The Pharma Innovation Journal 2024; 13(3): 175-183

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2024; 13(3): 175-183

www.thepharmajournal.com
Received: 11-01-2024
Accepted: 15-02-2024

Shesha Rao
Assistant Professor, BRIC Dornhalli Shahapur Yadgir, Dornahalli, Karnataka, India

Manjunatha KP
Assistant Professor, Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangaluru, Karnataka, India

Nagaraja K
Assistant Professor, Institute of Animal Health and Veterinary Biologicals (IAH & VB) Davengere Karnataka, Karnataka, India

Suguna Rao
Professor and Head Department of Veterinary Pathology, Veterinary College, Hebbal, KVAFSU, Bangalore, Karnataka, India

Sudharani
Assistant Professor, Institute of Animal Health and Veterinary Biologicals (IAH&VB) Hebbal Bangaluru, Karnataka, India

Corresponding Author:
Shesha Rao
Assistant Professor, BRIC Dornhalli Shahapur Yadgir, Dornahalli, Karnataka, India

Pathomorphological and hematologicals evaluation of antidiabetic effect of *trigonella foenum graecum* and *coccinia indica* in induced diabetes in Wister rats

Shesha Rao, Manjunatha KP, Nagaraja K, Suguna Rao and Sudharani

Abstract
The present study was taken up to evaluate the efficacy of *Trigonella foenum graecum* and *Coccinia indica* individualy and in combination along with glibenclamide in streptozotocin induced diabetic rats for a period of 90 days. The various groups in this study included normal control (Group-I), diabetic control (Group-II), diabetic rats treated with glibenclamide (Group-III), diabetic rats treated with *Trigonella foenum graecum* (Group-IV), diabetic rats treated with *Coccinia indica* (Group-V), diabetic rats treated with *Trigonella foenum graecum and Coccinia indica* (Group-VI), diabetic rats treated with *Trigonella foenum graecum* and glibenclamide (Group-VII), diabetic rats treated with *Coccinia indica* and glibenclamide (Group-VIII). Diabetic rats treated with *Trigonella foenum graecum*, *Coccinia indica* and glibenclamide (Group-IX) respectively. There was significant variation in Hematological and pathomorphological parameters of diabetic rats when compared to normal control rats. The alleviation of the diabetic and its complications induced by streptozotocin was observed in all the treatment groups with variable degree of improvement. *Trigonella foenum graecum* and *Coccinia indica* extracts were effective in alleviating streptozotocin induced diabetes and were comparable with glibenclamide. Combination of *Trigonella foenum graecum* with glibenclamide and *Coccinia indica* with glibenclamide showed better improvement in hematological parameters compared to individual extracts alone and improvement was statistically significant. However the combined treatment of *Coccinia indica* with glibenclamide half dose and *Trigonella foenum graecum* and *Coccinia indica* with glibenclamide half dose revealed a very good improvement in hematological parameters which indicated a synergistic effect between *Coccinia indica* and glibenclamide half dose.

Keywords: Diabetes, *Trigonella foenum gaecum*, *Coccinia indica*, glibenclamide, streptozotocin

Introduction
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. These metabolic abnormalities result either from a deficiency of the blood sugar-lowering hormone insulin or from insulin resistance, a defect in the body's capacity to respond to insulin (Chandra *et al*., 2004) [3]. The estimated global healthcare expenditure to treat and prevent diabetes is projected to exceed USD 490 billion by 2030 (Ramchandran *et al*., 2010) [17].
Diabetes is also associated with lifestyle factors and genetics (Craig *et al*., 2009) [4]. Chromosomal and mitochondrial DNA mutation, chemicals and drugs like pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, beta adrenergic agonists, thiazides, alpha interferon can cause diabetes.
Herbal medication has been used for the treatment of variety of ailments and a huge number of populations in the world are still entirely dependent upon traditional medicines. A number of medicinal plants and their formulations are being used for treating diabetes in Ayurvedic medicine system as well as in ethnomedicinal practices (Pareek *et al*. 2009) [15].
There is a need for studies on replacement of oral antidiabetic treatment with herbal medicines by experimental research in animal models. Hence, the present study was conducted with *Trigonella foenum graecum* and *Coccinia indica* commonly known as methi and Little gourd (kovai) respectively which are reported to possess hypoglycaemic effect (Khalki *et al*., 2010) [7] to evaluate their antidiabetic effect individually and in combination in comparison with an oral antidiabetic drug glibenclamide.

1. To evaluate the Antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in induced diabetes in rats.
2. To study pathomorphological and hematological changes in induced and treated diabetic rats.

3. To compare the hypoglycemic effects of *Trigonella foenum graecum* and *Coccinia indica* with an oral hypoglycemic agent glibenclamide.

Materials and Methods

Experimental animals

Healthy female albino Wistar rats weighing 190±20g obtained from R.L Instrument and lab animal supplier, Yeswanthpur, Bangalore, were used for the present investigation. The animals were maintained under standard laboratory conditions, with provision of standard laboratory animal feed (Amruth Feeds, Banglore) and clean drinking water *ad libitum*. The animals were acclimatized to the experimental conditions for two weeks after procurement. After acclimatization, animals were grouped and housed in polypropylene rat cages during the experimental period. The experiment was carried out for a period of 90 days. The study was carried out with a prior approval from the Institutional Animal Ethical Committee, Veterinary College, and Bangalore.

Sources: Drugs and chemicals

To induce experimental diabetes in rats, streptozotocin was used which was procured from Sigma Chemicals, St. Louis, USA. All the other chemicals used for the study were of analytical grade.

Preparation of STZ solution

Fresh 0.1 M citrate buffer having pH 4.5 was prepared and the same was maintained at 4-8°C. The STZ of required quantity was dissolved in ice-cold citrate buffer and injected intraperitoneally to rats immediately to avoid degradation.

Trigonella foenum graecum

The alcoholic seed extract of *Trigonella foenum graecum* used in the present study was obtained from Plantex Herbal Drug Company, Vijaywada. The powdered extract was weighed according to body weight and dissolved in distilled water to make the final concentration and given to the expimental animals.

Coccinia indica

The alcoholic plant extract of *Coccinia indica* used in the present study was obtained from Plantex Herbal Drug Company, Vijaywada. The powdered extract was weighed according to body weight and dissolved in distilled water to make the final concentration and given to the expimental animals.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg), an oral hypoglycaemic drug was dissolved in distilled water (82.33 ml) to give a concentration of 60 µg/ml. This was used as a stock solution and administered orally at a dose of 600 µg/ kg using clean and dry gavaging needles (Ramalingam *et al*., 2004)[18].

Administration of plant extracts and glibenclamide

Throughout the period of the experiment the plant extracts and glibenclamide were administered orally for their respective groups using clean gavaging needle attached to an appropriate disposable syringe during morning hours of the day for a period of 90 days.

Experimental design

One hundred and eight female albino Wistar rats were weighed and randomly distributed into nine groups of twelve rats each. Care was taken to maintain the intra-group weight variation to be less than 25 g and inter-group weight variation by 35 g.

The groups and treatments used were as follows

Group I (NC)	Normal control: Used for studying baseline values of the parameters
Group II (DC)	Diabetic control: Streptozotocin induced diabetic rats
Group III (GC)	Diabetic rats supplemented with glibenclamide at a dose of 600 µg / kg
Group IV (TFG)	Diabetic rats supplemented with extract *of Trigonella foenum graecum* at the dose rate of 1g/kg body weight.
Group V (CI)	Diabetic rats supplemented with extract *of Coccinia indica* at the dose rate of 200mg /kg body weight.
Group VI (TFG + CI)	Diabetic rats supplemented with extract *of Trigonella foenum graecum* and *Coccinia indica* at the dose rate of 1g/kg and 200 mg/kg body weight respectively.
Group VII (TFG +G)	Diabetic rats supplemented with extract *of Trigonella foenum graecum* and Glibenclamide at the dose rate of 1g/kg, and 300 µg /kg body weight respectively.
Group VIII (CI +G)	Diabetic rats supplemented with extract *of Coccinia indica* and Glibenclamide at the dose rate of 200mg/kg and 300 µg /kg body weight respectively
Group IX (TFG + CI +G)	Diabetic rats supplemented with extract *of Trigonella foenum graecum, Coccinia indica* and Glibenclamide at the dose rate of 1g/kg, 200mg/kg and 300 µg /kg body weight respectively.

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced in Groups II to IX by a single intra peritonial injection of a freshly prepared solution of streptozotocin (45 mg/kg body weight) in 0.1 M cold citrate buffer having a pH of 4.5 (Babu and Prince, 2004) [2]. The normal control animals received citrate buffer alone.

Confirmation of diabetes

The diabetic state was confirmed by estimating the blood glucose levels after 72 hours of STZ injection using ready to use Span diagnostic kit with semi-automatic biochemical analyzer. The animals that showed the blood glucose level above 200 mg/dl were considered as diabetic. After confirmation of diabetic state, the treatment was commenced.
The rats of group I and II were gavaged only with normal saline and the rats of all other groups with their respective treatments daily for 90 days. The animals were observed daily for recording treatment effect.

Clinical observation

Rats of all the groups were observed for feed and water

intake, general behaviour, alertness, urine output, diarrhoea and any other clinical signs.

Parameters analysed

Body weight

The rats were weighed on the day of the commencement of the experiment and on Day 3, 15, 30, 45 and 90 of the study to evaluate the effect of various treatments on body weight.

Hematological parameters

The blood samples collected at various intervals were subjected to Hematological estimation of RBCs, WBCs, Platelets, hemoglobin's using Semi-Automatic hematological Analyzer with commercial diluting kits (Span diagnostics, Bangalore). These parameters were estimated from blood, samples as per the procedure described by Tietz, (1976) [26].

Results and Discussion

The high cost of modern treatment of diabetes stresses upon an urgent need for the development of alternate strategies for the prevention and treatment of diabetes. Currently, the focus is on using traditional methods of treatment and prevention procedures by use of herbal preparations. Herbs for diabetes treatment are not new. Since ancient times, plants and plant extracts are used to combat diabetes. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, approximately 150 species of plants are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. Although hundreds of plants have been identified to possess antidiabetic effect, not many have been scientifically tested to prove their efficacy and safety. In this regard the current study was carried out to evaluate the efficacy of two plant extracts *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in comparison with an oral antidiabetic drug glibenclamide and in addition to know the performance and synergistic effects of these plants in combination with glibenclamide individually and in combination in the treatment of experimentally induced diabetes mellitus in rats as experimental model.

In the present study, the hypoglycaemic effect of *Trigonella foenum graecum* extract and *Coccinia indica* in diabetic rat models, was evaluated in comparision with glibenclamide, a reference Antidiabetic drug. The Antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* was confirmed with the help of pathomarphological and hematological analysis of various treatment groups and the results of the study are discussed as here under.

Normal control group (Group-I)

The animals belonging to control group remained healthy throughout the experimental period. All the values of various parameters analysed were within the normal range and indicated their healthy status.

Diabetic control group (Group-II)

The rats belonging to diabetic control group (Group-II) remained hyperglycaemic throughout the study period and revealed various pathomorphological and hematological changes indicative of diabetes.

The mean (± SE) haemoglobin values of diabetic control rats progressively decreased from 9.76±0.39 g/dL on 3rd day to 5.80±0.39g/dL on 90th day of experiment and the decrease was highly significant (P≤0.001) in comparison with those of normal control group as well as treatment groups (Groups III to IX) (Table 11; Figure 11).

The mean (± SE) TRC values of diabetic control rats progressively decreased from 6.33±0.58 cu mm on 3rd day to 4.30±0.50 cu mm on 90th day of experiment and the decrease was highly significant (p≤0.001) in comparison with those of normal control group (Table 12; Figure 12).

In the diabetic control animals a significant decrease in the mean body weight was observed. The decrease was statistically significant (p≤0.001) from day 3 of post STZ injection. A number of earlier workers also have encountered weight loss in diabetic animals after induction of diabetes with STZ (Soleimani *et al.*, 2007; Pragathi, 2011; Oyedemi *et al*., 2011; Nasreen, 2012 and Mallikarjun *et al*., 2013) [10, 12, 14, 16, 24].

The weight loss in diabetic rats could be attributed to hypoinsulinism that occurs in diabetes; decreased protein synthesis in the absence of insulin which is partly due to diminished transport of amino acids to the muscle (Warkins, 2003) [27]; loss of fluids leading to dehydration through glycosuric polyuria and altered uptake of glucose and glycogenesis by the target cells (Rubin and strayer 2008) [20]. Insulin, being an anabolic hormone which is functionally antidiabetic in nature, causes increased catabolism of carbohydrates, proteins and fat in its hypoinsulinaemic state contributing for weight loss.

Glibenclamide treatment group (Group-III)

The oral antidiabetic agents have been reported to exert their effects by various mechanisms such as stimulation of beta cells to produce more insulin (sulphonylureas and meglitinides) increase the sensitivity of muscles and other tissues to insulin (thiazolidinediones) decrease gluconeogensis by the liver (biguanides) and delay the absorption of carbohydrates from GIT (alpha glucosidase inhibitors). All these agents have their own drawbacks ranging from development of resistance to lack of responsiveness. Sulphonylureas lose effectiveness in 44 per cent of patients with in six years, may worsen heart disease, lower the glucose below the normal range and increase the body weight gain.

The rats of glibenclamide treatment group (Group-III) showed a consistent increase in the mean (± SE) Hb value from Day 3 to Day 90. The mean Hb values were 9.75±0.36, 11.79±0.27, 12.98±0.26, 13.26±0.27and 15.25±0.65 g/dL on Day 3, 15, 30, 45 and 90 post-treatment respectively. The improvement in the mean Hb was significant (p≤0.001) in comparison with those of diabetic control rats (Group-II) and were comparable to those of all other treatment groups (Groups-IV to IX) on 90th day of experiment (Table 11; Figure 11).

The mean (± SE) TLC values of Group-III rats treated with glibenclamide were observed to be progressively increased from 6.49±0.16 x 10^3/µL on 15th day to 8.75±0.15 x 10^3/µL on 90th day post-treatment. The values were significantly higher (p≤0.001) compared to diabetic control rats on 15th, 30th , 45th and 90th day of treatment and were comparable with that of normal control (Group I) on 45th and 90th day of observations (Table 13; Figure 13).

Similarly in glibenclamide treated group, there was a significant improvement in Hb concentration in comparison with the diabetic control group both on 30th and 45th day of the study. Improvement in the Hb concentration could be

attributed to antihyperglycaemic effect of glibenclamide and improvement in insulin level, thus reducing non-enzymatic glycosylation of haemoglobin (Rubin and Strayer, 2008) [20]. Group-III rats also showed a significant improvement in the blood parameters such as TRC, TLC, and platelets when compared to diabetic control animals. This could be due to amelioration of diabetic symptoms by glibenclamide.

***Trigonella foenum graecum* (Group IV) and *Trigonella foenum graecum* with Glibenclamide treatment groups (Group-VII)**

In the present study the mean body weights in *Trigonella foenum graecum* (Group IV) and *Trigonella foenum graecum* with half dose of glibenclamide (Group VII) treated rats significantly improved from 15th day onwards in comparison with the mean weights of diabetic control rats (Group-II). The mean body weights of rats of these two groups were comparable with those of other groups (Group III, V, VI, VIII and IX) on 45th and 90th day post-treatment.

The mean (± SE) Hb values of the Groups IV and VII treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively showed an improvement and the values were 10.20±0.34, 10.00±0.31 and 9.92±0.20 g/dL on 3rd day to 14.15±0.55, 13.80±0.40 and 14.60±0.30 g/dL on 90th day of treatment (Table 10; Figure 10).

The values were significantly higher compared to diabetic control animals ($p \leq 0.001$) throughout the study and were significantly lower ($p \leq 0.001$) compared to normal control animals on all the days of the study. The values were comparable with those of Group-III and did not significantly vary between the groups on 45th and 90th day of the experiment (Table 11; Figure 11).

The mean (± SE) values of TLC in Groups IV, V and VI treated with *Trigonella foenum graecum, Coccinia indica* and in their combination respectively were significantly increased ($p \leq 0.001$) when compared to diabetic control rats from Day 15 to Day 90 of the experiment. The mean (± SE) values were 5.60±0.24, 5.15±0.11 and 5.27±0.16 on 3rd day and increased to 7.30±0.90, 6.85±0.25 and 7.90± 0.40 x $10^3/\mu L$ on 90th day of the study respectively. However, the mean values were significantly lower ($p \leq 0.001$) compared to normal control on all the days of observations but comparable to glibenclamide group (III) on 90th day. Between the groups the mean TLC values did not differ significantly (Table 13; Figure 13).

Similar finding was also observed by Siddiqui *et al.,* 2006 [22] and Rafiq *et al.,* 2009 [19] who reported that progressive increase in weight could be due to attenuation of the toxicity of STZ by fenugreek seeds particularly at high dose and also a better digestibility and utilization of nutrients in the diet because of their high fibre content. 4-hydroxy isoleucine of fenugreek which increases adipogenesis as shown both in *in vitro* and *in vivo* studies by Shah *et al*. (2009) [21] and reduction in catabolic activity such as glycogenolysis, lipolysis, and gluconeogenesis during treatment with trigonella also could be attributed to the increase in the mean body weight gain (Nirmala *et al.,*2009) [13].

The analysis of blood parameters revealed a significant improvement in total TRC count, TLC count and platelet count in Group IV and group VII in the present study compared to diabetic control. It was also observed that there was no statistical difference in the improvement in the blood cell values between the groups though numerically the combined group had better values of TRC and platelets. This could be attributed to the antioxidants of Trigonella which quench the ROS produced during induction of diabetes with STZ and thereby improving the hemopoietic tissue. The findings of the study support the earlier reports by Sumiyoshi (1993) [23], that *T.foenum graecum* has immunomodulatory effect and stimulate immune functions by activating the natural killer cells, the function of T-lymphocytes and the level of interleukin- 2 (Tang *et al.,*1997) [25].

The significant improvement in the levels of circulating platelets in blood in Groups IV and VII could be attributed to inhibitory activity of certain constituents of *T. foenum graecum* on platelet aggregation as shown in previous studies in humans and animals (Apitz-castro *et al.,* 1988 and Lawson *et al.,* 2005) [1, 9].

***Coccinia indica* (Group-V) and *Coccinia indica* with glibenclamide half dose (Group VIII)**

The mean (± SE) Hb values of the Groups V and VIII treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination respectively showed an improvement and the values were 10.20±0.34, 10.00±0.31 and 9.92±0.20 g/dL on 3rd day to 14.15±0.55, 13.80±0.40 and 14.60±0.30 g/dL on 90th day of treatment.

The values were significantly higher compared to diabetic control animals ($p \leq 0.001$) throughout the study and were significantly lower ($p \leq 0.001$) compared to normal control animals on all the days of the study. The values were comparable with those of Group-III and did not significantly vary between the groups on 45th and 90th day of the experiment (Table 11; Figure 11).

There was a progressive increase in TLC towards normalcy in rats of Groups VII, VIII and IX treated with *Trigonella foenum graecum, Coccinia indica* individually and in their combination with glibenclamide at half dose respectively from 3rd day to 90th day of the experiment. The mean values were significantly improved ($p \leq 0.001$) compared to diabetic control rats from 30th day onwards. However, with respect to normal control rats the mean values were significantly lesser ($P \leq 0.001$) throughout the experiment except on 90th day. The mean TLC values of combined Group (IX) were comparable to those of control group on 45th and 90th day of the treatment (Table 13; Figure 13).

The Group V and Group VIII rats in the present study following induction of diabetes with STZ and treatment with Coccinia indica alone and in combination with gibenclamide revealed a significant increase in the body weight compared to diabetic rats from 15th day onwards and on 90th day it was comparable to that of control rats and did not differ significantly from those of glibenclamide alone and other treatment groups. The improvement in the body weight by feeding *Coccinia indica* also has been reported by many earlier workers (Kuriyan *et al.,* 2008 and Rafiq *et al.,* 2009) [8, 19]. The increase in the body weight could be attributed to the alleviation of diabetic symptoms and hyperglycemia by insulinomimetic effect of triterpene of *Coccinia indica* (Rafiq *et al.,* 2009) [19] and also to insulin stimulated glucose transport and anabolic effects of insulin (Kanetkar *et al.,* 2007) [6].

In the present study there was a significant improvement in the haemoglobin values from 15th day onwards in both *Coccinia indica* alone and in combination with glibenclamide groups in comparison with diabetic control group. The mean

values on 90th day did not significantly vary between these groups and also when compared with glibenclamide alone complete dose group. However the values failed to reach that of control group. The findings indicated that *Coccinia indica* alone and in combination with glibenclamide half dose has effective effect in alleviating the diabetic effect on Hb values and is equivalent to the glibenclamide complete dose. However, no synergistic effect was observed in the combined group.

The hematological values of TRC, TLC, and platelets which were significantly reduced in diabetic control rats showed significant improvement in *Coccinia indica* alone and in combination with glibenclamide half dose groups. There was no significant difference in the values of TLC and platelets among these treatment groups at different time intervals. However, total TRC values in the combined group had better effect compared to *Coccinia indica* alone group and was comparable to that of glibenclamide complete dose group. The values of TRC and TLC remained significantly lesser compared to control group.

The improvement in blood parameters in *Coccinia indica* treated groups could be attributed to the antioxidants of *Coccinia indica* which quench the ROS produced during induction of diabetes with STZ and thereby alleviating the damage in the hemopoietic tissue. In addition *Coccinia indica* has immunomodulatory effect and stimulate immune functions by activating the immune cells.

Combined treatment groups (Group- VI and IX)

In the present study the evaluation of Antidiabetic effect of combined herbal extract treatment revealed a significant increase in the mean body weight values in both group VI and group IX from 15th day onwards in comparison with diabetic control group. It was observed that the weight gain was better numerically in both the groups compared to other treatment groups and was comparable to that of control group. Between Group IV and Group IX, the weight gain was more in Group IX which indicated that combined treatment has better alleviating effect on diabetes induced changes. As discussed under respective individual herbal extract treatment groups, the weight gain could be attributed to the combined effect of active biological components of the Trigonella and Coccinia which improved insulin level and also increased glucose absorption at the periphery (Das *et al*., 2008 and Mishra *et al*., 2009) [5, 11]. There was a synergetic effect observed in increasing the mean body weight in combined treatment groups

The haemoglobin percentage of combined treatment groups (VI and IX) was significantly higher than diabetic control group from 15th day onwards. The mean Hb values between Groups VI and IX did not differ on 90th day though the value of Hb in group IX was numerically higher compared to any other treatment groups. This observation indicated that the combined treatment with plant extracts and glibenclamide has better hypoglycaemic effect by which the glycosylation of Hb was reduced and there by improved mean Hb values. The individual plant extracts have been reported to contribute for hypoglycaemia by their respective bioactive compounds and antioxidant property as discussed under respective plant extract treatment groups.

There was an improvement in all the blood cells counts (TRC, TLC and platelets) of the combined treatment groups (VI and IX) compared to diabetic animals. On 90th day of the experiment no statistical difference was observed between Groups VI and IX and did not differ from other treatment groups. Numerically the values were higher in combined treatment group IX which indicated that the Antidiabetic drug along with plant extracts with Antidiabetic effect has beneficial effect in counteracting the diabetes induced changes in the body. Though there was an increase in the cell counts, the values never reached values of control group at any interval of observation.

The means with at least one common superscript are not significantly different ($p \leq 0.001$)

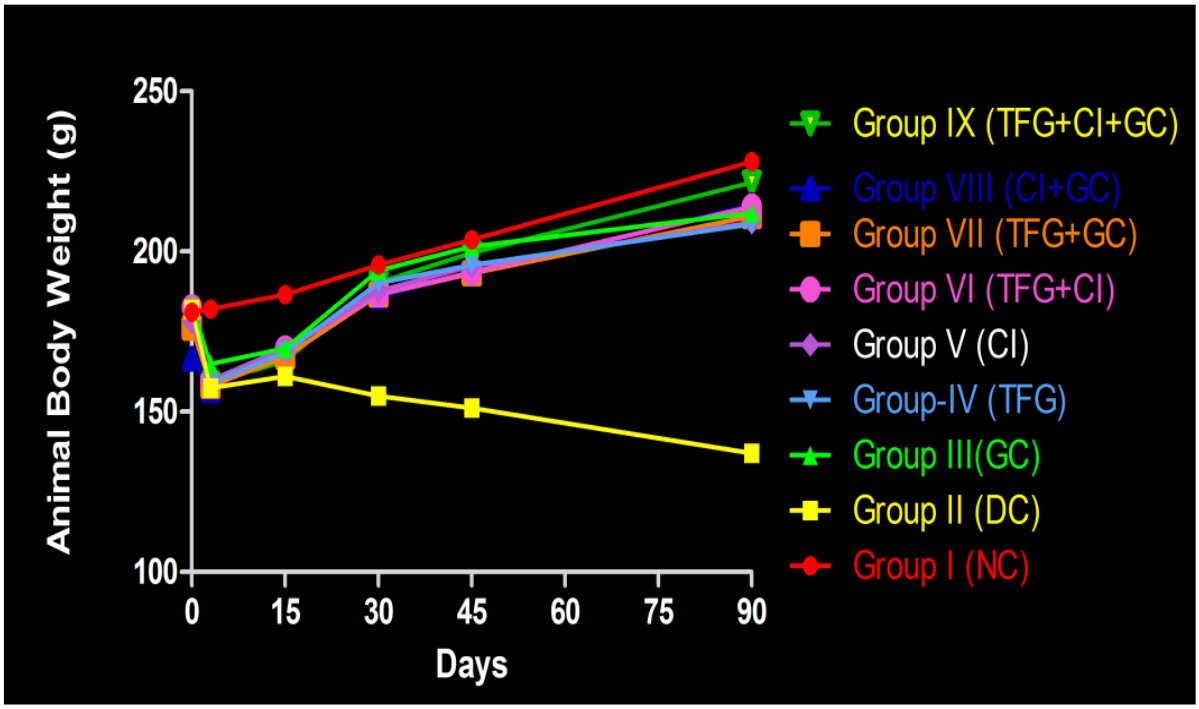

Fig 1: The Mean (± SE) animal body weight (g) values of different groups at different intervals of time

Table 1: The Mean (± SE) animal body weight (g) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	182.00±1.53 [a]	186.50±1.94[a]	195.80±1.62[a]	203.62±1.48[a]	228.00±3.00[a]
Group II	157.41±2.44[b]	162.91±2.00[b]	155.60±2.40[b]	151.12±5.43[b]	137.00±9.00[b]
Group III	164.91±2.39[b]	169.75±3.97[b]	194.10±3.21 [a]	201.50±2.99[a]	212.00±3.00[a]
Group IV	158.91±2.76[b]	168.50±2.30[b]	190.20±3.48[a]	195.75±1.90[a]	208.50±1.50[a]
Group V	159.83±2.50[b]	169.75±3.77[b]	187.70±3.57[a]	195.50±3.88[a]	209.00±2.00[a]
Group VI	159.16±1.55[b]	169.75±3.89[b]	186.50±3.73[a]	193.50±2.65[a]	214.00±3.00[a]
Group VII	158.00±1.73[b]	167.16±2.14[b]	186.60±2.72[a]	193.12±3.37[a]	211.00±4.00[a]
Group VIII	156.66±2.10[b]	168.08±2.36[b]	186.10±2.88[a]	193.12±3.96[a]	214.50±4.50[a]
Group IX	158.83±1.85[b]	165.91±2.98 [a]	190.30±3.11[a]	199.50±2.70[a]	221.50±4.50[a]

Table 2: The Mean (± SE) Haemoglobin (g/dL) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	14.16±0.46 [a]	13.72±0.39 [a]	14.58±0.35 [a]	15.87±0.36 [a]	17.05±0.55 [a]
Group II	9.76±0.39[b]	9.13±0.15 [e]	8.32±0.19[e]	7.00±0.29[d]	5.80±0.40[d]
Group III	9.75±0.36[b]	11.79±0.27 [c]	12.98±0.26[cd]	13.26±0.27[c]	15.25±0.65[bc]
Group IV	10.20±0.34[b]	10.91±0.25 [d]	12.35±0.34[d]	13.41±0.27[c]	14.15±0.55[bc]
Group V	10.00±0.31[b]	11.85±0.22 [c]	12.44±0.29[d]	13.37±0.30[c]	13.80±0.40[c]
Group VI	9.92±0.20[b]	11.53±0.18 [dc]	13.27±0.30[bc]	13.92±0.31[bc]	14.60±0.30[bc]
Group VII	9.95±0.24[b]	12.91±0.19 [b]	13.67±0.18[bc]	14.42±0.29[b]	14.70±0.10[bc]
Group VIII	9.75±0.21[b]	12.11±0.30 [c]	13.54±0.26[bc]	14.70±0.17 [b]	15.00±0.10[bc]
Group IX	9.75±0.22[b]	12.03±0.19 [c]	13.86±0.24[ab]	15.50±0.18[a]	16.00±0.80[ab]

The means with at least one common superscript are not significantly different ($p \leq 0.001$)

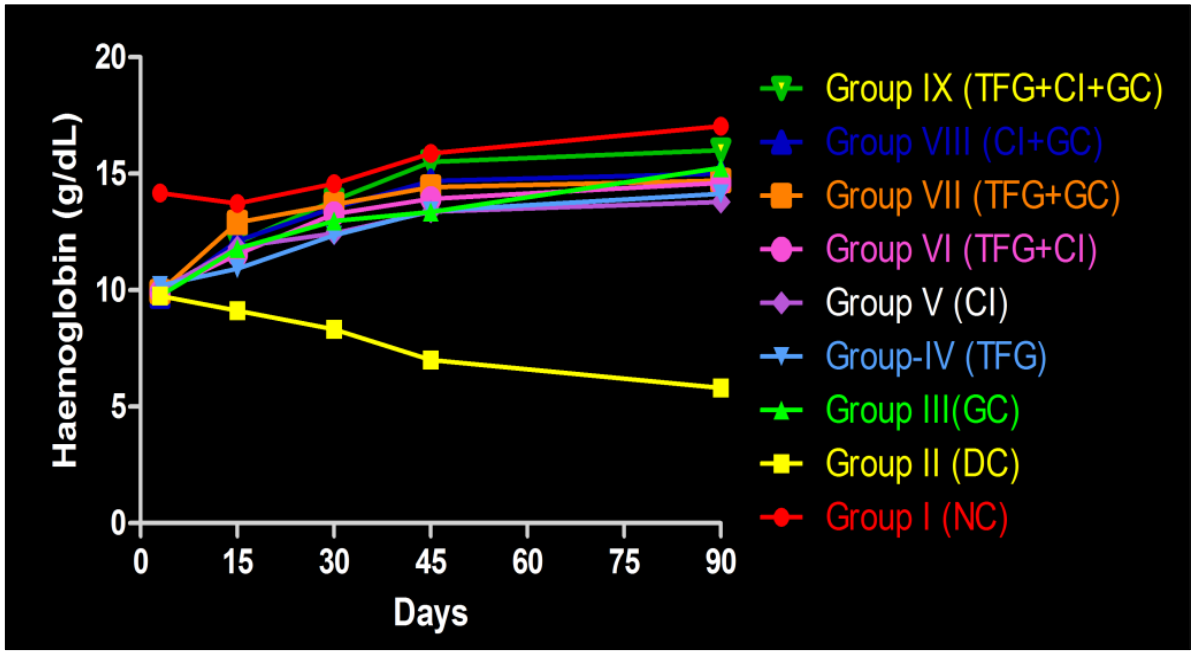

Fig 2: The Mean (±SE) Haemoglobin (grdL) values of deferent groups at different intervals of time

Table 3: The Mean (± SE) TRC count (x10^{12}/L) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	9.00±0.76 [a]	9.01±0.65 [a]	9.08±0.76 [a]	9.96±1.06 [a]	11.05±055 [a]
Group II	6.33±0.58[b]	5.79±0.43[b]	5.03±0.24[d]	4.73±0.33[f]	4.30±0.50[d]
Group III	5.35±0.12[bc]	6.36±0.13 [b]	7.34±0.10[b]	8.30±0.23[bcde]	9.60±0.20 [b]
Group IV	5.71±0.16[bc]	5.86±0.12[b]	6.29±0.13[c]	7.30±0.15[de]	7.55±0.50[c]
Group V	5.11±0.13[c]	5.82±0.17[b]	6.67±0.25[bc]	7.06±0.13[e]	7.60±0.30[c]
Group VI	5.65±0.15[bc]	6.12±0.15[b]	7.08±0.16[bc]	8.47±0.21[bcd]	8.35±0.75[bc]
Group VII	5.71±0.16[bc]	6.30±0.21[b]	7.01±0.20[bc]	8.65±0.14[bc]	8.75±0.15[bc]
Group VIII	5.70±0.10[bc]	6.26±0.10[b]	6.92±0.12[bc]	7.40±0.32[cde]	9.30±0.10 [b]
Group IX	5.76±0.19[bc]	6.78±0.22[b]	8.48±0.18[a]	9.00±0.28[ab]	9.50±0.10 [b]

The means with at least one common superscript are not significantly different ($p \leq 0.001$)

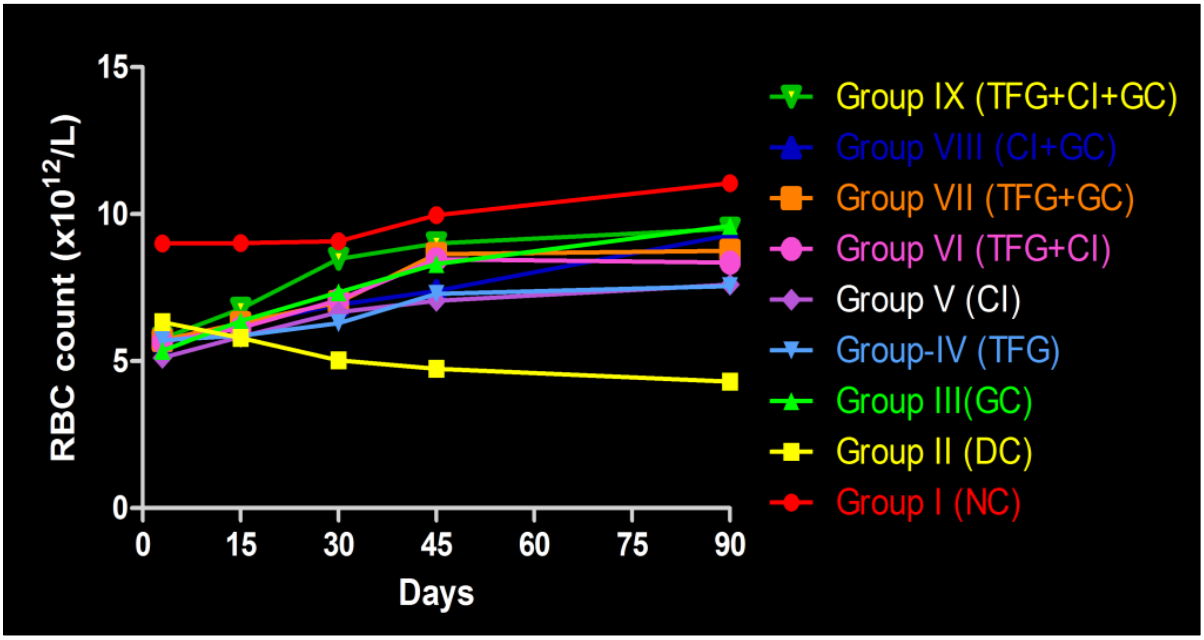

Fig 3: The Mean (±SE) TRC count (x10121/L) values ordifferen1 groups al different intervals of time

Table 4: The Mean (± SE) TLC count (x10^3/µL) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	8.51±0.30 ^{a}	8.07±0.43 ^{a}	8.30±0.39^{a}	8.66±0.48 ^{a}	10.05±0.45 ^{a}
Group II	6.01±0.15^{b}	5.70±0.13^{c}	4.75±0.12^{e}	3.76±0.14^{d}	3.05±0.15^{d}
Group III	4.97±0.15^{d}	6.49±0.16^{b}	7.59±0.22^{b}	8.42±0.34^{a}	8.75±0.15^{abc}
Group IV	5.60±0.24^{bc}	5.94±0.16^{bc}	6.21±0.18^{d}	6.70±0.23^{bc}	7.30±0.90^{c}
Group V	5.15±0.11^{dc}	5.95±0.14^{bc}	6.31±0.15^{d}	6.43±0.08^{c}	6.85±0.25^{c}
Group VI	5.27±0.16^{cd}	5.66±0.13^{cd}	6.12±0.10^{d}	7.12±0.31^{bc}	7.90±0.40^{bc}
Group VII	4.70±0.14^{d}	5.10±0.11^{d}	6.11±0.15^{d}	6.95±0.23^{bc}	7.35±0.45^{bc}
Group VIII	4.79±0.16^{d}	5.72±0.13^{c}	6.22±0.14^{d}	7.41±0.43^{b}	8.55±1.15^{abc}
Group IX	4.91±0.20^{d}	6.13±0.20^{bc}	6.94±0.13^{c}	8.42±0.19^{a}	9.45±0.75^{ab}

The means with at least one common superscript are not significantly different ($p \leq 0.001$)

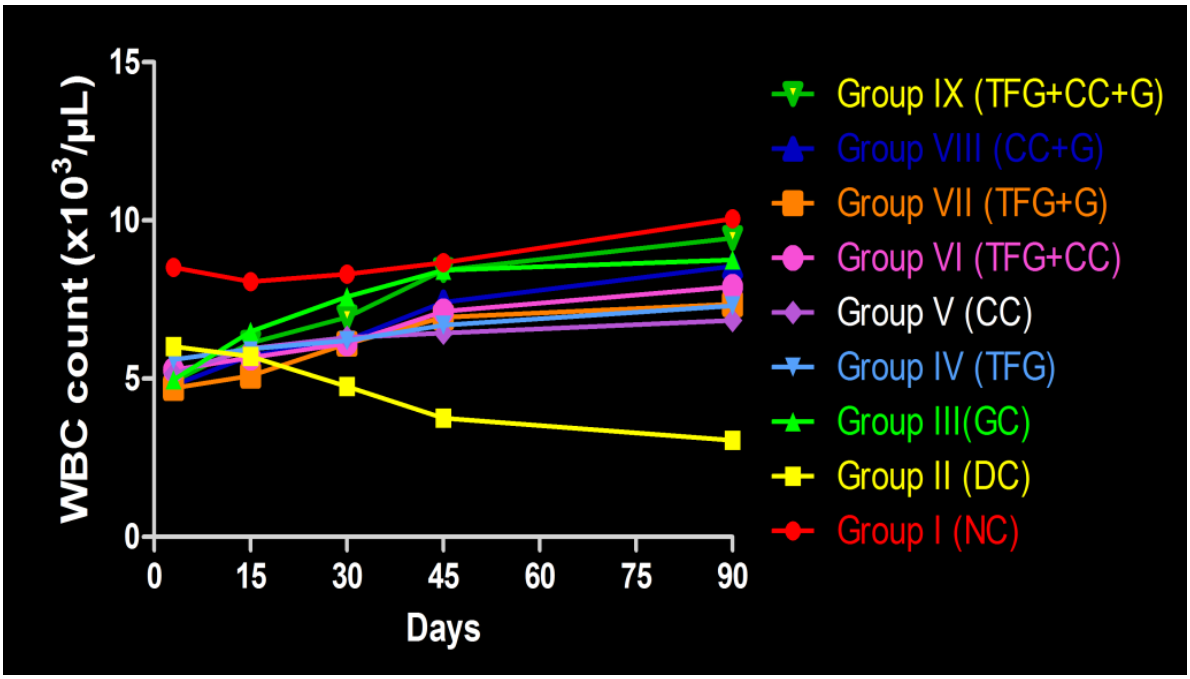

Fig 4: The Mean (±SE) TLC count (x103/ul) values of different groups at different interests of time

Table 5: The Mean (± SE) Platelet count (x10^9/L) values of different groups at different intervals of time

Groups	Days Post Treatment				
	3	15	30	45	90
Group I	442.58±23.18 [a]	436.50±23.26 [a]	440.70±22.89 [a]	467.12±27.50 [a]	453.50±24.50 [a]
Group II	281.08±16.61[b]	274.16±22.68[c]	301.40±17.35[c]	306.37±21.62[d]	277.50±29.50[b]
Group III	280.16±18.31[b]	296.83±23.64[bc]	333.30±17.03[bc]	361.12±10.23[cd]	404.50±20.50 [a]
Group IV	291.41±13.58[b]	303.58±12.39[bc]	319.00±12.93[bc]	355.12±13.23[bcd]	391.50±9.50 [a]
Group V	290.41±14.81[b]	321.16±14.19[bc]	353.70±15.37[b]	357.12±15.48[bcd]	385.00±23.00 [a]
Group VI	286.00±16.08[b]	323.00±17.00[bc]	342.10±13.67[bc]	391.25±16.74[b]	407.00±9.00 [a]
Group VII	283.41±14.65[b]	333.16±14.18[b]	359.00±10.41[b]	375.12±5.03[bc]	400.50±4.50 [a]
Group VIII	287.58±14.82[b]	324.41±11.53[bc]	336.10±13.70[bc]	361.62±13.02[bc]	415.50±21.50 [a]
Group IX	302.83±14.31[b]	347.41±15.93[b]	365.20±7.61[b]	388.37±14.08[bc]	434.50±22.50 [a]

The means with at least one common superscript are not significantly different ($p \leq 0.001$)

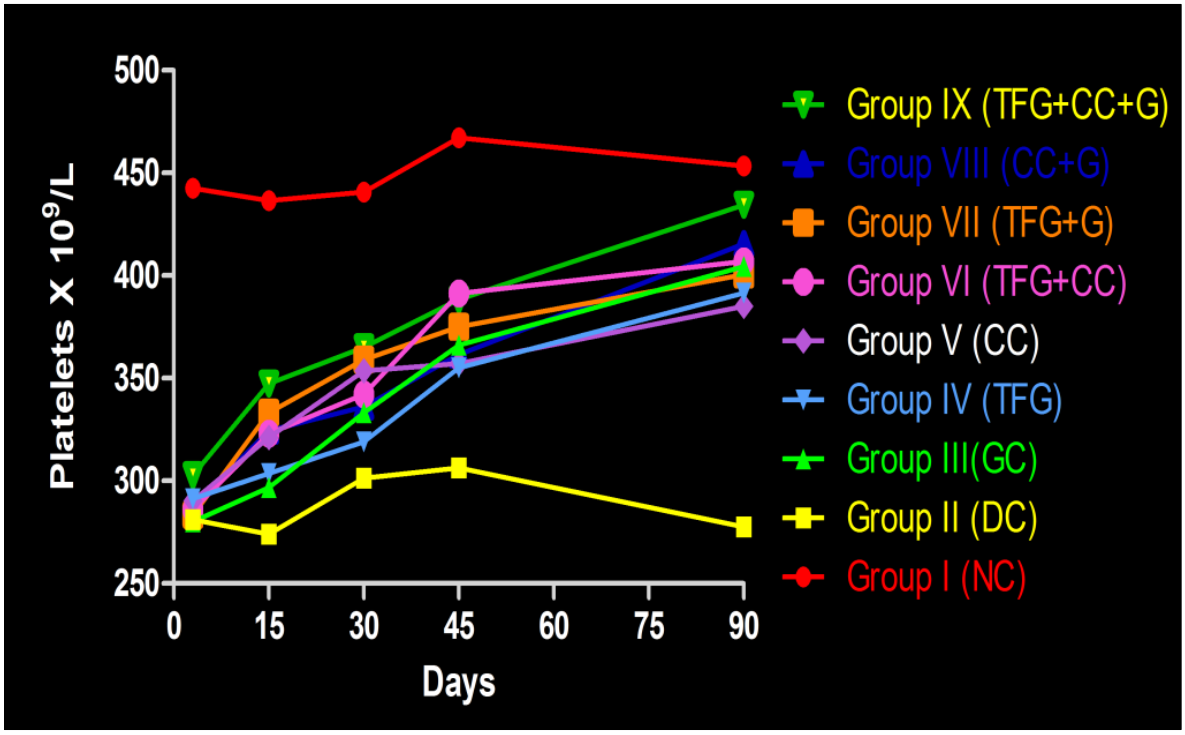

Fig 5: The Mean (±SE) Platekl count (x109/L) values of different groups at different intervals of time

Summary and Conclusion

The present study was focused on evaluation of Antidiabetic effect of *Trigonella foenum graecum* and *Coccinia indica* individually and in combination in induced diabetes in rats. The efficacy of these herbal extracts was also compared with that of glibenclamide, a novel cardinal Antidiabetic drug individually and in combination.

The rats belonging to diabetic control group (Group-II) showed significant decrease in the mean body weight throughout the experiment. The animals of all the treatment groups (Groups III to IX) revealed an improvement in their body weight. The mean body weight values in the combined treatment group were comparable with those of glibenclamide, *Trigonella foenum graecum* and *Coccinia indica* treatment groups on all the intervals of the study. There was a synergetic effect with increase in the mean body weight in combined treatment groups.

The diabetic control rats showed a decrease in mean haemoglobin percentage values as compared to normal control. Animals of all other groups showed a significant increase in Hb values as compared to diabetic control rats. The effect of herbal extracts both individually and in combinations was comparable to that of glibenclamide treatment group in improving Hb values.

The diabetic rats recorded a moderate decrease in TRC, TLC and platelet count compared to normal control animals. There was a improvement in blood cells counts of the treatment groups (III to IX) compared to diabetic animals. They were comparable with those of normal and glibanclamide control animals. Combined treatment groups showed better improvement in blood cell counts compared to the individual treatment groups of *Trigonella foenum graecum* and *Coccinia indica* extracts.

Both *Trigonella foenum graecum* and *Coccinia indica* extracts have potential antidiabetic activity but cannot be used as sole replacement therapeutic agents for conventional antidiabetic drugs. However can be used as adjunct to antidiabetic drugs.

Coccinia indica in combination group is better than T*rigonella foenum graecum in* providing hypoglycaemic effect and gives an opportunity to reduce the dose of glibenclamide, and may help in minimizing the adverse effect of glibenclamide as well as in achieving enhanced therapeutic effect.

Lastly the present study high lights that the indigenous medicinal plants can be used successfully as an alternative treatment in the management of diabetes with or without antidiabetic drugs.

References

1. Castro AR, Ledezma E, Esclante J, Jorquera A, Pinate FM, Rea MJ, et al. Reversible prevention of platelet activation by (E, Z)-4,5,9- trithiadodeca-1,6,11-triene 9-oxide (AJOENE) in dogs under extracorporeal circulation. Arzneimittel Forschung. 1988;38:901-904.
2. Babu PS, Prince SMP. Antihyperglycaemic and antioxidant effect of hyponidd, an ayurvedic herbomineral formulation in streptozotocin-induced diabetic rats. J Pharm Pharmacol. 2004;56(11):1435-1442.
3. Chandra A, Singh RK, Tewari L. Antioxidative potential of herbal hypoglycemic agents in diabetes - An overview. SFRR-India Bull. 2004;3:24-26.
4. Craig ME, Hattersley A, Donaghue KC. Definition, epidemiology and classification of diabetes, Definition, epidemiology and classification of diabetes in children and adolescents. Pediatr Diabetes. 2009;10:3-12.
5. Das PC, Mostofa M, Sarkar AK, Ali M. Comparative efficacy of two medicinal plants and Amaryl® tablet (Glimepiride) in induced diabetes mellitus in rat. J Bangladesh Agril Univ. 2008;6(2):297-300.
6. Kanetkar P, Singhal R, Kamat M. Gymnema sylvestre: A Memoir. J Clin Biochem Nutr. 2007;41:77-81.
7. Khalki L, M'hamed SB, Bennis M, Chait A, Sokar Z. Evaluation of the developmental toxicity of the aqueous extract from *Trigonella foenum graecum* L. in mice. J Ethnopharmacol. 2010;15:321-325.
8. Kuriyan R, Rajendran RC, Bantwal G, Kurpad AV. Effect of supplementation of Coccinia cordifolia extract on newly detected diabetic patients. Diabetes Care. 2008;31(2):216-220.
9. Lawson SR, Gabra BH, Guerin B, Neugebauer W, Nantel F, Battistini B, Sirois P. Enhanced dermal and retinal vascular permeability in streptozotocin-induced type 1 diabetes in Wistar rats: Blockade with an elective bradykininB1 receptor antagonist. Regul Pept. 2005;124(1-3):221-224.
10. Mallikarjuna G, Sugunarao, Satyenarayana ML. Pathomorphological and biochemical evaluation of hypoglycaemic effects of Momardica charantia and Gymnema sylvestre in experimentally induced diabetes in rats. IJABR. 2013;3(1):51-57.
11. Mishra A, Jaitly AK, Srivastava AK. Antihyperglycaemic activity of six edible plants in validated animal models of diabetes mellitus. Indian Journal of Science and Technology. 2009;2(4):0974-6846.
12. Nasreen A. Studies on hypoglycemic effect of aged garlic extract on experimentally induced diabetes mellitus in rats. M.V.S.c. thesis, KVAFSU, Bidar, Karnataka, India; c2012.
13. Nirmala A, Saroja HR, Vasanthi, Lalitha. Hypoglycemic effect of Basellaruba in streptozotocin induced diabetic albino rats. J Pharmacology and Phytotherapy. 2009;1(2):25-30.
14. Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of *Afzelia africana* (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pac J Trop Biomed. 2011;1:353-358.
15. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* Linn. BMC Complement Altern Med. 2009;9:48.
16. Pragathi BS. Evaluation of hypoglycaemic effect of Gymnema sylvestre and cow urine in diabetic rat model. M.V.S.c. thesis, KVAFSU, Bidar, Karnataka, India; c2011.
17. Ramachandran A, Wan RC, Snehalatha C. Diabetes in Asia. Lancet. 2010;375:408-18.
18. Ramalingam K, Saravanan SN, Leelavinothan. Effect of Eugenia jambolana seed kernel on antioxidant defense system in streptozotocin induced diabetes in rats. Life Sci. 2004;75:2717-2731.
19. Rafiq K, Sherajee SJ, Nishiyama A, Sufiun MA, Mostofa M. Effects of indigenous medicinal plants of Bangladesh on blood glucose level and neuropathic pain in streptozotocin-induced diabetic rats. Afr J Pharm Pharmacol. 2009;3(12):639-645.
20. Rubin R, Strayer DS. Rubin Pathology: Clinicopathological Foundation of Medicine. 5th Ed. Lippincott Williams and Wilkins publication; c2008, 961-968.
21. Shah SN, Bodhankar SL, Badole SL. Effect of trigonelline: An active compound from *Trigonella foenum graecum* Linn in alloxan induced diabetes in mice. J Cell Tissue Research. 2009;6:585-590.
22. Siddiqui MR, Moorthy K, Taha A, Hussain ME, Baquer NZ. Low doses of vanadate and trigonella synergistically regulate Na+/K+-ATPase activity and GLUT4 translocation in alloxan-diabetic rats. Mol Cell Biochem. 2006;285(1-2):17-27.
23. Sumiyoshi, Han Lk, M, Zhang J. Anti-obesity action of Salix matsudana leaves (part 1). Anti-obesity action by polyphenols of Salix matsudana in high fat-diet treated rodent animals. Phytother Res. 2003;17(10):1188-1194.
24. Soleimani S, Azarbaizani FF, Netaji V. The effect of Equisetrum arvense (Eqeisetraceae) in the histological changes of pancreatic beta cells in streptozotocin-induced diabetic rats. Pak J Biol Sci. 2007;10(23):4236-4240.
25. Tang Z, Sheng S, Liu X, Jian K, Yan M. Preventing function of garlic on experimental oral precancer and its effect on natural killer cells. Bulletin of Human Medical University. 1997;22:312-318.
26. Tietz. Fundamentals of Clinical Chemistry. WB Saunders Co., Philadelphia; c1976.
27. Warkins PJ. Diabetes mellitus, types and complications. In: Walkins PJ, editor. ABC of Diabetes, 5th Ed. BMJ Press; c2003, 75-95.