment option for the T2DM management in the patients with insulin treatment.

The present investigation has certain limitations. The primary limitation of this study is a single-arm study without placebo-control. Hence, study with placebo-control will be required to assess

comparative efficacy of IND-2 in T2DM subjects. Secondly, the sample size of the present investigation is relatively too small, and the study duration is also too short which provides only short-term effects. Hence, analysis of long-term effect with larger sample size remains to be evaluated. Lastly, in this study, we have not evaluated the parameters which give an insight to the possible mechanism of action of IND-2 such as serum insulin level, molecular markers in blood, level of antioxidants in blood, *etc*.

The findings of present investigation suggest that add-on therapy of IND-2 (herbal formulation rich in standardized fenugreek seed extract) with insulin in T2DM patients improved glycaemic control through a decrease in levels of glycated hemoglobin and number of insulin doses needed per day without a change in blood sugar, body weight and risk of hypoglycemia. Thus, IND-2 may provide a safe and well-tolerated add-on therapy option for the management of T2DM.

Conflict of interest statement

Amit Kandhare and Prasad Thakurdesai are the full-time employees of Indus Biotech Pvt. Ltd., Pune.

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References

[1] Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes: A patient-centered approach: Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2012; **35**(6): 1364-1379.

[2] Ghosh P, Kandhare AD, Raygude KS, Kumar VS, Rajmane AR, Adil M, et al. Determination of the long term diabetes related complications and cardiovascular events using UKPDS risk engine and UKPDS outcomes model in a representative western Indian population. *Asian Pac J Trop Dis* 2012; **2**(Suppl 2): S642-S650.

[3] Shivakumar V, Kandhare AD, Rajmane AR, Adil M, Ghosh P, Badgujar LB, et al. Estimation of the long-term cardiovascular events using UKPDS risk engine in metabolic syndrome patients. *Indian J Pharm Sci* 2014; **76**(2): 174-178.

[4] Lian F, Tian J, Chen X, Li Z, Piao C, Guo J, et al. The efficacy and safety of Chinese herbal medicine Jinlida as add-on medication in type 2 diabetes patients ineffectively managed by metformin monotherapy: A double-blind, randomized, placebo-controlled, multicenter trial. *PLoS One* 2015; **10**(6): e0130550.

[5] Seino Y, Kuwata H, Yabe D. Incretin-based drugs for type 2 diabetes: Focus on East Asian perspectives. *J Diabetes Investig* 2016; **7**(Suppl 1): 102-109.

[6] Stulc T, Sedo A. Inhibition of multifunctional dipeptidyl peptidase-Ⅳ: Is there a risk of oncological and immunological adverse effects? *Diabetes Res Clin Pract* 2010; **88**(2): 125-131.

[7] World Health Organization. *Diabetes key facts*. Geneva, Switzerland: World Health Organization; 2011.

[8] Acharya LD, Rau NR, Udupa N, Rajan MS, Vijayanarayana K. Assessment of cost of illness for diabetic patients in South Indian tertiary care hospital. *J Pharm Bioallied Sci* 2016; **8**(4): 314-320.

[9] International Diabetes Federation. IDF global guideline for type 2 diabetes. 2012. [Online]. Available from: http://www.idf.org/guideline-type-2-diabetes. [Accessed on July, 2016].

[10] American Diabetes Association. Summary of revisions to the 2011 clinical practice recommendations. *Diabetes Care* 2011; **34**(Suppl 1): S3.

[11] National Institute of Clinical Excellence. NICE CG28: Type 2 diabetes in adults: management. 2015. [Online]. Available from: https://www.nice.org.uk/guidance/ng28/resources/type-2-diabetes-in-adults-management-1837338615493. [Accessed on December 2015].

[12] Sharma M, Nazareth I, Petersen I. Trends in incidence, prevalence and prescribing in type 2 diabetes mellitus between 2000 and 2013 in primary care: A retrospective cohort study. *BMJ Open* 2016; **6**(1): e010210.

[13] Seino Y, Hiroi S, Hirayama M, Kaku K. Efficacy and safety of alogliptin added to sulfonylurea in Japanese patients with type 2 diabetes: A randomized, double-blind, placebo-controlled trial with an open-label, long-term extension study. *J Diabetes Investig* 2012; **3**(6): 517-525.

[14] Deacon CF, Lebovitz HE. Comparative review of dipeptidyl peptidase-4 inhibitors and sulphonylureas. *Diabetes Obes Metab* 2016; **18**(4): 333-347.

[15] Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: Progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA* 1999; **281**(21): 2005-2012.

[16] Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004; 351(11): 1106-1118.

[17] Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007; **356**(24): 2457-2471.

[18] Kuo IC, Lin HY, Niu SW, Hwang DY, Lee JJ, Tsai JC, et al. Glycated hemoglobin and outcomes in patients with advanced diabetic chronic kidney disease. *Sci Rep* 2016; 6: 20028.

[19] Marin-Penalver JJ, Martin-Timon I, Sevillano-Collantes C, Del Canizo-Gomez FJ. Update on the treatment of type 2 diabetes mellitus. *World J Diabetes* 2016; **7**(17): 354-395.

[20] International Expert C. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 2009; **32**(7): 1327-1334.

[21] Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; **32**(1): 193-203.

[22] Fonseca V, Gill J, Zhou R, Leahy J. An analysis of early insulin glargine added to metformin with or without sulfonylurea: Impact on glycaemic control and hypoglycaemia. *Diabetes Obes Metab* 2011; **13**(9): 814-822.

[23] Gross JL, Kramer CK, Leitao CB, Hawkins N, Viana LV, Schaan BD, et al. Effect of antihyperglycemic agents added to metformin and a sulfonylurea on glycemic control and weight gain in type 2 diabetes: A network meta-analysis. *Ann Intern Med* 2011; **154**(10): 672-679.

[24] Guariguata L, Nolan T, Beagley J. International Diabetes Federation. IDF

diabetes atlas. Brussels: International Diabetes Federation; 2014.

[25]Rodbard HW, Jellinger PS, Davidson JA, Einhorn D, Garber AJ, Grunberger G, et al. Statement by an American Association of Clinical Endocrinologists/American College of Endocrinology consensus panel on type 2 diabetes mellitus: An algorithm for glycemic control. *Endocr Pract* 2009; **15**(6): 540-559.

[26]Prabhakar PK, Doble M. Mechanism of action of natural products used in the treatment of diabetes mellitus. *Chin J Integr Med* 2011; **17**(8): 563-574.

[27]Yang LX, Liu TH, Huang ZT, Li JE, Wu LL. Research progress on the mechanism of single-Chinese medicinal herbs in treating diabetes mellitus. *Chin J Integr Med* 2011; **17**(3): 235-240.

[28]Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol* 2004; **92**(1): 1-21.

[29]Singh J, Cumming E, Manoharan G, Kalasz H, Adeghate E. Medicinal chemistry of the anti-diabetic effects of momordica charantia: Active constituents and modes of actions. *Open Med Chem J* 2011; **5**(Suppl 2): 70-77.

[30]Kumar SN, Mani UV, Mani I. An open label study on the supplementation of Gymnema sylvestre in type 2 diabetics. *J Diet Suppl* 2010; **7**(3): 273-282.

[31]Nahas R, Moher M. Complementary and alternative medicine for the treatment of type 2 diabetes. *Can Fam Physician* 2009; **55**(6): 591-596.

[32]Akhtar MS, Ramzan A, Ali A, Ahmad M. Effect of Amla fruit (*Emblica officinalis* Gaertn.) on blood glucose and lipid profile of normal subjects and type 2 diabetic patients. *Int J Food Sci Nutr* 2011; **62**(6): 609-616.

[33]Jayawardena MH, de Alwis NM, Hettigoda V, Fernando DJ. A double blind randomised placebo controlled cross over study of a herbal preparation containing *Salacia reticulata* in the treatment of type 2 diabetes. *J Ethnopharmacol* 2005; **97**(2): 215-218.

[34]Radha R, Amrithaveni M. Role of medicinal plant *Salacia reticulata* in the management of type ‖ diabetic subjects. *Anc* Sci *Life* 2009; **29**(1): 14-16.

[35]Tanimura C, Terada I, Hiramatu K, Ikeda T, Kasagi T, Kishino E, et al. Effect of a mixture of aqueous extract from *Salacia reticulata* (Kotala himbutu) and cyclodextrin on the serum glucose and the insulin levels in sucrose tolerance test and on serum glucose level changes and gastrointestinal disorder by massive ingestion. *Yonago Igaku Zasshi* 2005; **56**: 85-93.

[36]Sri KS, Kumari DJ, Sivannarayana G. Effect of Amla, an approach towards the control of diabetes mellitus. *Int J Curr Microbiol Appl Sci* 2013; **2**(9): 103-108.

[37]Pan A, Sun J, Chen Y, Ye X, Li H, Yu Z, et al. Effects of a flaxseed-derived lignan supplement in type 2 diabetic patients: A randomized, double-blind, cross-over trial. *PLoS One* 2007; **2**(11): e1148.

[38]Samani NB, Jokar A, Soveid M, Heydari M, Mosavat SH. Efficacy of *Tribulus terrestris* extract on the serum glucose and lipids of women with diabetes mellitus. *Iran J Med Sci* 2016; **41**(3 Suppl): S5-S5.

[39]Samani NB, Jokar A, Soveid M, Heydari M, Mosavat SH. Efficacy of the hydroalcoholic extract of *Tribulus terrestris* on the serum glucose and lipid profile of women with diabetes mellitus: A double-blind randomized placebo-controlled clinical trial. *J Evid Based Complementary Altern Med* 2016; **21**(4): NP91-97.

[40]Thakur G, Mitra A, Pal K, Rousseau D. Effect of flaxseed gum on reduction of blood glucose and cholesterol in type 2 diabetic patients. *Int J Food Sci Nutr* 2009; **60**(Suppl 6): 126-136.

[41]Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. *J Ethnopharmacol* 1997; **58**(3): 149-155.

[42]Madar Z, Abel R, Samish S, Arad J. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *Eur J Clin Nutr* 1988; **42**(1): 51-54.

[43]Sharma R, Raghuram T. Hypoglycaemic effect of fenugreek seeds in non-insulin dependent diabetic subjects. *Nutr Res* 1990; **10**(7): 731-739.

[44]Valette G, Sauvaire Y, Baccou JC, Ribes G. Hypocholesterolaemic effect of fenugreek seeds in dogs. *Atherosclerosis* 1984; **50**(1): 105-111.

[45]Sharma R, Sarkar A, Hazra D, Misra B, Singh J, Maheshwari B. Toxicological evaluation of fenugreek seeds: A long term feeding experiment in diabetic patients. *Phytother Res* 1996; **10**(6): 519-520.

[46]Hibasami H, Moteki H, Ishikawa K, Katsuzaki H, Imai K, Yoshioka K, et al. Protodioscin isolated from fenugreek (*Trigonella foenum graecum* L.) induces cell death and morphological change indicative of apoptosis in leukemic cell line H-60, but not in gastric cancer cell line KATO III. *Int J Mol Med* 2003; **11**(1): 23-26.

[47]Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A. Anti-inflammatory and antioxidative effects of mucilage of *Trigonella foenum graecum* (Fenugreek) on adjuvant induced arthritic rats. *Int Immunopharmacol* 2012; **12**(1): 205-211.

[48]Xue WL, Li XS, Zhang J, Liu YH, Wang ZL, Zhang RJ. Effect of *Trigonella foenum-graecum* (fenugreek) extract on blood glucose, blood lipid and hemorheological properties in streptozotocin-induced diabetic rats. *Asia Pac J Clin Nutr* 2007; **16** (Suppl 1): 422-426.

[49]Baquer NZ, Kumar P, Taha A, Kale RK, Cowsik SM, McLean P. Metabolic and molecular action of *Trigonella foenum-graecum* (fenugreek) and trace metals in experimental diabetic tissues. *J Biosci* 2011; **36**(2): 383-396.

[50]Sayed AA, Khalifa M, Abd el-Latif FF. Fenugreek attenuation of diabetic nephropathy in alloxan-diabetic rats: Attenuation of diabetic nephropathy in rats. *J Physiol Biochem* 2012; **68**(2): 263-269.

[51]Hfaiedh N, Alimi H, Murat JC, Elfeki A. Protective effects of fenugreek (*Trigonella foenum graecum* L.) upon dieldrin-induced toxicity in male rat. *Gen Physiol Biophys* 2012; **31**(4): 423-430.

[52]Sushma N, Devasena T. Aqueous extract of *Trigonella foenum graecum* (fenugreek) prevents cypermethrin-induced hepatotoxicity and nephrotoxicity. *Hum Exp Toxicol* 2010; **29**(4): 311-319.

[53]Xu LL, Zou K, Wang JZ, Wu J, Zhou Y, Dan FJ, et al. New polyhydroxylated furostanol saponins with inhibitory action against NO production from *Tupistra chinensis* rhizomes. *Molecules* 2007; **12**(8): 2029-2037.

[54]Sharma RD, Raghuram TC, Rao NS. Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *Eur J Clin Nutr* 1990; **44**(4): 301-306.

[55]Ribes G, Sauvaire Y, Baccou JC, Valette G, Chenon D, Trimble ER, et al. Effects of fenugreek seeds on endocrine pancreatic secretions in dogs. *Ann Nutr Metab* 1984; **28**(1): 37-43.

[56]Haeri MR, Izaddoost M, Ardekani MR, Nobar MR, White KN. The effect of fenugreek 4-hydroxyisoleucine on liver function biomarkers and glucose in diabetic and fructose-fed rats. *Phytother Res* 2009; **23**(1): 61-64.

[57]Narender T, Puri A, Shweta, Khaliq T, Saxena R, Bhatia G, et al. 4-hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent. *Bioorg Med Chem Lett* 2006; **16**(2): 293-296.

[58]Singh AB, Tamarkar AK, Narender T, Srivastava AK. Antihyperglycaemic effect of an unusual amino acid (4-hydroxyisoleucine) in C57BL/KsJ-db/db mice. *Nat Prod Res* 2010; **24**(3): 258-265.

[59]Sauvaire Y, Petit P, Broca C, Manteghetti M, Baissac Y, Fernandez-Alvarez J, et al. 4-Hydroxyisoleucine: A novel amino acid potentiator of insulin secretion. *Diabetes* 1998; **47**(2): 206-210.

[60]Kandhare AD, Rais N, Moulick ND, Deshpande A, Thakurdesai PA, Bhaskaran S. Efficacy and safety of herbal formulation rich in standardized fenugreek seed extract as add-on supplementation in patients with type 2 diabetes mellitus on sulphonylurea therapy: A 12-week, randomized, double-blind, placebo-controlled, multi-center study. *Pharmacogn Mag* 2018; **14**(57): S393–402.

[61]Weir DL, McAlister FA, Senthilselvan A, Minhas-Sandhu JK, Eurich DT. Sitagliptin use in patients with diabetes and heart failure: A population-based retrospective cohort study. *JACC Heart Fail* 2014; **2**(6): 573-582.

[62]Chon S, Gautier JF. An update on the effect of incretin-based therapies on beta-cell function and mass. *Diabetes Metab J* 2016; **40**(2): 99-114.

[63]Huyen VT, Phan DV, Thang P, Ky PT, Hoa NK, Ostenson CG. Antidiabetic effects of add-on *Gynostemma pentaphyllum* extract therapy with sulfonylureas in type 2 diabetic patients. *Evid Based Complement Alternat Med* 2012; **2012**: 452313.

[64]Parkin CG, Brooks N. Is postprandial glucose control important? Is it practical in primary care settings? *Clin Diabetes* 2002; **20**(2): 71-76.

[65]Banerji S, Banerjee S. A formulation of grape seed, Indian gooseberry, turmeric and fenugreek helps controlling type 2 diabetes mellitus in advanced-stage patients. *Eur J Integr Med* 2016; **8**(5): 645-653.

[66]Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016; **11**: 95-104.

[67]Khatun MA, kumar Prodhan U, Rahman N. Acute effects of green tea (*Camellia sinensis*) intake instead of anti-diabetic drug on hepatic enzymes and atherogenic risk factors in type 2 diabetic patients. *Int J Adv Res Biol Sci* 2017; **4**(3): 172-178.

[68]Dayer-Berenson L, Finckenor M, Tuzzolino M. Are add-on agents to statin therapy necessary in hypercholesterolemia? *Clin Lipidol* 2014; **9**(6): 695-707.

[69]Li FF, Shen Y, Sun R, Zhang DF, Jin X, Zhai XF, et al. Effects of vildagliptin add-on insulin therapy on nocturnal glycemic variations in uncontrolled type 2 diabetes. *Diabetes Ther* 2017; **8**(5): 1111-1122.

[70]Chan K, Zhang H, Lin ZX. An overview on adverse drug reactions to traditional Chinese medicines. *Br J Clin Pharmacol* 2015; **80**(4): 834-843.

[71]Zhang J, Onakpoya IJ, Posadzki P, Eddouks M. The safety of herbal medicine: From prejudice to evidence. *Evid Based Complement Alternat Med* 2015; **2015**: 316706.

[72]Davidson MB, Peters AL. An overview of metformin in the treatment of type 2 diabetes mellitus. *Am J Med* 1997; **102**(1): 99-110.

[73]Charpentier G, Fleury F, Kabir M, Vaur L, Halimi S. Improved glycaemic control by addition of glimepiride to metformin monotherapy in type 2 diabetic patients. *Diabet Med* 2001; **18**(10): 828-834.

[74]Mahajan S, Chauhan P, Subramani SK, Anand A, Borole D, Goswamy H, et al. Evaluation of "GSPF kwath": A *Gymnema sylvestre*-containing polyherbal formulation for the treatment of human type 2 diabetes mellitus. *Eur J Integr Med* 2015; **7**(3): 303-311.

[75]Ghorbani A. Clinical and experimental studies on polyherbal formulations for diabetes: Current status and future prospective. *J Integr Med* 2014; **12**(4): 336-345.

[76]Neelakantan N, Narayanan M, de Souza RJ, van Dam RM. Effect of fenugreek (*Trigonella foenum–graecum* L.) intake on glycemia: A meta-analysis of clinical trials. *Nutr J* 2014; **13**: 7.

[77]Roberts KT. The potential of fenugreek (*Trigonella foenum–graecum*) as a functional food and nutraceutical and its effects on glycemia and lipidemia. *J Med Food* 2011; **14**(12): 1485-1489.