Antihyperglycemic, antihyperlipidemic and antioxidant effect of flavonoid rich extract of Dikamali in Streptozotocin-Nicotinamide-induced type II diabetic rats

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Abstract

Background: Diabetes mellitus type II is the most prevalent metabolic disorder throughout the world and needs continuous development of pharmacotherapeutics for prophylaxis and treatment. Flavonoids are well known for their multifactorial mechanisms to treat diabetes mellitus. Dikamali, a gum resin from *Gardenia gummifera* is well known to contain the bunch of flavonoids. **Objective:** The present study was aimed to obtain the flavonoid-rich extract from the gum resin of *Gardenia gummifera* and evaluate its efficacy in streptozotocin-nicotinamide induced type II diabetic Wistar rats. **Materials and methods:** Evaluation of antidiabetic activity was done at the different dose level of extract viz. 100, 200 and 400 mg/kg body weight of an animal. Parameters such as fasting blood glucose, insulin, Insulin resistance (IR), food and water intake, body weight, lipid profile, urea, creatinine and, antioxidant status of organs were checked. Gluconeogenic enzymes activity was also estimated. **Results:** Studies indicated that the test extract reduced the fasting blood glucose and improved the glucose tolerance in diabetic rats by mitigating insulin resistance, lipid imbalance, and oxidative stress. The test extract also alleviated the enhanced activities of gluconeogenic enzymes in diabetic rats. The micrographs of pancreas histopathology were also supportive of the above findings. The extract also reduced the urea and creatinine levels in diabetic rats which are indicative of its potential in treating diabetic complications also. **Conclusion:** Overall, the test extract showed attractive findings and thus in future, it can be a promising candidate for developing alternate therapeutic moiety for the treatment of diabetes, hyperlipidemia and associated complications.

Keywords: Antidiabetic, *Gardenia gummifera*, streptozotocin, nicotinamide, flavonoids

Introduction

Diabetes is a well known complex metabolic disorder manifested by fasting or postprandial hyperglycemia. Most common etiologies are insulin deficiency, impaired actions of insulin, and impairment in metabolic processes of the body. Secondary manifestation includes glucosuria, polyuria, polyphagia and muscle wasting. The disease is also associated with secondary complications such as nephropathy, retinopathy, hyperlipidemia, and cardiovascular diseases which play a major role in morbidity and mortality in diabetic patients (Bell, 1991; Bell and Polonsky, 2001). For a long time, many antidiabetic drugs are available to society however they present the concern of safety and thus make the disease treatment challenging to the medical professional (Holman and Turner, 1991). Ethnopharmacology witnessed the use of various herbs and their isolated constituents in diabetes treatment. Ayurvedic system of medicine in India has used various indigenous plants to treat diabetes in the era of Charaka and Sushruta. There are many phytoconstituents which are gaining popularity due to their ability to deal with various edges of chronic disorders such as diabetes. Additionally, they claim to have lesser adverse events (Grover et al., 1991; Swathi et al., 2018). Flavonoids are one of the phytoconstituents in the herbs which have proved their efficacy in the diabetes treatment through multiple mechanisms such as inhibition of glucose absorption, enhancement of glucose uptake, insulin mimetic effect, increase insulin secretion, antioxidant potential or by regulating the enzyme involved in carbohydrate metabolism (Babu and Gilbert, 2013). *Gardenia gummifera* (family- Rubiaceae) is a shrub widely

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distributed throughout India. The gum resin of the plant is greenish yellow in color with sharp pungent taste and odor and marketed in the forms of tears, cakes or irregular masses. It is commonly known as dikamali in India. The gum resin is useful in neuropathy, wounds, infections, skin diseases, cardiac complications, splenomegaly, impaired metabolism, obesity etc in the Ayurvedic medicinal system (Ayurvedic Pharmacopoeia of India, 2008; Nadkarni, 1976). Several researchers extracted flavonoids from gum resin (Chhabra et al., 1977; Gupta et al., 1975; Krushnamurthi et al., 1971, 1972). Gum resin showed antioxidant and DNA damage preventing activity in one of the scientific study (Kalim et al., 2010). Other researchers also proved the antihypercholesterolemic effect of gum resin in animals (Gajjar et al., 2008). By taking this into consideration, the present study was aimed at evaluating flavonoid-rich extract of *G. gummifera* gum resin in streptozotocin-nicotinamide induced type II diabetic rat model.

Materials and methods

Drugs and chemicals

Streptozotocin (STZ) was purchased from Sigma-Aldrich, India, and Nicotinamide (NA) was purchased from SDFCL, India. Metformin was provided as a gift sample by Alkem Laboratories LTD, Dabhel, Daman, India. Other chemicals and reagents used in this experiment were of analytical grade.

Collection of plant material and its authentication

Dikamali a gum resin from *G. gummifera* was collected from a local market in Navi Mumbai. The sample was authenticated by Dr. Ganesh Iyer, H.O.D. Botany Department, Ramnarain Ruia College, Matunga, Mumbai, India.

Preparation of extracts

The gum was pulverized to a coarse powder and was subjected to sohxlet extraction. Firstly, the powdered gum was defatted using petroleum ether and subjected to methanolic extraction using soxhlet apparatus. The methanolic extract was dried in a rotary evaporator under reduced pressure till the extract attained constant weight. The extract was further successively fractionated using 1:1 ethyl acetate and water to obtain the ethyl acetate and water fraction of methanolic extract, separately. The water fraction was further fractionated using n-butanol to obtain n-butanol and a water fraction. Each fraction was then concentrated in the rotary evaporator. Finally, different extracts of powdered gum resin of *G. gummifera* were obtained namely methanolic extract, ethyl acetate extract, butanolic extract, and aqueous extract.

Phytochemical analysis

The preliminary phytochemical screening of all the extracts was carried out for the detection of flavonoids (Khandelwal and Sethi, 2014; Gokhale, 2011). The extracts showing the presence of flavonoids qualitatively were subjected to estimation for total flavonoids content.

Determination of total flavonoids content

Total flavonoids content in methanol, ethyl acetate, and butanol extract was analyzed spectrophotometrically (UV-1800 Spectrophotometer by SHIMADZU) (Kamtekar et al., 2014). The extract showing maximum flavonoids content was subjected to further analysis.

High performance thin layer chromatography analysis of Ethyl acetate extract of *G. gummifera* gum resin (GGEAE)

High-performance thin-layer chromatography (CAMAG Linomat 5) was performed in Anchrom Lab Pvt Ltd. Mumbai, India on aluminum-backed plates coated with silica gel 60F254 (E. Merck KGaA) The CAMAG glass twin-trough chamber was saturated using a mobile phase comprising of Chloroform: Ethyl acetate (1:1) (V/V), wherein the development was done in the ascending front at room temperature. After development, detection was done using Natural products - Polyethylene glycol reagent. Scanning was done using CAMAG TLC scanner 4 and documentation was done with WIN CATS software (Wagner and Bladt, 1996).

Animals

Male albino Wistar rats weighing (200-300 g) were used for the study and are maintained in an air-conditioned room (25±1°C) with a 12-h light: 12-h dark cycle, 35-60% humidity. The rats were acclimatized to the laboratory conditions for a week. Feed and water were provided ad libitum to all the animals. All studies were conducted in accordance with the CPCSEA guidelines (Reg. No. 1090/PO/ac/07/CPCSEA). , and the study was approved by the Institutional Animal Ethics Committee (Approval Number: IAEC/TKCP/2015/16).

Acute toxicity study of GGEAE

The acute oral toxicity study was performed as per OECD guidelines 423 (Mir et al., 2013). Male Wistar rats were used for the study and were observed for 14 day study period.

Effect of GGEAE on Oral Glucose Tolerance Test (OGTT) in normoglycemic rats

OGTT was performed in overnight fasted normal rats (Barik et al., 2008). These rats were divided into five groups (n = 6); Group 1 rats were considered as normal rats and group 2, 3, 4, and 5 were administered with GGEAE at different dose levels, viz. 100, 200 and 400 mg/kg body weight and standard drug (metformin, 100 mg/kg),

respectively. Prior to the administration of GGEAE and a standard drug (metformin, 100 mg/kg), the blood glucose level for all the animals were estimated by withdrawing blood from the tail vein as basal readings (0 min reading). Glucose solution (2 g/kg) was fed after the administration of the test compounds. Blood was withdrawn again from the tail vein at 30, 60, 90 and 120 min of glucose administration. The blood glucose levels were estimated using glucometer (Accu-Chek Active Roche Diagnostics, USA).

Preparation of STZ and NA solution

The STZ was prepared freshly by dissolving in 0.1 M citrate buffer (pH 4.5) and NA was prepared by dissolving it in normal saline solution.

Induction of Type II Diabetes in rats

Diabetes was induced in the rats by injecting STZ (55 mg/kg; i.p.) followed by NA (180 mg/kg i. p.) with a gap of 15 minutes. After 7 days, rats with fasting blood glucose (FBG) more than 126 mg/dl were considered diabetic and included in the study (Masiello et al., 1998 and Ananda et al., 2012).

Evaluation of GGEAE for antidiabetic activity

The rats were grouped (n = 6) into 6 groups and were treated for 21 days according to the following protocol:

Normal Control: Healthy rats

Diabetic Control: Diabetic rats

GGEAE 100 mg/kg: Diabetic rats treated with GGEAE (100mg/kg),

GGEAE 200 mg/kg: Diabetic rats treated with GGEAE (200mg/kg),

GGEAE 400 mg/kg: Diabetic rats treated with GGEAE (400mg/kg),

Standard Control: Diabetic rats treated with Metformin (100mg/kg).

Body weight, FBG, food intake and water intake of all animals was measured prior to disease induction (basal) and on Day 0, 7, 14, and 21 of the treatment with test compounds. The blood glucose levels were estimated using glucometer (Accu-Chek, Roche Diagnostics, USA). On day 22, after overnight fasting, the blood sample of rats were collected via intra-cardiac puncture using EDTA as anti-coagulants, following which the rats were sacrificed under ketamine xylazine (i.p.) anesthesia.

Plasma was utilized for estimation various biochemical parameters. Insulin was estimated using insulin Elisa kit for rats (Krishgen Biosystems). The insulin resistance was measured as Homeostatic model assessment- Insulin resistance (HOMA-IR) by the following formula [insulin (µIU/ml) x glucose (mmol/L)/22.5] (Nandhini et al., 2005).

Triglycerides, cholesterol, High density lipoproteins (HDL), urea, and creatinine were estimated using the commercially available kit (Pathozyme Diagnostics, Maharashtra, India). Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were estimated using Friedewald's equation (Friedewald et al., 1972).

Soon after sacrificing the animals, Pancreas, liver, and kidney were immediately isolated, washed in ice-cold saline to remove blood. Thiobarbituric acid reactive substances (TBARS) (Niehius and Samuelson, 1968), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (Banerjee et al., 2010) and reduced glutathione content (Ellman, 1965) in these tissues were estimated. Liver glycogen content was estimated by anthrone reagent method (Yadav et al., 2007) and activities of Glucose 6 phosphatase and fructose 1, 6 bisphosphatase in the liver were estimated by method described by Baginsky and Gancedo. (Ananda et al., 2012)

Portions of the pancreas were stored in 10% formalin solution. Histopathologies of the pancreas were studied by staining sectioned slices of tissue with hematoxylin and eosin.

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 6. Values are expressed as mean ± SEM (n=6) and statistical analysis was carried out by using by one way ANOVA followed by Dunnett's test. The values were considered significant when $P < 0.05$.

Results

Percentage yields, qualitative and quantitative analysis of extracts for total flavonoids content

The extract yields were methanolic (31.25), ethyl acetate (20), butanolic (1.15) % of dry plant material. Phytochemical screening showed the presence of flavonoids in methanolic, ethyl acetate and butanolic extracts. Total flavonoid content was 179.28±16.31, 604.93±9.93and 62.87±8.49 mg of Quercetin equivalent/gm of extract in methanolic, ethyl acetate and butanolic extract respectively. The flavonoid content was determined from calibration curve of quercetin (y = 0.052x + 0.296, R^2 = 0.949).

HPTLC analysis of GGEAE

As shown in Figure 1 Ethyl acetate extract of *G. gummifera* shows 8 quenching zones in the Rf range of 0.04-0.76. After treating with Natural products ‒ Polyethylene glycol reagent 10 bright yellow zones are observed in the visible region (540 nm) in the Rf range of 0.05-0.78.
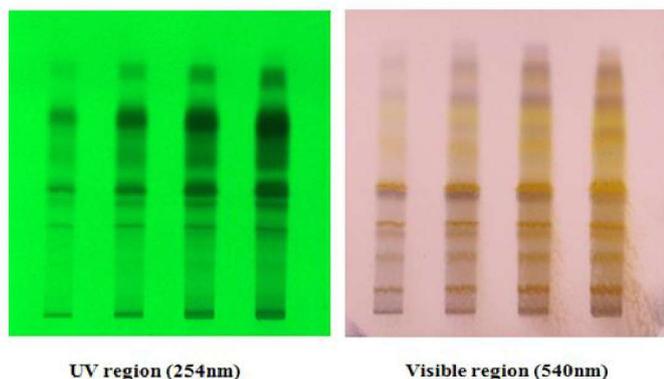
Figure 1. HPTLC analysis of GGEAE

Acute Oral Toxicity study of GGEAE

Acute oral toxicity study of GGEAE was carried out up to an elevated concentration of 2,000 mg/kg. However, at this dose, the test compound did not exhibit any sign of toxicity, behavioral changes, and mortality. Thus, GGEAE was found to be nontoxic and safe to use in further evaluations.

Effect of GGEAE on OGTT in normoglycemic rats

The glucose-induced hyperglycemia reached a maximum at 30 min in the normal rats and glucose level drops gradually over 120 minutes. In the rats administered with standard drug metformin and GGEAE at different dose levels, a drop in glucose level was significantly high at all time points (P<0.001) when compared with the normal control (Figure 2). The area under the curves (AUC) was 15813, 14953, 14473, 13513, 13508 for rats of group 1,2,3,4 and, 5 respectively. This indicates decrease in AUC in extract treated rats when compared to normal rats.

Effect of GGEAE on blood glucose levels of diabetic rats

As showed in figure 3 STZ - NA injection caused a significant rise in Blood glucose level in diabetic rats when compared with
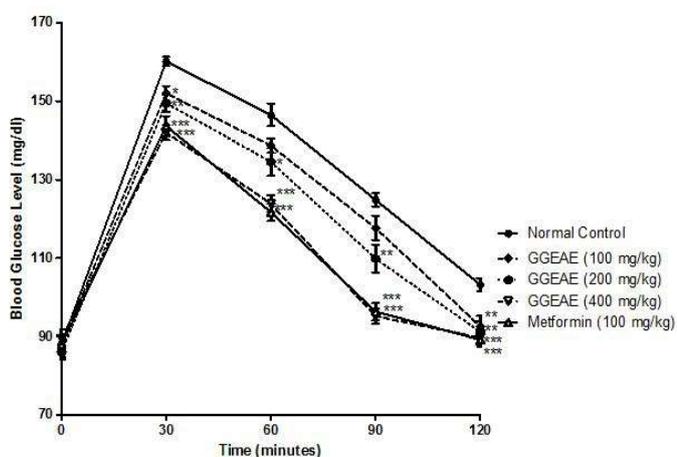
normal control (P-value <0.05). Administration of GGEAE at a dose of 100, 200 and 400 mg/kg body weight of animal for 21 days caused 11.4%, 27.8% and 40.3% decrease in glucose level in diabetic rats. Whereas standard drug metformin caused 42.2 % decrease.

Effect of GGEAE on plasma insulin and HOMA-IR

Insulin level was decreased in diabetic rats when compared with normal control rats (P<0.001). However, treatment with GGEEA and standard drug metformin did not show any significant rise in insulin level in diabetic rats. Although insulin resistance alleviated significantly after treatment with test extract and a standard drug (Table1).

Effects of GGEAE on body weight, food, and water intake

Intoxication with STZ- NA causes a decrease in the body weight and an increase in feed and water intake in animals. Treatment with test compounds reduced the feed and water intake. Weights of the animals were decreasing in diabetic rats as seen from % change in the body weight of animal from day 0 to day 21 of treatment. Treatment with extract reduced this weight loss at 100 and 200 mg/kg dose, however no significant effect was observed at 400 mg/kg (Table 1).

Effect of GGEAE on urea and creatinine

Plasma Urea and creatinine level significantly rose (P<0.001) in diabetic rats compared to normal control rats. Treatment with GGEAE reduced these renal parameters at a dose of 200 and 400 mg/kg and the standard drug also showed this potential (Table1).

Effects of GGEAE on triglycerides, cholesterol, LDL, VLDL, HDL

Lipid parameters such as triglyderides, cholesterol, LDL, VLDL were increased and HDL was decreased in diabetic
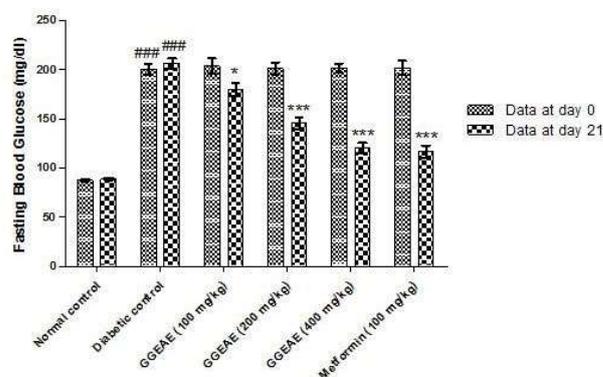

Figure 2. Effect of administration of GGEAE on OGTT in normoglycemic rats. *p<0.05; **p <0.01; ***p <0.001, significantly different compared to Normal control.

Figure 3. Effect of administration of GGEAE on fasting blood glucose levels of diabetic rats. #p<0.05; ##p <0.01; ###p <0.001, significantly different compared to Normal control.
*p<0.05; **p <0.01; ***p <0.001, significantly different compared to Diabetic control.

Table 1. Effect of administration of GGEAE on biochemical parameters

Parameters	Normal control	Diabetic control	GGEAE (100 mg/kg)	GGEAE (200 mg/kg)	GGEAE (400 mg/kg)	Metformin (100 mg/kg)
Blood Glucose (mg/dl)	89.000±1.183	206.333±4.951###	180.167±6.585*	145.333±6.189***	120.500±4.951***	116.667±6.243***
% change in Blood glucose	↑1.597±1.776	↑3.062±1.223	↓11.421± 0.603***	↓27.826± 1.111***	↓40.309± 1.125***	↓42.287± 1.761***
Plasma Insulin (µIU/ml)	23.833±1.151	14.583±0.666###	14.450± 0.526	14.883± 0.643	14.967± 0.471	14.917±0.340
HOMA-IR	5.239±0.267	7.423±0.361###	6.427± 0.328*	5.309± 0.209***	4.442± 0.184***	4.282± 0.193***
% Body weight change	↑6.884±0.380	↓9.824±0.936###	↓6.650± 0.709*	↓5.900± 0.169**	↓6.942± 1.129	↓7.280 ± 0.515
Food intake (gm/day)	21.217± 1.491	30.617± 1.277##	28.400± 1.956	26.050± 1.741	22.750± 1.582*	22.067± 1.872**
Water intake (ml/day)	58.000± 3.606	97.833± 4.799###	82.000± 3.830*	76.167± 2.167**	65.500± 5.439***	58.333± 3.333***
Urea	25.033±1.820	81.950±3.248###	73.850±2.379	67.350±1.796**	56.450±2.609***	33.066±1.996***
Creatinine	0.562±0.055	2.643±0.204 ###	2.430±0.193	2.283±0.123	1.922±0.085#**	0.780±0.0380***

#p<0.05; ##p <0.01; ###p <0.001, significantly different compared to Normal control. *p<0.05; **p <0.01; ***p <0.001, significantly different compared to Diabetic control. ↑: % increase; ↓: % decrease

Table 2. Effect of administration of GGEAE on lipid profile

Parameter	Normal control	Diabetic control	GGEAE (100 mg/kg)	GGEAE (200 mg/kg)	GGEAE (400 mg/kg)	Metformin (100 mg/kg)
Triglycerides	76.333±3.029	123.0±1.897 ###	100.833±3.341 ***	93.167±1.352***	82.500±1.176***	97.167±1.352***
Total Cholesterol	60.500±2.814	90.333±2.716 ###	86.833±2.088	80.833±2.857	70.667±3.148***	78.833±2.857*
HDL	40.500±2.473	25.333±2.305 ###	27.000±1.897	30.500±1.996	36.833±2.548**	31.333±1.706
VLDL	15.267±0.606	24.600±0.379 ###	20.167±0.66***	18.633±0.270***	16.500±0.235***	19.433±0.270***
LDL	4.733±0.523	40.400±0.937 ###	39.667±0.971	31.700±0.939**	17.333±3.061***	28.067±3.176***

#p<0.05; ##p <0.01; ###p <0.001, significantly different compared to Normal control. *p<0.05; **p <0.01; ***p <0.001, significantly different compared to Diabetic control.

rats (Table 2). Treatment with GGEAE lowered the triglycerides and VLDL at a dose of 100 mg/kg when compared with diabetic control (p<0.001) and the effect is also comparable to metformin (p <0.001). Lowering of LDL level was achieved at 200 mg/kg dose. To reduced cholesterol and increase HDL level, 400 mg/Kg dose was required (Table 2).

Effect of GGEAE on oxidative stress markers

The oxidative stress marker TBARS was increased whereas antioxidant enzymes namely catalase and SOD decreased in diabetic rats. Reduced glutathione level was also dropped in diabetic rats. All these effects were significant (p<0.001) when compared with normal rats. GGEAE was able to mitigate the changes occurred in TBARS, SOD, and catalase in all organs at different dose levels. However, there was no change observed in reduced glutathione content at all the doses (Table 3).
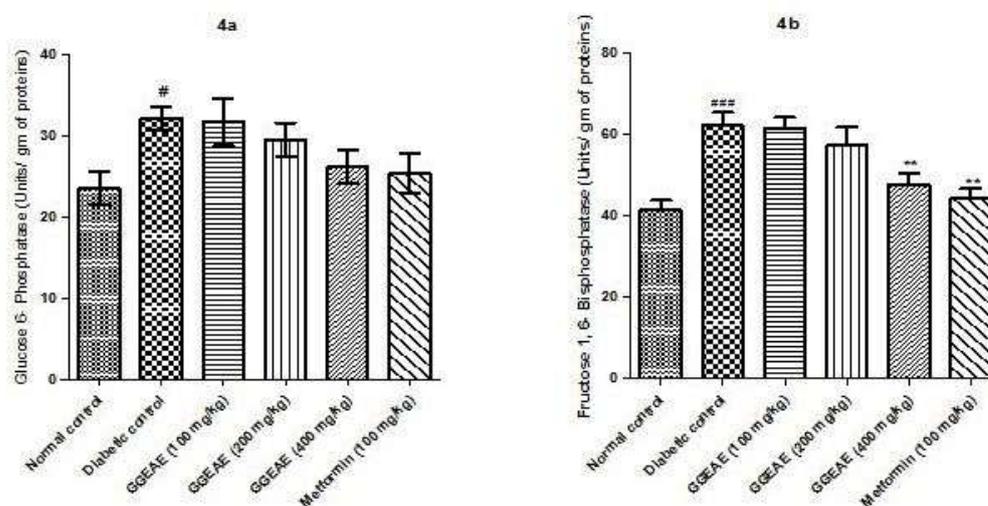

Figure 4. Effect of administration of GGEAE on gluconeogenic Liver enzymes: 4a- effect on glucose 6 phosphatase; 4b- effect on fructose 1,6 bisphosphatase. #p<0.05; ##p <0.01; ###p <0.001, significantly different compared to Normal control. *p<0.05; **p <0.01; ***p <0.001, significantly different compared to Diabetic control.

Table 3. Effect of administration of GGEAE on oxidative stress markers

Parameter	Tissue	Normal control	Diabetic control	GGEAE (100 mg/kg)	GGEAE (200 mg/kg)	GGEAE (400 mg/kg)	Metformin (100 mg/kg)
TBARS (nMoles/mg proteins)	Pancreas	0.743±0.032	3.412±0.172[###]	3.105±0.225	2.758±0.102[**]	1.300±0.127[***]	1.135±0.050[***]
	Liver	4.222±0.393	7.660±0.353[###]	7.152±0.218	6.565±0.356	5.765±0.228[**]	5.133±0.324[***]
	Kidney	1.163±0.059	4.265±0.196[###]	4.003±0.152	3.582±0.166[*]	3.413±0.097[**]	2.195±0.164[***]
Catalase (mMole H_2O_2/min/mg protein)	Pancreas	11.277± 0.574	5.192± 0.383[###]	5.550± 0.390	6.323± 0.374	7.673± 0.342[***]	8.543± 0.666[***]
	Liver	91.833±2.768	52.150±2.755[###]	56.183±1.804	61.367±1.887[**]	80.500±1.108[***]	75.067±1.970[***]
	Kidney	24.750±1.014	14.267±0.993[###]	15.950±0.437	18.133±0.507[**]	18.617±0.801[***]	19.750±0.773[***]
SOD (nMoles/mg proteins)	Pancreas	16.500±1.241	7.223± 0.630[###]	8.845± 0.715	9.740± 0.839	11.610± 0.693[**]	13.133± 0.909[***]
	Liver	32.833± 1.161	18.750± 1.320[###]	20.700± 1.580	22.383± 1.401	26.017± 1.728[**]	26.383± 1.718[**]
	Kidney	21.150± 1.262	11.288± 0.908[###]	11.747± 0.786	14.300± 1.039	17.217± 1.213[**]	16.933± 1.440[**]
Reduced Glutathione (µMole/mg protein)	Pancreas	27.533± 0.801	14.183± 0.909[###]	14.333± 0.270	14.467± 0.445	15.383± 0.478	18.217± 1.040[**]
	Liver	31.633± 0.869	15.917± 0.582[###]	15.567± 0.681	15.500± 0.642	15.950± 0.694	20.683± 0.473[**]
	Kidney	31.233± 1.323	18.167± 0.779[###]	18.383± 0.862	18.450± 0.521	18.567± 0.694	23.200± 0.944[**]

#p<0.05; ##p <0.01; ###p <0.001, significantly different compared to Normal control. *p<0.05; **p <0.01; ***p <0.001, significantly different compared to Diabetic control.

Effect of GGEAE on gluconeogenic liver enzymes

As showed in figure 4 enzyme activity of glucose 6 phosphatase and fructose 1,6 bisphosphatase was enhanced in diabetic rats when compared with normal control rats (p<0.05). GGEAE was able to reduce the activity of fructose 1,6 bisphosphatase when compared with diabetic rats (p<0.05) at dose 0f 400 mg/kg. Both GGEAE and metformin failed to reduce the activity of glucose 6 phosphatase significantly when compared with diabetic control.

Histopathology of Pancreas

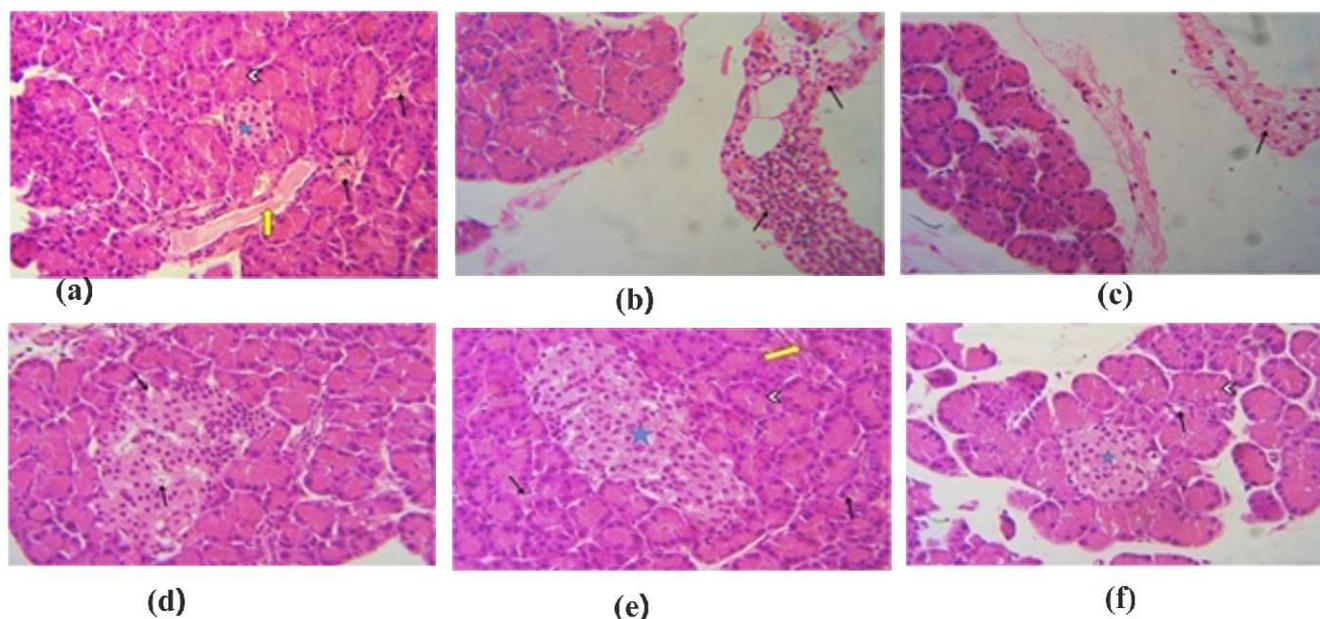Microscopic examination of pancreatic tissue of all the groups was done (Figure 5).

Figure 5. Histopathology of pancreas: 5a: Normal control showing normal histology, acinus (arrow head), interlobular duct (large arrow), intercalated duct (small arrow), Islet of Langerhans (star) {H & E, 400X}. 5b: Showing necrosis of adipose tissues (arrow) {H & E, 100X}. 5c: Diabetic rats treated with GGEAE (100mg/kg) showing necrosis of adipose tissues (arrow) {H & E, 100X}. 5d: Diabetic rats treated with GGEAE (200mg/kg) showing cytoplasmic vacuolation at endocrine pancreas- Islet of Langerhans (arrow) {H & E, 400X}. 5e: Diabetic rats treated with GGEAE (400mg/kg) showing normal histology, acinus (arrow head), interlobular duct (large arrow), intercalated duct (small arrow), Islet of Langerhans (star) {H & E, 400X}. 5f: Standard control howing normal histology, acinus (arrow head), intercalated duct (small arrow), Islet of Langerhans (star) {H & E, 400X}.

No6rmal control rat's pancreas exhibited normal histology (Figure 5a). The pancreas of diabetic rats showed focal mild cytoplasmic vacuolation at endocrine pancreas (Figure 5b). Treatment of diabetic rats with GGEAE 100 mg/kg and 200mg/kg showed focal mild necrosis of perilobular adipose tissues and focal mild cytoplasmic vacuolation at endocrine pancreas respectively (Figure 5c, 5d). The pancreas of rats treated with GGEAE 400 mg/kg (Figure 5e) and metformin 100 mg/kg (Figure 5f) did not unveil any markers of pathological importance.

Discussion

Results of phytochemical analysis and HPTLC studies support the presence of flavonoids in GGEAE where as quantitative estimation of flavonoids suggest abundance of flavonoids in the GGEAE ((Khandelwal and Sethi, 2014; Gokhale, 2011; Wagner and Bladt, 1996; Kamtekar et al., 2014).

At present Type II Diabetes mellitus is a disease with the very high prevalence and associated with severe complication too. Insulin deficiency or impaired functioning, altered metabolism and oxidative stress are some of the root causes of the disease (Hamden et al., 2011). In the present study, we observed that STZ- NA administration caused hyperglycemia, Insulin deficiency, with some degree of insulin resistance and increase in food and water intake which confirms induction of type II diabetes mellitus. Oxidative stress markers associated with hyperglycemia such as TBARS, SOD, Catalase were also raised in multiple organs whereas antioxidant, reduced glutathione level dropped (Nayak et al. 2014; Oyedemi et al., 2012). Moreover, raised levels of urea, creatinine, triglycerides, cholesterol were suggestive of the hazardous effect of diabetes and metabolic disturbances (Kumar and Janardhana, 2011; Szkudelski et al., 2013). The activity of gluconeogenic enzyme namely glucose 6 phosphatase and fructose 1, 6 bisphosphatase were also raised in diabetic animals which may be due to lack of suppressor effect of insulin (Ananda et al., 2012).

Our work showed that the flavonoids rich extract from *G. gummifera* was effective in curing diabetes and associated complications. This antidiabetic potential of GGEAE could be due to i) Enhance insulin sensitivity and improve glucose utilisation as insulin levels are not raised significantly however glucose levels were decreased, which resembles the observation done in other studies (Paoli et al., 2013; Sendrayaperumal et al., 2014; Zhang et al., 2012). ii) Improved lipids and carbohydrate metabolism as discussed by other authors (Prasath and Subramanian, 2014; Srinivasan and Pari, 2013). iii) Antioxidant activity which is evident from elevated levels of SOD, catalase and alleviated levels of TBARS in pancreas, liver, and kidney of extract treated groups which are in agreement with others observations (Pietta, 2000). iv) Inhibition of gluconeogenic enzyme and thus reduction in de novo glucose synthesis as shown by other scientists (Sharma et al., 2008). v) Decrease glucose absorption through inhibition of intestinal enzymes such as alpha amylase and/or alpha-glucosidase as seen in other works (Musa et al., 2012). The antioxidant effect of GGEAE might have improved the cell membrane integrity and able to mitigate the organ damage as seen in histopathological micrographs which may indirectly contribute towards glucose lowering effect (Adewole et al., 2006). Additionally, GGEAE also reduced the level of triglycerides, cholesterol, LDL, VLDL in diabetic rats which indicates the potential of extract to reduce liver dysfunctioning and metabolic complication in diabetes in accordance with other workers (Gajjar et al., 2008). Further, reduction in the level of urea and creatinine in diabetic rats treated with GGEAE indicates its protective effect on kidney dysfunctions in diabetes (Sheela et al., 2013).

From the above findings, we can conclude that Ethyl acetate extract of *G. gummifera* gum resin (GGEAE) has anti-diabetic prospects. It has exerted this effect either through enhancing insulin sensitivity, antioxidant activity or through some other extra pancreatic mechanisms. The activity could be imputed to flavonoids which are present in an abundant amount in the extract. The extract has succeeded to mitigate the variety of parameters associated with liver, kidney, and metabolic dysfunction with the remarkable whack of lipid abnormalities associated with diabetes, hence proved latent quality to reverse diabetic complications too.

Acknowledgement

Conflicts of interest

The authors declare that they have no conflict of interest.

References

Adewole SO, Caxton-Martins EA, Ojewole JA. 2006. Protective effect of quercetin on the morphology of pancreatic β-cells of streptozotocin-treated diabetic rats. African Journal of Traditioanl, Complementary and Alternative Medicine 4(1):64-74.

Ananda Prabu K, Kumarappan CT, Christudas S, Kalaichelvan VK. 2012. Effect of *Biophytum sensitivum* on Streptozotocin and Nicotinamide induced diabetic rats. Asian Pacific Journal of Tropical Biomedicine 2(1):31-5.

Babu VA, Liu D, Gilbert ER. 2013. Recent advances in understanding the anti-diabetic actions of dietary flavonoids. Journal of Nutritional Biochemistry 24(11):1777-89.

Banerjee M, Banerjee N, Ghosh P, Das JK, Basu S, Sarkar AK, States JC, Giri AK. 2010. Evaluation of the serum catalase and myeloperoxidase activity in the chronic arsenic exposed individuals and concomitant cytogenetic damage. Toxicology and Applied Pharmacology 49(1):47-54.

Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. 2008. Antidiabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. Indian Journal of Pharmacology 40(1):19-22.

Bell GI, Polonsky KS. 2001. Diabetes mellitus and genetically programmed defects in-cell function. Nature 414(6865):788-91.

Bell GI. 1991. Molecular defects in diabetes mellitus. Diabetes 40(4):413-22.

Chhabra SC, Gupta SR, Sharma CS, Sharma ND. 1977. A New Wogonin Derivative from *Gardenia* Gum. Phytochemistry 16(7):1109.

Ellman GL. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82(1):70-7.

Friedewald WT, Levi RI, Fredrickson DJ. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the ultracentrifuge. Clinical Chemistry 18(6):499 -502.

Gajjar AV, Jaiswal SJ, Patel JA. 2008. Ethnopharmacology. Indian Journal of Pharmacology 40(Supplement 2): S66-S91.

Gokhale SB, Gokhale A, Kulkarni Y, Yele S. 2011.Chemical test to screen phytochemical nature of herbal drugs. In: Experimental Pharmacognosy, pp. 2.1-2.8, Pune, Nirali Prakashan.

Grover JK, Vats V, Rathi SS, Dewar RJ. 2001. Traditional Indian anti diabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice Journal of Ethnopharmacology 76(3):233-8.

Gupta SR, Seshadri TR, Sharma CS, Sharma ND. 1975. Chemical investigation of dikamali gum: isolation of a new flavones 4'hydroxy wogonin. Indian Journal of Chemistry 13:785-8.

Hamden K, Jaouadi B, Carreau S, Aouidet A, Elfeki A. 2011. Therapeutic effects of soy isoflavones on α-amylase activity, insulin deficiency, liver–kidney function and metabolic disorders in diabetic rats. Natural Product Research 25(3):244–55.

Holman R, Turner RC. 1991. Oral agents and insulin in the treatment of NIDDM. In: Pickup, J., Williams, G. (Eds.), Text Book of Diabetes, pp. 467-469, UK, Blackwell, Oxford.

Kalim MD, Bhattacharyya D, Banerjee A, Chattopadhyay S. 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Complementary and Alternative Medicine 10:77.

Kamtekar S, Keer V, Patil V. 2014. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. Journal of Applied Pharmaceutical Science 4 (09):061-5.

Khandelwal KR, Sethi VK. 2014. Preliminary phytochemical screening. In: Practical Pharmacognosy, pp. 25.1-25.9, Pune, Nirali Prakashan.

Krishnamurthi M, Seshadri TR, Sharma ND. 1971. Some minor components of dikamali gum. Indian Journal of Chemistry 9:189-90.

Krishnamurthi M, Seshadri TR, Sharma ND. 1972. Chemical investigation of dikamali gum: isolation of two new flavones, dimethoxy- and trimethoxy wognin. Indian Journal of Chemistry 10:23-5.

Kumar Ek, Janardhana GR. 2011. Antidiabetic activity of alcoholic stem extract of *Nervilia plicata* in streptozotocin-nicotinamide induced type 2 diabetic rats. Journal of Ethnopharmacology 133 (2):480-3.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. 1998. Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 47:224–9.

Mir AH, Sexena M, Malla MY. 2013. An acute oral toxicity study of methanolic extract from *Tridex procumbens* in Sprague Dawley's rats as per OECD guidelines 423. Asian Journal of Plant Science and Research 3(1):16-20.

Misra HP, Fridovich I. 1972. The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. The Journal of Biological Chemistry 247(10):3170-5.

Musa MY, Griffith AM, Michels AJ, Schneider E, Frei B. 2012. Inhibition of α-Amylase and α-Glucosidase Activity by tea and grape seed extracts and their constituent catechins. Journal of Agricultural and Food Chemistry 60(36):8924-9.

Nadkarni KM. 1976. Indian Materia Medica, vol I, pp.569, Mumbai, Popular Prakashan.

Nandhini AT, Thirunavukkarasu V, Ravichandran MK, Anuradha CV. 2005. Effect of taurine on biomarkers of oxidative stress in tissues of fructose-fed insulin-resistant rats. Singapore Medical Journal 46(2):82-7.

Nayak Y, Hillemane V, Daroj VK, Jayashree BS, Unnikrishnan MK. 2014. Antidiabetic activity of benzopyrone analogues in nicotinamide-streptozotocin induced type 2 diabetes in rats. The Scientific World Journal 2014, 854267:1-12.

Niehius WG, Samuelson B. 1968. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. European Journal Biochemistry 6(1):126-130.

Oyedemi S, Bradley G, Afolayan A. 2012. Antidiabetic activities of aqueous stem bark extract of *Strychnos henningsii* Gilg in Streptozotocin-nicotinamide type 2 diabetic rats. Iranian Journal of Pharmaceutical Research 11 (1):221-8.

Paoli P, Cirri P, Caselli A, Ranaldi F, Bruschi G, Santi A. 2013. The insulinmimetic effect of morin: A promising molecule in diabetes treatment. Biochimica et Biophysica Acta. 1830(4):3102-11.

Pietta P. 2000. Flavonoids as Antioxidants. Journal of Natural Products 63(7):1035-42.

Prasath GS, Subramanian SP. 2014. Antihyperlipidemic effect of fisetin, a bioflavonoid of strawberries, studied in streptozotocin-induced diabetic rats. Journal of Biochemical and Molecular Toxicology 28(10):442-9.

Sendrayaperumal V, Iyyam PS, Subramanian S. 2014. Design, synthesis and characterization of zinc-morin, a metal flavonol complex and evaluation of its antidiabetic potential in HFD-STZ induced type 2 diabetes in rats. Chemico-Biological Interactions 219:9-17.

Sharma B, Viswanath G, Salunke R, Roy P. 2008. Effects of flavonoid-rich extract from seeds of *Eugenia jambolana* (L.) on carbohydrate and lipid metabolism in diabetic mice. Food Chemistry 110(3):697-705.

Sheela N, Jose MA, Sathyamurthy D,6 Kumar BN. 2013. Effect of silymarin on streptozotocin-nicotinamide-induced type 2 diabetic nephropathy in rats. Iranian Journal of Kidney Diseases 7(2):117-23.

Srinivasan S, Pari L. 2013. Antihyperlipidemic effect of diosmin: A citrus flavonoid on lipid metabolism in experimental diabetic rats. Journal of Functional Foods 5(1):484-92.

Swathi NP, Gowtham M, Paridhavi M. 2018. Some promising medicinal plants with anti-diabetic wound healing activity: A review. Asian Journal of Pharmacy and Pharmacology 4(4):379-85.

Szkudelski T, Zywert A, Szkudelska K. 2013. Metabolic disturbances and defects in insulin secretion in rats with streptozotocin-nicotinamide induced diabetes. Physiological Research. 62(6):663-70.

The Ayurvedic Pharmacopoeia of India. 2008. Vol VI, part I, 1st (Eds) pp.113-114, New Delhi, Controller of Publication.

Wagner H, Bladt S. 1996. Flavonoid drugs including *Gingko biloba* and *Echinaceae* species. In: Plant Drug Analysis A Thin layer Chromatography Atlas, pp. 195-245, New York, Springer.

Yadav H, Jain S, Sinha PR. 2007.Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. Nutrition 23:62–68.

Zhang WY, Lee JJ, Kim Y, Kim IS, Han JH, Lee SG. 2012. Effect of eriodictyol on glucose uptake and insulin resistance in vitro. Journal of Agricultural and Food Chemistry 60(31):7652-8.