Antidiabetic and Antilipidemic activity of Poly herbal formulation (PHF) in STZ-NA induced diabetes in rats

Uma P¹, Venkatachalam V V², Mani Chandrika P³, Sorabh Kumar Agrawal⁴

¹Research Scholar, Department of Pharmacology, Annamalai University, Chidambaram-608002, Tamil Nadu, India
²Department of Pharmacy, Annamalai University, Chidambaram-608002, Tamil Nadu, India
³Bojjam Narasimhulu Pharmacy College for Women, Sayeedabad-500059, Telangana State, India
⁴Anwarul Uloom College of Pharmacy, New Mallepally-500008, Telangana State, India

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ABSTRACT

The effects of polyherbal formulations were studied in the streptozotocin-nicotinamide (STZ-NA) induced diabetes rat model. The present study was undertaken to assess the effects of polyherbal formulations on the blood sugar level (BSL) as well as blood lipid level (BLL) of STZ-NA diabetic rats. The leaves of *Moringa oleifera* and roots of *Raphanus raphanistrum* were used for the study due to the presence of various phytoconstituents such as alkaloids, saponins, tannins, steroids, phenolic acids, flavonoids. Three polyherbal formulations were prepared from different portions of leaves of *Moringa oleifera* and roots of *Raphanus raphanistrum* and titled PHF-I, PHF-II and PHF-III. Diabetes in experimental animals was induced by STZ injection intraperitoneally (i. p) after 30 min of Nicotinamide injection i. p in all animal groups except normal control group animals. Group I served as normal control received no treatment. Group II served as negative control received streptozotocin-nicotinamide. Group III rats were treated with Metformin, Group IV, Group V and Group VI rats treated with PHF-I, PHF-II and PHF-III respectively. Physical parameters (body weight, feed and water intake), Biochemical parameters (Blood Glucose, Serum Insulin, Serum C-Peptide Level, Serum Leptin, Serum Total cholesterol, Serum Triglycerides, LDL and VLDL) were measured on 0th, 14th and 28th day. The study results and histopathology of the pancreas indicate that oral administration of polyherbal formulation- II proved as a more effective, safe anti-diabetic agent in comparison to Polyherbal formulation I and III by Decrease in body weight, fasting blood glucose, serum glucose level. Increase in serum insulin level, serum C-peptide with a significant decrease in blood serum lipid level.

INTRODUCTION

Diabetes Mellitus (DM) is a major metabolic disorder characterized by increased blood glucose and disturbance in carbohydrate, lipid and protein metabolism and insulin secretion (Valiathan, 1998). The major cause of Diabetes Mellitus is the shortage of insulin secretion and decline in cell response toward insulin. As per a recent WHO Report, almost 580 million adults living with diabetes around the globe. India also has more than 80 million diabetic
individuals which are currently diagnosed with the disease (Kaveeshwar, 2014). The spread of DM is more in recent years due to modern lifestyle linked with an increase in overweight and sedentary population. Diabetes increases dyslipidemia inpatient which enhances the risk of stroke and myocardial infarction (Brown, 1994). Earlier research indicates that abnormalities of lipoprotein metabolism are also observed in diabetic patients. Hyperlipidemia is the primary factor, increase the risk of the earlier development of atherosclerosis and cardiovascular complications (Goldstein et al., 1973). As per American Heart Association (AHA), elevated levels of total cholesterol (TC) and triglycerides [TG] in serum are the primary risk factor, associated with the progression of atherosclerotic lesions (Kaur et al., 2002). Delay in management of diabetes may increase the risk and complications in diabetic patients like diabetic ketoacidosis, cardiovascular disease, stroke, nephropathy, foot ulcers and retinopathy.

Many research investigations suggested that medicinal plants are the best alternative to treat diabetic conditions like lowering lipid and glucose levels and management of diabetic complications.

The present study was planned to investigate the effect of different polyherbal formulations in Streptozotocin and Nicotinamide induced diabetic rats.

**MATERIALS AND METHODS**

**Collection and authentication of plant material**

The medicinal plants were collected from authenticating suppliers and authenticated by scientists of the Botanical Survey of India. About 200 g of leaves powder of each plant was taken in an RBF and infused in 500 ml D.W. Chloroform (10 ml) was added in the poly formulation as a preservative. Then the residue was removed by filtering the extract and preserved in an airtight container for further use.

**Preparation and standardization of Polyherbal formulations**

Three different formulations were prepared and named as A, B and C in which Formulation A contains 50% of *Moringa oleifera* and 50% *Raphanus raphanistrum*, Formulation B contains 70% of *Moringa oleifera* and 30% *Raphanus raphanistrum* and Formulation C contains 30% of *Moringa oleifera* and 70% *Raphanus raphanistrum* (Uma et al., 2020). Physicochemical properties such as colour, odour, taste, pH, particle size, viscosity, specific gravity and stability testing were done to standardize the Poly Herbal Formulations (Nitin et al., 2010; Rekha and Amrish, 2013).

**Acute Toxicity Testing**

The acute oral toxicity of polyherbal formulation was determined as per OECD guidelines at doses of 50, 500, 1000, 2000 and 5000 mg/kg. Behaviours changes in rats were observed for 14 days after dosing. Mortality of rats was used to calculate the mean lethal dose (LD50) value (Arulselvan et al., 2006; Lanjhiyana et al., 2011).

**Experimental Animals Setup**

We housed Thirty-six (36) healthy albino Wistar rats of either sex weighing between 180-200 g in an animal facility. The experiment was conducted according to the approved methods of the institutional committee. The animals were kept for two weeks on a normal diet and water ad libitum prior to the start of the experiment. After the adjustment period, rats were randomly divided into the following groups.

- **Group I Non-diabetic (ND) rats**: received Distilled water and served as normal control
- **Group II Streptozotocin-Nicotinamide diabetic rats**: received Distilled water and served as a negative control
- **Group III Streptozotocin-Nicotinamide diabetic rats**: received Metformin-100 mg/kg/day, p. o and served as the standard group
- **Group IV Streptozotocin-Nicotinamide diabetic rats treated with polyherbal formulation- A**: (500 mg/kg/day, p. o)
- **Group V Streptozotocin-Nicotinamide diabetic rats treated with polyherbal formulation- B**: (500 mg/kg/day, p. o)
- **Group VI Streptozotocin-Nicotinamide diabetic rats treated with polyherbal formulation- C**: (500 mg/kg/day, p. o)

**Induction of Diabetes and Administration of Polyherbal Formulation**

The study was designed for 28 days by repeated oral administration. Induction of diabetes in overnight fasted rats of Group II to VI was done with a single dose of Nicotinamide (110 mg/kg b w) first followed by a single dose of Streptozotocin (55 mg/kg b w) once (Ng et al., 2015). Then rats of Group II to VI were fed with 5% glucose solution for the next 24 h to prevent fatal hypoglycemia and examined for 72 h to record diabetes by measuring BSL with help of a glucometer (Szkudelski, 2012). The rats have elevated levels of blood glucose levels marked as Diabetic rats while normal untreated marked as non-diabetic (ND) rats.

Rats body weight was measured with a digital
The blood glucose test is done with an Accu Chek glucometer (Kumar et al., 2011). The blood glucose test was performed using a commercially available glucometer (Accu-Chek Active Blood Glucose Meter Kit). Serum glucose was determined by using a commercially available kit (Accu-Chek Active Blood Glucose Meter Kit). Serum Insulin, Serum C-Peptide and Serum Leptin was determined by using a commercially available ELISA kit.

**Monitoring of Lipid level**

Serum total cholesterol, HDL, LDL, VLDL and triglycerides levels were determined on the 1st, 14th, and 28th day of treatment (Steel et al., 1997).

**Statistical Analysis**

Data were analyzed statistically by using the analysis of variance (ANOVA). Duncan multiple range tests was applied in case of significant difference among the experimental groups at a level of significance (Steel et al., 1997).

**RESULTS**

**Acute oral Toxicity**

Acute oral toxicity studies results revealed the non-toxic nature of the polyherbal formulation. The rats treated with polyherbal formulations were normal and did not exhibit any significant fluctuations in behavioural or neurological responses.

There was no mortality or toxicity reaction up to 5000mg/kg body weight of polyherbal formulation. So LD₅₀ of Polyherbal formulation was considered 1/10 of 5000 mg/kg (Lanjhiyana et al., 2011).

**Histopathology**

Tissue section of pancreas collected from sacrificed rats and processed for histopathological examinations (Figure 1).

**Histopathology of Pancreas**

(A) Histopathology of control group animals exhibit normal pancreatic cells with fully active islets of Langerhans in pancreatic parenchyma (B) Histopathology of negative control exhibit a number of tiny islets of Langerhans, the ruination of β cells and loss of cellular contents (C) Histopathology of the Metformin treated group exhibit almost normal structure of pancreas and regular nuclei of cells (D) Histopathology of polyherbal formulation treated group I exhibit delicate necrotic changes in pancreatic β cells (E) Histopathology of polyherbal formulation treated group II exhibit typical pancreatic parenchyma and active β cells in islets of Langerhans. (F) Histopathology of polyherbal formulation treated group III exhibit a less number of islets of Langerhans and cell inflammation in β cells of pancreas.

**DISCUSSION**

In the present study, STZ-NA was used as an inducer of diabetes in albino rats to understand the pathophysiology of diabetes in order to develop and design new drugs for its cure and management (Viana et al., 2004; Soliman, 2013). Acute oral toxicity was performed according to OECD guidelines. Based upon acute oral toxicity the therapeutic dose was selected to assess the anti-diabetic potential of polyherbal formulations in STZ-NA induced diabetes in rats.

In the present study, many physical variables like B.W., food and water intake was noted. Results have exhibited a decrease in B.W. of diabetic rats in comparison to the normal control group, results shown in Table 1. A decrease in body weight may be due to increasing gluconeogenesis and lipolysis of triglycerides and structural proteins degradation (Eubehi et al., 2010). The results revealed in Table 2, that Polyherbal formulation- II significantly (P≤0.01) elevated the body weight in comparison to Metformin treated group (Rout et al., 2013; Agarwal et al., 2012). An increase in fasting blood glucose and serum glucose level was observed in diabetic rats, results shown in Table 3. Polyherbal formulation-II produces significant negative effects on fasting blood glucose and serum glucose level (Ezeigbo et al., 2016; Subhasree et al., 2015). The polyherbal formulation II significantly (P≤0.01) increased serum insulin level in diabetic rats up to
### Table 1: Feed intake (mg/day) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Days | Treatments          | Control | - ve control | Standard | PHF I (500 mg/kg) | PHF II (500 mg/kg) | PHF III (500 mg/kg) |
|------|---------------------|---------|--------------|----------|-------------------|-------------------|---------------------|
| 0    |                     | 11.05±0.7 | 31.00±0.86   | 31.90±0.58 | 33.90±0.71       | 33.50±0.58       | 32.8±0.51           |
| 14   |                     | 17.60±0.55 | 36.00±0.84   | 27.80±0.51 | 30.40±0.55       | 28.00±0.55       | 29.40±0.51           |
| 18   |                     | 25.80±0.55 | 39.20±0.68   | 26.20±0.37 | 30.00±0.32       | 27.80±0.33       | 27.80±0.37           |

Mean values represented in table in Mean±SE, where n= 6

### Table 2: Body weight (gm) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Day  | Treatments          | Control | -ve control | Standard | PHF I (500 mg/kg) | PHF II (500 mg/kg) | PHF III (500 mg/kg) |
|------|---------------------|---------|--------------|----------|-------------------|-------------------|---------------------|
| 0    |                     | 190.55±4.90 | 188.02±4.96   | 188.47±4.28 | 186.28±3.23       | 187.50±3.30       | 188.00±3.86           |
| 14   |                     | 221.18±3.54 | 166.39±6.15   | 210.62±4.80 | 195.35±3.50       | 204.00±3.85       | 204.00±4.24           |
| 18   |                     | 259.64±5.41 | 149.68±3.7   | 238.16±4.38 | 203.88±3.90       | 236.00±3.41       | 211.00±3.29           |

Mean values represented in table in Mean±SE, where n= 6

### Table 3: Fasting Blood Glucose(mg/dl) level after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Day  | Treatments          | Control | -ve control | Standard | PHF I (500 mg/kg) | PHF II (500 mg/kg) | PHF III (500 mg/kg) |
|------|---------------------|---------|--------------|----------|-------------------|-------------------|---------------------|
| 0    |                     | 108.61±4.37 | 443.82±4.37  | 437.55±3.24 | 430.91±4.37       | 441.85±3.21       | 427.30±4.38           |
| 14   |                     | 113.80±3.48 | 486.57±4.81  | 231.41±2.64 | 347.37±5.49       | 262.51±4.38       | 316.72±4.87           |
| 28   |                     | 112.80±4.77 | 531.81±4.29  | 147.38±2.83 | 256.58±3.27       | 158.62±4.51       | 246.37±4.28           |

Mean values represented in table in Mean±SE, where n= 6

### Table 4: Serum Glucose (mg/dL) level after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments          | Day 0       | Days Day 14 | Day 28   | Over all Mean |
|---------------------|-------------|-------------|----------|---------------|
| Control             | 106.82±4.84 | 111.61±4.13 | 109.81±4.28 | 109.42±2.43  |
| -ve control         | 444.20±8.11 | 476.20±6.71 | 519.00±5.31 | 479.8±6.20   |
| Metformin           | 428.00±4.20 | 221.00±3.67 | 138.20±2.65 | 261.40±22.32 |
| PHF I (500 mg/kg)   | 429.40±4.12 | 326.40±2.73 | 233.60±2.62 | 329.80±16.20 |
| PHF II (500mg/kg)   | 436.20±3.81 | 244.20±3.43 | 143.00±3.07 | 274.8±20.01  |
| PHF III (500 mg/kg) | 418.40±3.49 | 292.80±3.97 | 224.20±3.38 | 311.80±15.85 |

Mean values represented in table in Mean±SE, where n= 6
### Table 5: Serum Insulin (U/L) level after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Day 0        | Day 14      | Day 28      | Over all Mean |
|------------------|--------------|-------------|-------------|---------------|
| Control          | 15.85±0.59   | 16.94±0.54  | 17.59±0.69  | 17.34±0.62    |
| -ve control      | 7.50±0.67    | 6.81±0.45   | 6.58±0.75   | 6.96±0.32     |
| Metformin        | 7.42±0.84    | 13.95±0.77  | 17.65±0.73  | 13.01±1.23    |
| PHF I (500 mg/kg)| 7.66±0.85    | 10.38±0.75  | 12.23±0.71  | 10.09±0.69    |
| PHF II (500 mg/kg)| 7.34±0.82  | 12.76±0.79  | 15.78±0.64  | 11.96±1.15    |
| PHF III (500 mg/kg)| 7.47±0.52  | 11.94±0.64  | 14.65±0.67  | 11.35±0.89    |

Mean values represented in table in Mean±SE, where n= 6

### Table 6: Serum C-peptide (ng/mL) level after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Day 0        | Day 14      | Day 28      | Over all Mean |
|------------------|--------------|-------------|-------------|---------------|
| Control          | 1.57±0.06    | 1.48±0.06   | 1.53±0.05   | 1.52±0.03     |
| -ve control      | 0.68±0.05    | 0.51±0.06   | 0.47±0.06   | 0.55±0.04     |
| Metformin        | 0.71±0.05    | 1.27±0.05   | 1.49±0.03   | 1.16±0.12     |
| PHF I (500 mg/kg)| 0.70±0.05    | 0.94±0.04   | 1.03±0.05   | 0.89±0.04     |
| PHF II (500 mg/kg)| 0.69±0.06  | 1.07±0.05   | 1.21±0.05   | 0.99±0.06     |
| PHF III (500 mg/kg)| 0.65±0.06  | 1.18±0.05   | 1.34±0.06   | 1.05±0.09     |

Mean values represented in table in Mean±SE, where n= 6

### Table 7: Serum leptin (ng/mL) level after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Day 0        | Day 14      | Day 28      | Overall Mean |
|------------------|--------------|-------------|-------------|--------------|
| Control          | 4.09±0.08    | 4.16±0.15   | 4.11±0.15   | 4.12±0.07    |
| -ve control      | 2.25±0.07    | 1.87±0.30   | 1.72±0.21   | 1.94±0.12    |
| Metformin        | 2.16±0.11    | 2.74±0.23   | 3.07±0.21   | 2.65±0.14    |
| PHF I (500 mg/kg)| 2.09±0.10    | 2.36±0.18   | 2.41±0.16   | 2.28±0.09    |
| PHF II (500 mg/kg)| 2.17±0.20  | 2.62±0.17   | 2.87±0.17   | 2.55±0.12    |
| PHF III (500 mg/kg)| 2.21±0.21  | 2.43±0.21   | 2.64±0.18   | 2.42±0.12    |

### Table 8: Serum Total Cholesterol (mg/dL) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Day 0        | Day 14      | Day 28      | Overall Mean |
|------------------|--------------|-------------|-------------|--------------|
| Control          | 121.60±4.30  | 120.00±3.90 | 120.20±3.62 | 120.60±2.12  |
| -ve control      | 206.60±6.27  | 230.60±4.57 | 266.80±5.48 | 234.67±7.23  |
| Metformin        | 200.20±4.08  | 168.20±3.76 | 140.20±3.25 | 169.20±9.38  |
| PHF I (500 mg/kg)| 198.80±3.77  | 175.20±3.31 | 142.00±2.47 | 172.00±6.46  |
| PHF II (500 mg/kg)| 197.00±3.75 | 144.80±2.75 | 111.00±2.70 | 150.60±9.40  |
| PHF III (500 mg/kg)| 197.20±3.77 | 166.80±2.92 | 128.20±3.06 | 164.07±7.75  |

Mean values represented in table in Mean±SE, where n= 6
Table 9: Serum Triglycerides (mg/dL) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Days        | Overall Mean |
|------------------|-------------|--------------|
|                  | Day 0       | Day 14       | Day 28       |               |
| Control          | 68.80±1.28  | 69.80±1.16   | 69.20±1.28   | 69.27±0.67   |
| -ve control      | 120.40±4.21 | 148.40±3.37  | 164.60±3.03  | 144.47±5.24  |
| Metformin        | 120.60±1.36 | 107.60±1.86  | 91.00±1.95   | 106.40±3.37  |
| PHF I (500 mg/kg)| 121.60±2.32 | 119.20±1.20  | 110.40±2.27  | 116.66±1.58  |
| PHF II (500mg/kg)| 117.40±1.91 | 88.40±1.50   | 70.80±1.32   | 91.60±5.21   |
| PHF III (500 mg/kg)| 119.60±1.91| 112.20±2.15  | 109.40±2.69  | 113.73±1.67  |

Mean values represented in table in Mean±SE, where n= 6

Table 10: Mean±SE High Density Lipoproteins Cholesterol (mg/dL) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Days        | Overall Mean |
|------------------|-------------|--------------|
|                  | Day 0       | Day 14       | Day 28       |               |
| Control          | 39.40±0.81  | 40.20±0.86   | 40.60±0.93   | 40.07±0.48   |
| -ve control      | 23.60±1.50  | 19.60±1.75   | 17.80±1.83   | 20.33±1.12   |
| Metformin        | 20.80±0.86  | 27.40±0.51   | 32.40±0.68   | 26.33±1.75   |
| PHF I (500 mg/kg)| 21.80±1.20  | 24.80±0.86   | 27.80±0.58   | 24.80±0.82   |
| PHF II (500mg/kg)| 23.80±1.43  | 30.60±1.29   | 34.20±0.73   | 29.53±1.75   |
| PHF III (500 mg/kg)| 21.20±0.86 | 27.40±0.51   | 31.40±0.51   | 26.67±1.17   |

Mean values represented in table in Mean±SE, where n= 6

Table 11: Mean±SE Serum low density lipoproteins (mg/dL) after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Days        | Overall Mean |
|------------------|-------------|--------------|
|                  | Day 0       | Day 14       | Day 28       |               |
| Control          | 68.49±0.94  | 65.93±1.87   | 65.12±1.60   | 66.52±0.90   |
| -ve control      | 161.34±1.82 | 189.82±7.19  | 221.04±7.51  | 190.73±7.28  |
| Metformin        | 158.00±3.08 | 106.80±2.82  | 65.60±0.51   | 110.13±10.18 |
| PHF I (500 mg/kg)| 156.00±4.30 | 126.20±2.67  | 90.20±0.73   | 124.13±7.36  |
| PHF II (500mg/kg)| 157.53±4.57 | 94.00±1.05   | 60.40±1.03   | 103.98±10.8  |
| PHF III (500 mg/kg)| 157.80±6.51| 114.40±3.39  | 74.06±0.42   | 115.42±9.42  |

Mean values represented in table in Mean±SE, where n= 6

Table 12: Serum very low density lipoproteins g/dL after oral administration of STZ-NA, Metformin and Polyherbal formulations at 0, 14th and 28th day

| Treatments       | Days        | Overall Mean |
|------------------|-------------|--------------|
|                  | Day 0       | Day 14       | Day 28       |               |
| Control          | 13.76±0.33  | 13.86±0.37   | 13.86±0.37   | 13.83±0.19   |
| -ve control      | 23.11±0.64  | 29.68±0.87   | 32.96±0.83   | 28.92±1.06   |
| Metformin        | 24.12±0.41  | 20.52±0.24   | 19.23±0.13   | 21.29±0.66   |
| PHF I (500 mg/kg)| 25.32±0.61  | 22.83±0.30   | 21.30±0.27   | 23.48±0.32   |
| PHF II (500mg/kg)| 24.49±0.81  | 17.64±0.23   | 15.16±0.20   | 18.43±1.06   |
| PHF III (500 mg/kg)| 26.94±0.47 | 21.45±0.22   | 21.89±0.34   | 22.76±0.30   |

Mean values represented in table in Mean±SE, where n= 6
28 days of treatment, results shown in Table 4. An increase in the level of Insulin may be due to discharge from existing β cells as well as increased release of glucose into peripheral tissues (Broadhurst et al., 2000). Serum C-peptide level plays a key role in insulin secretion (Table 5). The results of the present study revealed a significantly (P ≤ 0.01) decrease in serum C-peptide and serum insulin levels in polyherbal formulation I treated diabetic animals (Hossain et al., 2016). Metformin and Polyherbal formulations significantly (P ≤ 0.01) raised serum C-peptide levels resulting in improved glucose metabolism (Kang et al., 2010).

Elevation in lipid parameters is a common complication arise with diabetes mellitus. The present study results are shown in Table 7, Table 8, Table 9, Table 10, Table 11 and Table 12 exhibit the significant (P ≤ 0.01) rise in serum cholesterol, triglycerides, LDL and VLDL levels in the Streptozotocin-nicotinamide induced diabetic rats. Treatment of diabetic rats with different polyherbal formulation extracts resulted in a gradual decrease in serum cholesterol, triglycerides, VLDL and LDL levels and an increase in High-density lipid levels at the 14th and 28th days of treatment (Vidhya and Udayakumar, 2016).

The lipid-lowering effect of polyherbal formulation arises due to the presence of flavonoids which have already been reported as cholesterol and triglycerides lowering potential by the action of HMG-CoA reductase and cholesterol-regulating enzyme (ACAT). HMG-CoA decreases the biosynthesis of cholesterol while cholesterol-regulating enzyme plays a critical role in the absorption and esterification of cholesterol (Pandey et al., 2012). The Current study demonstrates the increase of serum HDL, known as good cholesterol plays an important role in coronary disease management (Youl et al., 2010).

**CONCLUSION**

The result indicates that Poly herbal formulation-II demonstrate as an effective, safe anti-diabetic agent. Development of Hyperglycemia and hyperlipidemia in diabetes and oxidative stress and effect of Poly herbal formulation-II should be analyzed in detail to understand the cellular level changes.

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Conflict of Interest
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