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Research Article

EVALUATION OF ANTIDIABETIC ACTIVITY OF WHOLE PLANT OF 70% ETHANOLIC EXTRACTS OF *INDIGOFERABARBERI GAMBLES*

Jaya Sankar Reddy.V[1]*, Ramesh Reddy.K[1], G. Sindhu[2]

[1]*Department of Pharmacology, KrishaTeja Pharmacy College, Tirupati, Andhra Pradesh, India.*

[2] *Academic Consultant, Division of Pharmacy, S.V.University, Tirupati, Andhra Pradesh, India*

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ABSTRACT

Various herbs have been found beneficial in the management of NIDDM and are gaining considerable recognition in the management of NIDDM worldwide. The present study was planned to search, standardize, and evaluate the efficacy of indigenous herbal ingredient ***IndigoferaBarberi Gambles***which is used in some areas for the treatment of Diabetes mellitus and whose hypoglycemic effects have not yet been scientifically studied. The whole of ***IndigoferaBarberiGambles***collected, shade dried and ground to get powder and extracted with ethanol in soxhlet extractor and concentrated in rotary flash evaporator under vaccum. Preliminary phytochemical & toxicological were carried out on the extract. Studies were under taken to evaluate anti diabetic activity of 70% ethanolic extract of ***IndigoferaBarberi Gambles***on both normal and streptozotocin induced diabetic rats.

INTRODUCTION

Indigofera is a large genus of over 750 species of flowering plants belonging to the family Fabaceae. They are widely distributed throughout the tropical and subtropical regions of the world (Wills, 1985). The species are mostly shrubs, through some are herbaceous and few can become small trees up to 5-6 m tall. Most are dry season or winter deciduous. *Indigoferabarberi* Gamble is a rare, endemic plant mainly found in Sheshachalam hills belongs to Fabaceae family. Vernacular names in telugu is Thambajalari&Thummajalari. The population of this plant species is rapidly declining due to various factors such as habitat

***Address for correspondence**

Jaya Sankar Reddy.V*
Department of Pharmacology,
KrishaTeja Pharmacy College, Tirupati,
Andhra Pradesh, India.
E-mail:shankarpharmacology@gmail.com

Destruction, fragmentation of population and lower percentage of seed germination etc.Diabetes mellitus (DM) was recognized as early as 1500 B.C. by Egyptian physicians who described it as a disease associated with "the passage of much urine". The term "diabetes" was coined by the Greek physician Aretaeus, who noticed that patients with diabetes had a disease that used the siphoning of the structural components of the body into the urine (White, 1996). Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Barcelo and Rajpathak, 2002). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood and blood glucose levels increases, which are termed as hyperglycemia. If the glucose level in the blood remains high over a long period, this can result in long-term damage to organs such as the kidneys, liver, eyes, nerves, heart and blood vessels.

Complications in some of these organs can lead to death.

2. Aim & objectives

Aim: Evaluations of 70% ethanolic extracts of graded doses of 100, 200 & 400 mg/kg b. w of whole plant of *Indigoferabarberi* for antidiabetic activity in normal and STZ induced diabetic rats.

Objectives

- Glucose levels in normoglycemic rats
- Glucose levels in STZ induced diabetic rats
- Body weight
- Lipid profile (TC, TG & HDL)
- Liver marker enzymes (AST, ALT & ALP).
- Estimation of Proteins, urea &creatinine.
- Estimation of TNF - α
- *In vivo* antioxidants (SOD, CAT & GSH)
- Histopathology of pancreas

3. Materials & method

3. 1: Extraction

The whole plant of*Indigoferabarberi* (Fabaceae) were collected from seshachalam hills of Tirumala in chittoor district of AP, India. The pant of *Indigoferabarberi* (Fabaceae) was authenticated by Dr.Madhavachetty, Asst Prof., Dept of Botany, S.V.University, 1 kg of whole plant of *Indigoferabarberi* (Fabaceae) was collected & washed with distilled water (d H_2O)) to remove dirt and soil. The collected plant materials were dried under shade at room temperature for 10 days. The dried plant materials were powdered by using a grinding mill to obtain a coarse powder and then passed through 40-mesh sieve. The powdered material was subjected to extraction with chloroform, ethyl acetate & 70% ethanol by successive solvent extraction method based on the increasing order of polarity of solvent. The aqueous fraction was obtained by hot percolation method. The extract was then filtered with whatman filter papers (No.1) and the filtrate was evaporated to dryness in rotary evaporator at 40^0C. The obtained crude extract was stored in a refrigerator at 4^0C until time of use.

3.2: Preliminary qualitative phytochemical analysis: Preliminary qualitative phytochemical analysis was carried out according to the standard methods of Harbone,(1973); Kokate*et al*., (1997).

3.3: Acute toxic class method

Alcoholic extracts were studied for the acute oral toxicity according to the guidelines set by organization for Economic Co-operation and Development (OECD) guidelines number 423.

***3.4: In vitro* antioxidant studies**

The scavenging activity for DPPH free radicals was measured according to the procedure described by Blios, (1958), inhibition of nitric oxide radical generation *in vitro* was followed as per the method reported by Green *et al*., (1982), Scavenging activity of H_2O_2 activity of extract and its sub-fractions were evaluated by Jayaprakasha*et al*., (2004), measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions.

3.5: Antidiabetic activity

Effect of IBEE on normoglycemic rats (Turner, 1965), on oral glucose tolerance test (OGTT) (Khan *et al*., 2010) & on hyperglycemic in STZ induced diabetic rats(Purohi and Sharma *et al*., 2006).

Effect of graded doses of IBEE on hyperglycemic activity in Streptozotocin (STZ) Induced diabetes rats.

Induction of diabetes –Steptozotocin (STZ) Induced diabetes

Diabetes was induced in all groups except control group rats, aged 4 months by single intraperitoneal injection of STZ (50 mg/kg) after overnight fasting for 16 h STZ was dissolved in citrate buffer at PH of 4.5. Diabetes was confirmed by estimations made after third day of STZ injection for serum glucose by a semi auto analyzer (Screen Master 3000, USA). Diabetic rats showing more than 220 mg/dl were used for the experiment (Kesari, 2006)).

Experimental protocol

Evaluation of antidiabetic activity (Purohi and Sharma *et al*., 2006) the animals were kept fasting for 16 h with ad libitum, on first day blood serum glucose levels were estimated after administering streptozotocin (STZ). The STZ (Sigma chemical Co., U.S.A) freshly was dissolved in citrate buffer (PH 4.5) and made diabetic by injection of a single dose 50 mg/kg b.w;i.p. STZ treated rats were given 2% of glucose in drinking water for the first 24 h encounter any initial hypoglycemia. On the third day the animals were checked for serum blood

glucose levels, higher than 220 mg/dl were used for the experiments and animals were divided into 6 groups. During the treatment period, the animals were checked for blood glucose levels at regular intervals for assessing its anti-hyperglycemic activity.

Serum analysis

Using commercial diagnostic kits, from Randox-Laboratories, San Diego-USA, total protein,urea, creatininel, TNF - α, lipid profile (TC, TG & HDL), Liver marker enzymes (aspartateaminotransferase (AST), alanine aminotransferase(ALT) & alkaline phosphate), invivo antioxidant enzyme levels were analyzed

Statistical analysis

Results are expressed as the mean±SEM.Statistical differences between control and treatedgroups were tested by one way analysis of variance(ANOVA) followed by Dunnett's test using GraphPad 5.0 software**.**

RESULTS

4.1: Percentage yield of 70% ethanolic extracts of Indigoferabarberi

Part used	: Whole plant
Weight of dried leaves	: 1kg

Extracted with : Chloroform, ethyl acetate, 70% ethanol & water

DISCUSSION

Alloxan and STZ are widely used to induce experimental diabetes in animals. Streptozotocin induced diabetic rat model is considered as a suitable experimental model for diabetes mellitus, as it causes diabetes by rapid depletion of β-cells, which leads to a reduction of insulin release. Administration of 50 mg/kg b.w of STZ effectively induces diabetes in normal rats as reflected by glycosuria, hyperglycemia, polyphagia, and polydipsia and body loss when compared with normal rats. In normoglycemic rats, 70% ethanolic extract of whole plant of *Indigoferabarberi* (IBEE) showed dose dependent hypoglycemic effect at 4 h. When these were administered to glucose loaded normal rats (OGTT) fasted for 18 h, reduction in blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h in the fasting blood glucose due to increase in the serum insulin levels. Hence, the possible mechanism by which 70% ethanolic extract whole plant *of Indigoferabarberi* (IBEE) brings about its hypoglycemic action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents. The mechanism by which STZ brings about a diabetic state includes selective destruction of pancreatic beta cells, leading to hypoinsulinemia which as a result decreased glucose uptake and hyperglycemia which is the characteristic feature of diabetes mellitus (Marles and Farnsworth, 1995). STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of 70% ethanolic extracts of whole plant of *Indigofera*for 30 days caused a significant decrease in blood glucose levels. The possible mechanism by plant extracts mediated its anti-diabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as an evident by the significant increase in the level of insulin in the extract treated animals.

STZ-induced diabetes is characterized by a severe loss in body weight (Al-Shamaony*et al*., 1994). The animals treated with extract and reference drug glibenclamide 5 mg/kg b.w showed a gradual and significant increase in the b.w after 7 days of the treatment. The increase in the b.w was observed till the end of the study (30 days). Decrease in b.w of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Due to insulin deficiency protein content is decreased in muscular tissue by proteolysis. A significant reduction in b.w observed in diabetic rats also due to increased excretion of glucose and reduced uptake of glucose by peripheral tissues and glycogen synthesis. Diabetes is associated with hyperlipidemia (Schoenfelder*et al*., 2010). It is well known that under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses the triglycerides under normal conditions. Destruction of β cells results in decreased plasma insulin and ultimately hyperlipidemia & hypertriglyceridemia. Diabetic rats which suggested that HDL is inversely related to the total body cholesterol (Reshma and Sushma, 2002).Oral administration of graded doses of 100, 200 & 400 mg/kg b.w of different extracts of 70% IBEE showed significant increase in serum insulin level. A marked decrease in triglycerides and total cholesterol while increase in HDL. Serum enzymes including AST and ALT are used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage (Egesie*et al*., 2006). Inflammatory hepatocel-

lular disorders result in extremely elevated transaminase. In accordance with these findings, streptozotocin treatments has a significant role in the alteration of liver functions since the activity of AST and ALT were significantly higher than those of normal value. The increase of AST and ALT will increase the incidence of heart and liver diseases. Serum concentration of AST is in proportion to the amount of cellular leakage or damage. It is released into serum in larger quantities when any one of these tissues is damaged. Its increased levels are usually associated with heart attacks or liver disease. The significance of ALT, an enzyme found primarily in the liver, is far greater. Its enhanced release into the blood stream is the result of liver abnormality. It therefore serves as a fairly specific indicator of liver status and its elevated levels in serum indicate liver damage. Oral administration of graded doses of 100, 200 & 400 mg/kg b.w of different extracts of 70% IBEE showed reduction in the activity of these enzymes in plasma was compared to the values of the diabetic group's levels (Foreston*et al*., 1985). The decrease in the AST level, which is an indication of the protective effect on liver and heart. Proteins are synthesized in liver. Inhibition of protein synthesis indicates disruption and dissociation of polyribosomes from endoplasmic reticulum. In untreated diabetic rats, decreased levels of plasma total proteins, albumin and globulins were observed when compared with normal rats. Treatment with oral administration of graded doses of 100, 200 & 400 mg/kg b.w of different extracts of 70% IBEE and reference drug glibenclamide 5 mg/kg b.w fraction prevented the altered total proteins levels in a highly significant manner.

Oxidative stress in diabetes is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals. The SOD and CAT are the two major scavenging enzymes that remove free radicals. Reduced activities of these antioxidant enzyme in liver, kidney and pancreas tissues have been observed in diabetic rats and this activity may result in a number of deleterious effects due to accumulation of superoxide anion (O^-) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (OH), resulting in initiation and propagation of LPO. SOD protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. CAT was shown to be responsible for the detoxification of H_2O_2, and protects the tissues from highly reactive hydroxyl radicals (Mahboob*et al*., 2005). This decrease in CAT activity could result from inactivation by glycation of enzyme (Yan and Harding, 1997). In present study oral administration of graded doses of 100, 200 & 400 mg/kg b.w of different extracts of 70% IBEE and reference drug glibenclamide 5 mg/kg b.w showed a significant increase in the hepatic and renal SOD and CAT activities of the diabetic treated rats. Glutathione is a tripeptide, intracellular antioxidant and protects the cellular system from adverse effects of lipid peroxidation. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Winterbourn*et al*., 1995). Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased GSH content. Treatment with oral administration of graded doses of 100, 200 & 400 mg/kg b.w of different extracts of 70% IBEE and reference drug glibenclamide 5 mg/kg b.w resulted in the elevation of the GSH levels, which protects the cell membrane against oxidative damage by regulating the redox status of protein in the membrane (Inove*et al.,* 1987).

Tremendous increase in lipid peroxidation observed in diabetic rats was attributed to chronic hyperglycemia which causes increase production of reactive oxygen species (ROS) due to the autooxidation of monosaccharide which leads to the production of superoxide and hydroxyl free radicals (Wolff and dean 1987) which cause tissue damage by reacting with polyunsaturated fatty acids in membranes (Das *et al*., 2007). Reductases and polyphenols present in ethanolic extracts might be responsible for scavenging of hydroxyl free radicals and breaking the free radical chain by donating a hydrogen atom. The present study clearly supports prominent antioxidant protection by ethanolic extracts *alone* and glibenclamide against STZ induced oxidative stress (Zhang *et al*., 2009). In the present study, the studies on histology and ultra-structure demonstrated that most of the islets were affected and showed changes in their structure. The cellular integrity architecture were intact in the normal control group, It showed normal acinic and normal cellular population in the islets of Langerhans in pancreas, whereas diabetic animal showed severe necrotic changes of pancreatic islets, especially in the center of islets, relative reduction in size, number of islets especially around the central vessel & severe reduction of beta cells were clearly seen. The β-cells showed degranulation and swelling of the intracellular organelles. All these vital intracellular structures were affected thus inhibiting the synthesis and production of insulin and the microscopic examination showed abundant patches of β-cells in pancreas (Anil *et al*., 1996).

S No	Groups	Induction & treatment (mg/kg b.w for 30 days)
1	Group I (Normal control)	Normal control group rats were received saline 5 ml/kg.
2	Group II (Diabetic control)	Diabetic rats received normal saline 5 ml/kg b.w
3	Group III (Standard)	Diabetic rats were received glibenclamide 5 mg/kg.
4	Group IV (Test -1)	Diabetic rats were treated with IBEE (100 mg/kg p.o.)
5	Group V (Test - 2)	Diabetic rats were treated with IBEE (200 mg/kg b.w, p.o)
6	Group VI (Test - 3)	Diabetic rats were treated with IBEE (400 mg/kg b.w, p.o,)

Table 1: Percentage yield of different extracts of Indigofera*barberi* (whole plant) with different solvents and results were given in Table 1.

S No	Parameters	Chloroform	Ethyl acetate	70% Ethanol	water
1	Consistency	Oily	Oily	Viscous	Viscous
2	Color	Green	Brownish green	Radish black	Cream
3	% of yield	4.75%	3.50%	9.20%	4.20%

4.2: Preliminary phytochemical screening of IBEE

Table 2:Preliminary phytochemical screening of the different extracts of *Boswellia ovailifoliata (A), Indigo-ferabarberi (B) &Rhynchosiabeddomei (C).*

S. No	Phytoconstituents	Chloroform	Ethyl acetate	70% Eth-anol	water
1	Alkaloids	-	-	-	-
2	Glycosides	+	-	+	+
3	Carbohydrates	-	-	+	-
4	Proteins	-	-	-	-
5	Amino acids	-	-	-	-
6	Flavonoids	+	+	+	+
7	Steroids	+	+	+	-
8	Triterpenoids	+	+	+	-
9	Tannins	+	+	+	+
10	Phenols	+	+	+	+
11	Saponins	-	-	-	-

4.3: *Invitro antioxidant* activity of IBEE

Table 3: The absorbance and scavenging activity or % inhibition of DPPH, NO & H_2O_2

***Indigoferabarberi* ethanolic extract (IBEE – B) on DPPH**			
Conc. µg/ml	***Indigoferabarberi***	**Ascorbic acid**	**BHT**
50	15.38±0.65	20.12±0.51	18.16±0.99
100	19.25±0.96	28.12±0.89	21.36±1.11
150	28.36±1.21	39.46±1.16	33.16±1.32
200	37.34±1.75	56.23±1.52	47.19±1.58
250	45.28±1.84	79.16±1.98	72.38±1.81
IC_{50}	274.44	168.62	193.32
B. *Indigoferabarberi*ethanolic extract (IBEE – B)			
Conc. µg/ml	***Indigoferabarberi***	**Ascorbic acid**	**BHT**
50	16.18±0.46	28.36±0.76	20.17±0.75
100	22.39±0.94	39.38±0.89	31.25±0.91
150	32.25±1.98	48.16±1.13	42.36±1.27
200	39.38±1.56	59.24±1.09	53.19±1.65
250	44.16±1.71	70.36±1.89	64.29±1.17
300	49.06±1.32	84.29±1.87	72.39±1.69
IC_{50}	283.24	161.33	190.55
B. *Indigoferabarberi* ethanolic extract (IBEE – B)			
Conc. µg/ml	***Indigoferabarberi***	**Ascorbic acid**	**BHT**
50	10.11±0.38	26.48±0.63	18.36±0.95
100	19.25±0.70	39.38±0.74	27.42±0.56
150	24.36±1.36	47.19±1.64	34.28±1.74
200	32.28±0.71	59.28±2.11	42.36±1.36
250	38.39±0.89	70.38±2.81	62.38±1.64
300	44.65±2.11	84.09±1.36	76.43±1.48
IC_{50}	328.68	162.9	203.46

Data expressed as mean ± SEM. Each sample was analyzed in triplicate.

Table 4: Effect of graded doses of IBEE on normoglycemic blood glucose levels

B. IBEE - Blood glucose level (mg/dl) h.					
Groups & dose	**Pre treat**	**0.5**	**1.0**	**2.0**	**4.0**
I	78.1±3.98	79.32±2.79	79.94±3.41	79.56±2.86	79.32±3.84
II-Glibenclamide	76.59±3.33	56.35±2.86[c] (26.42)	47.26±8.36[c] (38.29)	50.15±6.26[c] (34.52)	54.08±4.96[c] (29.39)
III-100 IBEE	78.26±2.75	62.78±3.22[b] (19.78)	52.88±2.35[b] (32.42)	56.60±6.86[b] (27.67)	60.63±2.36[b] (22.52)
IV-IBEE 200	80.57±3.81	62.49±2.88[b] (22.43)	52.49±2.37[b] (34.85)	55.96±2.96[b] (30.54)	59.63±2.24[c] (25.98)
V-IBEE 400	74.62±2.76	55.60±2.87[c] (25.89)	47.31±4.73[c] (36.59)	50.56±4.06[c] (32.23)	54.18±1.92[c] (27.38)

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 5: Effect of graded doses of IBEE on OGTT in normal rats.

S NO	Groups	Dose	Blood glucose level (mg/mL)			
			0 min	**60 min**	**120 min**	**240 min**
1	Control	5 ml/kg	79.56±3.73	182.98±3.74	156.86±3.81	129. 38±3.56
2	Standard	0.2 mg/kg	83.82±3.42	171.16 ±3.89**	149.65±5.23	92.77±3.57
3	Test 1	100 mg/kg	81.27±3.23	162.12 ±4.43***	140.59±4.65	124.16±4.85
4	Test -2	200 mg/kg	79.93±3.76	141. 44±4.98***	121.22±3.38	118.7±4.42
5	Test -3	400 mg/kg	80.47±3.86	148.17±3.06***	116.79±3.28	106.95±3.38

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 6: Effect of graded doses of IBEE on***antihyperglycemicactiivty***in STZ induced diabetes and treated animals over 30 days

S NO	Groups	Blood glucose level (mg/mL)				
		1 [th] day	10 [th]day	20[th] day	30 [th]day	%
1	Control	79.56±3.29	83.65±3.96***	88.38±3.89***	87.12±3.43***	-
2	Positive	269.92±8.32	274.76±6.84	279.98±7.4***	272.64±8.26	-
3	Standard	274.71±6.16	121.18±4.30***	112.65±4.2***	86.42±3.21***	68.54
4	Test 1	267.43±8.81	145.45±5.73***	139.78±5.3***	124.92±4.54***	53.28
5	Test 2	271.89±6.26	136.17±4.86***	121.52±5.2***	113.61±5.76***	58.21
6	Test 3	272.48±7.65	128.62±4.21***	116.38±3.4***	102.58±3.05***	62.35

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 7:Effect ofgraded dosesof IBEEon body weights in sreptozotocin induced diabetic treated albino rats

S.no	Groups	After induction Body WT (GRAMS)				% of reduc-tion
		1 day	10 day	20 th day	30 th day	
1	Control	169.49±6.87	175.45±6.89***	179.97±7.26***	184.07±8.91***	-
2	Positive	187.62±7.46	146.64±5.65	141.37±5.87	130.18±4.25	-30.61
3	Standard	174.73±4.78	154.85±5.47	162.69±6.44***	169.58±4.86***	-2.94
6	Test -1	170.48±7.57	142.96±5.65	146.73±5.32	149.49±4.23***	-12.31
5	Test -2	181.62±7.84	151.31±4.86	158.37±5.14***	163.52±5.57***	-9.46
6	Test -3	177.86±6.37	152.73±5.36	159.81±4.89***	162.93±6.72***	-8.39

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 8: Effect of graded doses of BOEE/IBEE/RBEE on TC, TG, HDL& LDL elsin STZ induced diabetes and treated animals over 30 days

S NO	Groups	TC	TG	HDL	LDL
1	Control	134.14±4.78***	79.44±3.58***	54.78±2.95***	67.85±2.98***
2	Positive	286.33±8.74	178.17±5.72	32.36±1.21	152.49±4.38
3	Standard	142.48±5.87***	85.19±3.96***	50.56±2.87 ***	78.49±2.89***
4	Test -1	165.84±4.65***	112.18±5.86***	36.76±1.20 **	87.38±3.76***
5	Test -2	154 .26±4.83***	104.37±3.05***	43.13±1.58***	82.11±3.56***
6	Test -3	150.12±4.31***	98.48±3.91***	46.98±1.72***	79 .32±3.29***

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 9: Effect of graded doses of BOEE/IBEE/RBEE on liver marker enzymes s in STZ induced diabetes and treated animals over 30 days

S NO	Groups	SGPT	SGOT	ALP
1	Control	41.23±1.87***	69.75±3.70***	112.55±4.85***
2	Positive	98.93±4.62	138.85±5.69	259.18±4.31
3	Standard	51.84±2.96***	76.48±3.37***	118.74±3.16***
4	Test -1	63.49±3.49***	104.23±4.98***	142.64±4.83***
5	Test -2	56.74±3.87***	92.73±3.13***	138.41±3.69***
6	Test -3	53.49±3.19***	88.86±3.06***	131.74±3.09***

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 10: Effect of graded doses of BOEE/IBEE/RBEE on total protein, urea & uric acid in STZ induced diabetes and treated animals over 30 days

S NO	Groups	Protein (mg/dl)	Serum urea (mg/dl)	Serum creatinine (mg/dl)
1	Control	7.68±0.65***	33.18±1.97***	0.82±0.021***
2	Positive	3.2±0.26	74.69±2.79	1.61±0.05
3	Standard	7.10±0.68***	32.96±1.78***	0.86±0.042***
4	Test -1	5.29±0.36***	41.58±2.93***	1.0±0.62**
5	Test -2	6.18±0.47***	37.84±2.11***	0.94±0.08***
s6	Test -3	6.42±0.35***	35.28±2.06***	0.89±0.04***

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 11:Effect of graded doses of EEBO (A), EEIB (B)& EERB (C) on TNF –Alpha, IL -6 & NO in Streptozotocin induced diabetic treated albino rats

S NO	Groups	TNF-· α(pg ml –1)
1	Control	45.11±2.34***
2	Positive	97.84±3.98
3	Standard	51.68±2.61***
4	Test -1	62.24±3.72***
5	Test -2	58.68±2.79***
6	Test -3	54.17±2.47***

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Table 12: Effect of graded doses of (A) EEBO, (B) EEIB & (C) EEBR on anti-oxidant enzymes in streptozotocin induced diabetic treated albino rats

S.no	Groups	CAT	SOD	GSH
1	Control	47.73±3.04***	9.86±1.05***	11.32±0.93***
2	Diabetic	26.34±2.26	4.72±0.45	3.21±0.74
3	Standard	43.58±3.23***	8.24±0.62***	11.10±0.96***
4	Test -1	35.93±2.74***	6.96±0.48***	7.48±0.84***
5	Test -2	39.69±2.10***	7.16±0.56***	8.15±0.89***
6	Test -3	40.14±3.13***	7.98±0.74***	9.89±0.92***

Data represent the mean ± SEM, (n=6). Statistical significance: [a]P< 0.05, [b]P< 0.01,[c]P< 0.001, versus with respect to diabetic control groups (One way ANNOVA followed by Dennett's multiple comparison test).

Effect of graded doses of IBEE (B) on histopathology of pancreas in STZ induced diabetes

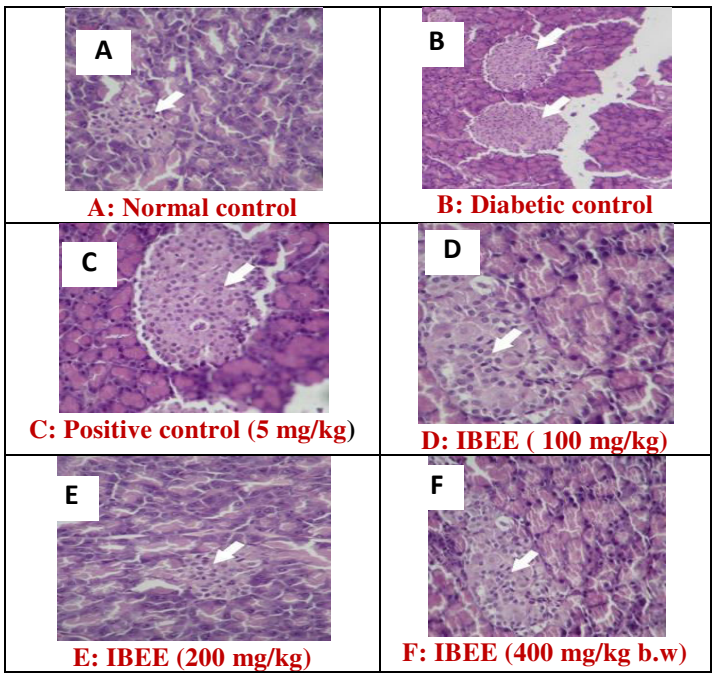

Fig.1. Histological observation on pancreas sections from STZ-induced pancreas damage rats with or without IBEE treatment. Photomicrographs of histological changes of rat pancreas.

Selective destruction of β-cells was observed in STZ induced diabetic rats (Susan Bonner *et al*., 1994). Small and shrunken islets and destruction of β-cells was observed in the diabetic condition (Kesavulu*et al*., 2000).The oral administration of 70% ethanolic extracts of whole plant of *Indigoferabarberi,* at doses of 100, 200 & 400 mg/kg b.w for 30 days significantly restored the pathological changes in STZ induced diabetic animals by the enhancement of sections of pancreatic islets, ability to regenerate the damaged endocrine tissue and increase β-cell numbers due to the regeneration/proliferation in the pancreatic β-cells. Medicinal plants and herbal extracts containing glycosides, flavonoids, tannins etc. have been reported to demonstrate antidiabetic activities (Suba*et al*., 2004), also previous studies have shown that *Axonopuscompressus* contains all the above phytoconstituents (Ogie-Odia*et al*., 2010). It is therefore possible that the phytochemicals present in the whole plant of *Indigoferabarberi*may be responsible for the observed antidiabetic activity.

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