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ANTIDIABETIC ACTIVITY OF *FICUS DALHOUSIAE* MIQ LEAVES ETHANOLIC EXTRACT ON STREPTOZOTOCIN INDUCED DIABTES IN WISTAR ALBINO RATS

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ABSTRACT

Recently, a substantial body of literature provides strong evidence to support the potentials of *Ficus dalhousiae* (FD), plant and its extracts in various pathological conditions. Thus, the FD (Verbenaceae), plant was selected for this study and extract at various concentrations were prepared and antidiabetic efficacy was evaluated in laboratory animals. The fresh leaves and bark of ***FD*** were collected and n-Hexane, Chloroform, Ethanol and Aqueous extract were prepared. The preliminary phytochemical studies showed the presence of various phytochemical and presence of total phenolic, tannin, and flavonoid compounds. Moreover, ethanolic leaves extract of FD (ELEFD) and water leaves extract of FD (WLEFD), showed dose dependent increased in scavenging activity on free radicals and reducing power assay in our present study. Moreover, plant extracts normalizes the glucose level similar to standard drug and exhibited potent antidiabetic effect. Oral administration of ELEFD, also improved body weight in diabetic rats. In addition, administration of plant extracts effectively increased serum HDL and reduced the level of TC, TG, LDL and VLDL cholesterol. In this study, treatment with ELEFD reduced TBARS level and increased SOD, CAT as well as GSH levels. In the light of the above consideration, it can be concluded that with the support of the phytochemical studies and the in- vitro and in-vivo pharmacological studies were done and ELEFD showed promising antioxidant and anti-diabetic activities in a dose dependent manner.

KEYWORD: Diabetes; Anti-diabetic activity; Lipid profile; Oxidative stress.

1. INTRODUCTION

DM is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] It is classified into insulin dependent DM [IDDM] or Type 1, and non-insulin dependent DM [NIDDM] or Type 2. Type 1 diabetes encompasses cases due to pancreatic islet beta-cell destruction and is prone to ketoacidosis. Type 2 diabetes includes the common major form of diabetes which results from defect[s] in insulin secretion with a major contribution from insulin resistance.[1]

In 2000, an estimated 171 million people in the world had diabetes and this is projected to increase to 366 million by 2030.[3] An alarming trend in the last few decades has been the constant rise in the prevalence of diabetes across globally. The implications of these on the healthcare industry are also significant, with more and more healthcare costs emerging with the increase in the diabetes epidemic.

The mainstay of non-pharmacological treatment of diabetes is diet and physical activity.[3] Other methods of treatment include; acupuncture, hydrotherapy, mineral supplementation and conventional drugs which include; exogenous insulin, oral hypoglycemic agents and transplantation.[3] In conventional medical practice, the present therapies of DM are reported to have side effects.

Medicinal plants are used for hundreds of years as remedies for human diseases and animals. In previous few decades, there has been growing interest in exploiting biological activities of plant-based medicines because of their natural origin, cost effectiveness and lesser facet effects. Medicinal plants are used by almost 80% of the world's population for their basic health care because of their low cost and ease in availability.[4] Numerous encouraging leads have come up with the convergence of empirical uses of various plant species in various parts of the globe showing potential antidiabetic property.

Recently, a substantial body of literature provides strong evidence to support the Pharmacological potentials of *Ficus dalhousiae* (FD), plant and its extracts in various pathological conditions. According to the Ayurvedic literature of India, FD has been explored for its various medicinal properties viz. haemostatic, anti-inflammatory, antiseptic, diarrhea,

dysentery, skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation.[5] In this respect, different extract of this plant are expected to act as a potential strategy to treat diabetes.

2. MATERIALS AND METHODS

2.1. Plant Material

2.1.1. Collection and Authentication of *Ficus dalhousiae* Plant

Fresh leaves and bark of **FD** were collected from Tirumala hills, Chittoor district from the state of Andhra pradesh. The plant materials were taxonomically identified and authenticated by Dr. Madhava Chetty, Asst Professor, Dept. Of Botany, S.V. University, Tirupathy Andhra Pradesh, India and the sample voucher specimen and herbarium have been preserved in the Dept. Of Pharmacognosy, Luqman College of Pharmacy Gulbarga, Karnataka.

2.2. Experimental animals

The Institutional Animal Ethical Committee approved the experimental protocol used in the present study. Age-matched young Wistar rats weighing about 200-250 g were employed in the present study. The animals were housed in the room maintained at approximately 24±1°C temperature and humidity of 55±5% with 12-hour light/dark cycle. Free access to food (standard chow from Ashirwad Industries, Ropar, India) and water was allowed. The animals were acclimatized for at least 3-4 days before the initiation of the experiment and were observed for any sign of disease. The animals were maintained under proper conditions until the termination of the experiment. The animals were sacrificed after a predetermined period of the treatment as per the study design to evaluate various parameters.

2.3. Standard drugs and Chemicals

Glucose, insulin, total cholesterol, triglycerides, LDL, HDL, VLDL, serum creatinine, urea and uric acid estimation kits were procured from Erba diagnostics Pvt. Ltd.

2.4. Preparation of leaf and bark extracts of FD

The fresh leaves and bark of ***FD*** were collected. The collected leaves and stembark were washed thoroughly under running water. After, the leaves and barks were kept for 5-6 hours for the water to dry off. After that leaves and barks cut into smaller pieces and air dried for eight days. Then the dried leaves and barks were coarsely powdered using grinder. The powder obtained was successively extracted with solvents of increasing polarity (hexane, chloroform, and ethanol) using Soxhlet apparatus. The resultant mixture then magnetically

stirred in a round bottom flask for overnight at room temperature. Then the resultant mixture was heated in a water bath at 70 °C for 6 hours with constant stirring. Then the mixture was filtered using cotton and Whatman filter paper. After filtration, the filtrates of the extracts were lyophilized with a lyophilizer. The resultant lyophilized extracts were found as brown colored solid material. Moreover, aqueous extract was also made by soaking 10 g of the weighed plant leaves and barks powder in 100 ml of boiled hot water. That mixture was boiled for thirty minutes into a conical flask and put for 24 h. The extract was filtered using filter paper and evaporated. The extract above obtained named as: n-Hexane extract, Chloroform extract, Ethanol extract and Aqueous extract.

2.5. Preliminary phytochemical screening of FD, plant extracts[6-7]

All the extracts of three plants were subjected to preliminary phytochemical investigation for the detection of the following metabolites: (1) alkaloids, (2) carbohydrates, (3) glycosides, (4) phenolic compounds, (5) flavonoids, (6) protein and free amino acids, (7) saponins (8) sterols (9) acidic compound, (10) steroids, (11) fixed oil and fats and (12) terpenoids.

2.6. Quantitative estimation of leaf and bark extracts of FD plants

2.6.1. Estimation of total phenolic content

According to the Folin–Ciocalteau method, the total phenolic (soluble) content was estimated using the Folin–Ciocalteau reagent. This method is based on the oxidation reaction. Gallic acid was used as standard reagent in this procedure. Extract solution (1.0 g/ml) was taken in the flask and then dilution of extract was made up to 46 ml with distilled water. After dilution, Folin–Ciocalteau reagent (1 ml) was added and mixed. After proper mixing, the solution was allowed to stand for 3 minutes. Further sodium carbonate was mixed into the above mixture solution and allowed to stand for 180 minutes by occasional shaking. Blue color developed was then noted at 760 nm. Phenolic contents in the extract were expressed in terms of mg of GAE /g of extract.[8,9]

2.6.2. Estimation of tannin content

The tannin content in a sample was determined by Folin - Ciocalteu method. About 0.1 ml of the extract was taken in a (10 ml) volumetric flask containing 7.5 ml of distilled water, and 0.5 ml of Folin Phenol reagent, 1 ml of 35 % Na2CO3 solution and makeup the volume to 10 ml with distilled water. The mixture was well shaken and kept for 30 min in room temperature. A series (20, 40, 60, 80 and 100 µg/ml) of reference standard solutions of gallic acid was prepared in the same method as described earlier. Absorbance for test and standard

solutions was measured against the blank at 725 nm in an UV/Visible spectrophotometer.[10,11]

The tannin content and total phenol content was expressed in terms of mg of GAE/g of extract.

2.6.3. Determination of total flavonoid content

The aluminium chloride colorimetric method was employed to measure the total flavonoid content of a sample. In a 10 ml volumetric flask 1 ml of extract and 4 ml of distilled water along with 0.3 ml of 5 % sodium nitrite solution and 10 % aluminium chloride was added at an interval of 5 minutes. To this reaction mixture, 2 ml of 1M sodium hydroxide and 10 ml with distilled water was added after 5 minutes interval. A series (20, 40, 60, 80 and 100 µg/ml) of reference standard solutions of quercetin was prepared in the same method as described earlier. The absorbance for standard and test solutions was determined against the reagent blank at 510 nm in an UV/Visible spectrophotometer. The total flavanoid content was expressed in terms of mg of RE/g of extract.[12,13]

2.7. *In-Vitro* antioxidant assay

2.7.1. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of leaf and bark extracts of FD plant

The in-vitro antioxidant activity of all plants extracts was measured by DPPH free radical scavenging assay method described earlier.[14,15] DPPH, a stable free radical has been extensively used to measure free radical scavenging activity of compounds. DPPH solution (0.1 mM) was prepared in ethanol and 1.0 ml of this solution was added to 3.0 ml of extract solution prepared in water at different concentrations (1-5 µg/ml). Mixture was incubated in dark for thirty minutes and the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. The result of this method was expressed in the form of % DPPH scavenging effect and was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was absorbance of blank and A_t absorbance in presence of extract. Test was carried out in triplicate.

2.7.2. Reducing power assay of leaf and bark extracts of FD plant for antioxidant activity

According to Oyaizu[16], reducing power was evaluated as follows, 2.5 ml volume of various concentrations of extracts (10-320 µg/ml) were mixed with sodium phosphate buffer (2.5 ml, 200 mM) and potassium ferricyanide (2.5 ml, 1%) at pH 6.6. Mixture was incubated (50 ˚C for 20 min). After incubation trichloroacetic acid (2.5 ml of 10% w/v) was added into the above solution. After proper stirring and mixing this solution was centrifuged (8 min at 1000 rpm) for separation of layers. After centrifugation of 8 min, upper layer was separated. This upper layer was taken for estimation. 5 ml of upper layer was added into deionized water (5 ml) and ferric chloride (1 ml, 0.1%). After proper mixing, absorbance was measured at 700 nm using double beam spectrophotometer. This procedure was repeated three times and means values ± S.D. were calculated. EC_{50} value was calculated from concentration-absorbance graph and ascorbic acid was used as standard.

In in-vitro antioxidant study, ethanolic leaf extract of FD, displayed highest antioxidant activity. So based on the results of DPPH free radical scavenging activity and reducing power activity, ethanolic leaf extract of FD were further used for anti-diabetic activity in animal models

2.8. Preparation of extract doses for hypoglycemic studies

Oral dose of ***FD*** extract at 100-500 mg/kgbwt was prepared in CMC for evaluation of hypoglycemic effect. Doses of the extract were selected on the basis of pilot studies reported in the literature.

2.9. Oral administration to Wistar rats

The required amount of plant extracts were dissolved in minimum amount of CMC & water (preferably 0.5 to 2 ml) and administered orally to the rats by using a 2 ml syringe with the help of polythene tubing fixed to the tip of a long needle. All plants extract at different doses and standard drugs such as glipizide were administered to diabetic rats after 7 days of injection of STZ and their treatment was continued for 4 weeks. All the parameters were assessed at the end of 4 weeks of treatment i.e. 4 weeks of diabetes induction in all groups.

2.10. Induction of diabetes

Diabetes was induced by administration of streptozotocin (STZ; 65 mg/kg, *i.p.*) prepared in fresh citrate buffer (pH 4.5). Determining fasting blood glucose (FBG) level after 72 h of

STZ injection confirmed development of diabetes. The rats with FBG level ≥ 250 mg/dl were included in the study.[17]

2.11. Experimental Design

Experimental animals were divided into seven different groups comprising eight animals each. All plant extracts were evaluated for their antidiabetic effect at dose of 100-500 mg/kg per oral (*p.o.)* and glipizide (4 mg/kg, p.o.).

Group I (Untreated normal control rats): Normal control rats received only normal diet and water during the experimental period but without any therapy.

Group ll (Plant extract treated normal rats): Normal rats treated with a single dose of aqueous extract of ***FD*** orally at a dose of 500 mg/ kgbwt daily one time.

Group III (Diabetic control rats): Rats of this group were STZ-induced diabetic model and were served as diabetic controls throughout the experimental period but without any therapy.

Group IV (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of ***FD*** orally at a dose of 100 mg/kgbwt daily one time

Group V (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of ***FD*** orally at a dose of 200 mg/kgbwt daily one time

Group VI (Plant extract treated diabetic rats): Diabetic models of rats treated with a single dose of aqueous extract of ***FD*** orally at a dose of 500 mg/kgbwt daily one time

Group VII (Glipizide Treated Diabetic Group): The diabetic rats after 1 week of STZ administration were treated with glipizide (4 mg/kg, p.o.).

2.12. Assessment of STZ-induced Diabetes

2.12.1. Estimation of body weight

Each animal body weight was measured before induction of STZ. Body weight was measured periodically till the end of study.

2.12.2. Blood samples for biochemical estimation

Blood samples were withdrawn (under light anesthesia) by retro orbital puncture method in the morning after overnight fasting and analyzed for measurement of Blood glucose level, lipid profile [Serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL)]. Blood was allowed to clot and centrifuged at 4000 rpm for 15 min at 4˚C and serum was separated. The serum samples were frozen until analyzing the biochemical parameters. Biochemical estimation was carried out using available laboratory kits of Erba diagnostics Pvt. Ltd.

2.12.2.1. Estimation of serum glucose

Blood glucose level was estimated after 72 hours of STZ administration to confirm diabetes. Fasting blood glucose level was estimated on 0th day, 30th day and 75th day.

The glucose concentration was estimated by glucose oxidase peroxidize GOD-POD method using the commercially available kit.[18] 1000 µl of working glucose reagent was added to 10 µl of serum, 10 µl of standard glucose (100 mg/dl) and 10 µl of purified water to prepare the test, standard and blank, respectively. All the test tubes were incubated at room temperature for 30 min. The absorbance of test and standard samples were noted against blank at 505 nm spectrophotometrically (Thermo Double Beam Spectrophotometer, Thermo Electron Corporation, United Kingdom).

2.12.2.2. Assessment of Blood lipid profile

2.12.2.2.1. Estimation of serum total cholesterol (TC)

The total cholesterol was estimated by cholesterol oxidase peroxidase CHOD-POD (cholesterol oxidase-peroxidase) method.[19] 1000 µl of cholesterol reagent was added to 10 µl of serum, 10 µl of standard cholesterol (200 mg/dl) and 10 µl of purified water to prepare the test, standard and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbances of test and standard samples were noted against blank at 505 nm spectrophotometrically.

2.12.2.2.2. Estimation of serum triglycerides (TG)

The serum triglyceride was estimated by glycerophosphate oxidase peroxidase GOD-POD method.[20] 1000 µl of enzyme reagent was added to 10 µl of serum, 10 µl of standard (200 mg/dl) and 10 µl of purified water to prepare the test, standard and blank, respectively. All the test tubes were incubated at room temperature for 15 min. The absorbance of test and standard samples were noted against blank at 546 nm spectrophotometrically.

2.12.2.2.3. Estimation of high-density lipoprotein (HDL)

The HDL was estimated by cholesterol oxidase peroxidase CHOD-POD method. HDL level in serum was measured by following manufacturer protocol mentioned in kit.[21] The overall method for determining HDL can be divided into two steps.

Step 1. 200 µl of serum and 300 µl of precipitating reagent were taken into the centrifuge tube, mixed well and were incubated at room temperature for 5 min and then centrifuged at 3000 rpm for 10 min to get clear supernatant.

Step 2. 1000 µl of cholesterol reagent was added to 100 µl of supernatant (from step1), 100 µl of HDL cholesterol standard (50 mg/dl) and 100 µl of purified water to prepare the test, standard and blank, respectively. All the test tubes were incubated at room temperature for 10 min. The absorbance of test and standard samples were noted against blank at 505 nm spectrophotometrically. On addition of the precipitating reagent to the serum, followed by centrifugation, HDL fraction remains in the supernatant while the lipoprotein precipitates out.

2.12.2.2.4. Serum LDL and VLDL

Serum VLDL and LDL concentrations were calculated according to Friedewald equation.[22]

LDL cholesterol = Total cholesterol (TC) – High density lipoprotein (HDL) -Triglycerides (TG)/5.

2.13. Assessment of oxidative stress in serum samples

The oxidative and antioxidant parameters in serum samples were assessed by estimating TBARS (thiobarbituric acid reactive substance), GSH (glutathione), CAT (catalase) and SOD (superoxide dismutase) levels.

2.13.1. TBARS level in serum

1 ml of 20% trichloroacetic acid was added to 100 µl of serum and 1% thiobarbituric acid (TBA) reagent (1.0 ml) which were mixed and incubated at 100°C for 30 min. After cooling on ice, samples were centrifuged at 1000g for 20 min. Serum concentration of TBARS was measured spectrophotometrically at 532 nm. A standard curve using 1, 1, 3, 3-tertramethoxyopropane (1 µM to 10 µM) was plotted (Fig. 10) to calculate the concentration of TBARS.[23]

2.13.2. Estimation of Serum Reduced Glutathione

The GSH level was estimated using the methods described by Ellman.[24] Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB] is a chemical used for measuring the amount of thiol group. Thiols react with this compound, cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (NTB-), which ionizes to the NTB2- dianion in water at neutral and alkaline pH. This NTB2- ion has a yellow color. The NTB2- is quantified in a spectrophotometer by measuring the absorbance at 412 nm.

100 µl of serum was mixed with 2 ml of 0.3 M disodium hydrogen phosphate buffer (pH 8.4) and 0.4 ml of distilled water. Then, 0.25 ml of 0.001 M freshly prepared DTNB [5,5' -

dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v sodium citrate] was added to the reaction mixture, and then incubated for 10-min. The absorbance of the yellow colored complex was noted spectrophotometrically at 412 nm. A standard curve was plotted using the reduced form of glutathione (0.1–1 μM), and the results were expressed as mM/g protein.

2.13.3. Estimation of Superoxide Dismutase Activity

SOD activity was measured by the method of Misra and Fridovich.[25] Auto oxidation of epinephrine at pH 10.4 was spectrophotometrically measured. In this method, supernatant of the kidney and sciatic nerve tissues was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and the reaction was started by the addition of 0.02 ml (−)-epinephrine. After 5 min the absorbance was measured at 480 nm (UV-1800 Spectrophotometer, Shimadzu, Japan). The activity of SOD was expressed as % activity of normal control.

2.13.4. Estimation of Catalase Activity

Serum CAT activity was assayed by the method described earlier.[26] The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 mol/l phosphate buffer (pH 7), 0.1 ml of serum and 0.4 ml of 2 mol/l hydrogen peroxide. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The absorbance was measured at 620 nm using Spectrophotometer (UV-1800, Shimadzu, Japan) and expressed as micromoles of hydrogen peroxide decomposed /min/milligram protein.

2.14. Statistical Analysis

Data were presented as mean± S.EM. For continuous variables, student t-test was used to differentiate mean difference. For comparison between more than 2 group, the data were processed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. *P < 0.05 was considered significant. Statistical analysis was performed using SPSS version 21.

3. RESULTS

3.1. Preliminary Phytochemical screening in the leaves and barks extracts of FD, Plant

Preliminary phytochemical screening of the crude, 1:10 and 1:100 extracts of all the four solvents (n-hexane, chloroform, ethanol and water) was performed in order to characterize the classes of compounds which are present in the leaf and bark of FD plant (table 1). The results of the phytochemical screening revealed that, all the bark and leaf extract of the plant

showed the presence of flavonoids, Saponins, steroids, alkaloids, tannins, phenolic compounds, triterpenoids and carbohydrates. Moreover, ethanolic extract of leaf of FD (ELEFD), aqueous extract of leaf of FD (WELFD), ethanolic extract of bark of FD (EBEFD) and aqueous extract of bark of FD (WEBFD) found more rich in these phytoconstituents as compare to hexane and chloroform extracts. Glycosides, anthraquinones, and reducing sugars are not present in either of the extracts. Phytochemical constituents were identified and their presence / absence of the in different solvent extracts were scored as +++/+ + / + / - based on color change/precipitation

Table 1: Preliminary phytochemicals constituents in the FD plant.

Phytochemicals	Plants extracts							
	N-hexane		Chloroform		Ethanol		Water	
	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf	Stem bark	Leaf
Tannins	+	+	+	+	++	++	+++	++
Phenols	+	+	++	++	++	++	+++	++
Saponins	+	+	++	++	++	++	++	++
Phlobatannins	-	-	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	++	++	+++	++
Steroids	+	+	++	++	++	+++	+++	+++
Alkaloids	+	+	+	+	++	+++	+++	+++
Glycosides/ Reducing Sugar	-	-	-	-	-	-	-	-

(-) Phytochemicals not detected, Trace: Phytochemicals detected in low amounts; + Phytochemicals detected slightly high than trace, ++ Phytochemicals detected in moderate levels, +++ or ++++ Phytochemicals detectable in high amount

3.2. Quantitative estimation of total phenolic, flavonoids and tannin content in the leaf and bark extracts of FD plant

The results for the total phenol, tannin and flavonoid estimation of all four extracts of **FD** are tabulated in **table 2.** The total phenolic, tannin and flavonoids content of n-hexane, Chloroform, ethanol and aqueous extract s in leaf part was ranged from 1.2 -22.4, 0.8-12 and 2.3-14.2 g GAE/100 g extract respectively (table 2). Whereas, the total phenolic, tannin and flavonoids content of n-hexane, chloroform, ethanol and aqueous extract s in bark part was ranged from 3.1 -19.7, 1.1-11.3, and 2.4-24.18 g GAE/100 g extract, respectively. In the leaf and bark, ethanol extracts demonstrating highest total phenolic, tannin and flavonoid content followed by water, chloroform and n-hexane extracts.

Table 2: Quantitative estimation of Phytoconstituents in leaf and bark extract of FD plant.

Sample	Total Phenolic mgGAE/g of extract	Total Tannins mgGAE/g of extract	Total Flavonids mgRE/g of extract
Leaf			
n-hexane	1.2 ± 0.5	0.8 ± 0.2	2.3 ± 0.5
Chloroform	3.5 ± 1.2	1.7 ± 0.4	4.2 ± 0.9
Ethanol	22.4±0.4	12±4.5	24.18±0.33
Water	16.8 ± 0.5	10.65 ± 0.6	9.4 ± 0.2
Bark			
n-hexane	3.1 ± 0.6	1.1 ± 0.1	2.4 ± 2.7
Chloroform	2.3 ± 0.7	1.6 ± 0.4	10.7 ± 2.8
Ethanol	19.7±2.3	11.3±1.7	12.12±0.12
Water	10.4 ± 1.5	4.3 ± 1.6	9.2 ± 3.3

3.3. In-vitro Antioxidant activity

3.3.1. DPPH radical-scavenging activity of ELEFD, WLEFD, EBEFD and WBEFD

The extracts of FD exhibited significant ($p<0.05$) antioxidant activity assayed by DPPH scavenging activity (table 3). The antioxidant activity of FD leaves and bark extracts shown in Table 3. Ascorbic acid was used as a reference compound. All the estimations were done in triplicates and free radical scavenging activity was expressed in terms of IC_{50}. $IC_{50,}$ which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

Ascorbic acid had shown IC_{50} value of 33.50 ± 0.74 µg/ml, whereas the IC_{50} of EBEFD at a dose range of 1-600µg/ml was 115.19±7.92, 94.42 ± 11.30 and 89.46 ± 8.25 µg/ml, respectively (figure 1). Whereas, the IC_{50} of ELEFD at a dose range of 1-600µg/ml was 97.14± 11.74, 81.22 ± 7.29, 63.99 ± 10.25 µg/ml, respectively. IC_{50} of WBEFD at a doses range of 1-600 µg/ml was 127.39 ± 4.56, 113.24 ± 10.13, 96.64 ± 11.13, respectively whereas the IC_{50} of WLEFD was 122.41 ± 7.47, 110.23 ± 5.32, 102.56 ± 12.52at doses of 1-600 µg/ml, respectively. The treatment with extracts produced dose dependent antioxidant activity and indicates the less antioxidant effect of the leaf was lower than the stem bark. But these values are found to be lower than the standard ascorbic acid.

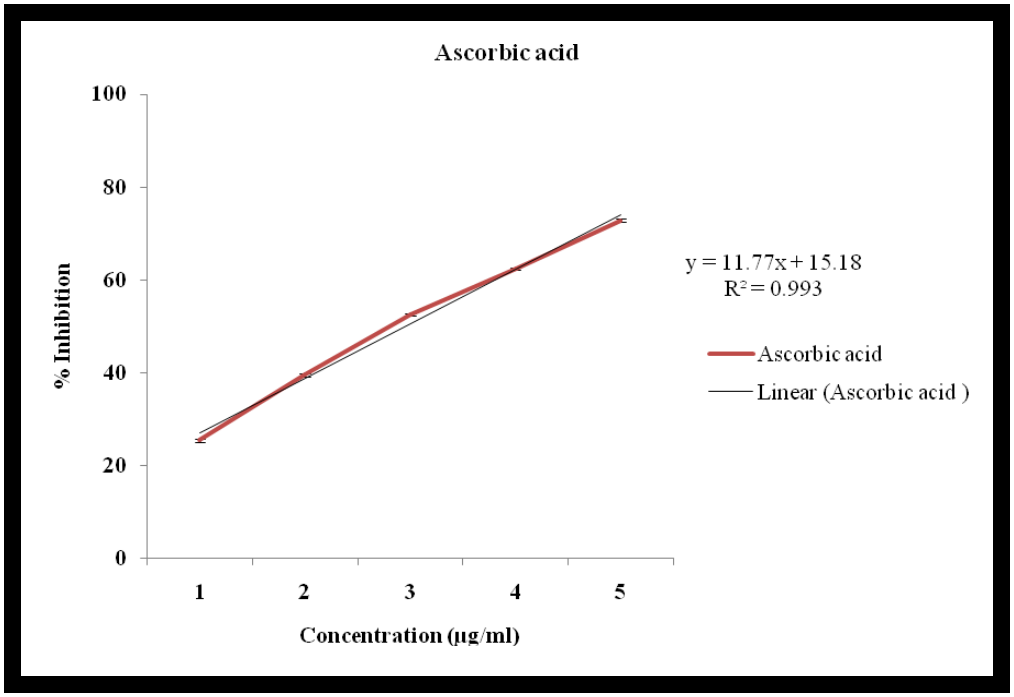

Figure 1: Standard curve between percentage inhibition (DPPH radical-scavenging activity) and ascorbic acid concentration.

Table 3: DPPH radical-scavenging activity of ELEFD, WLEFD, EBEFD and WBEFD.

Samples	Treatment	Dose Concentration	IC50 Values
1	Ascorbic Acid	(1-10µg/ml)	33.50 ± 0.74 µg/ml
2	EBEFD	(1-200µg/ml	115.19 ± 7.92µg/ml
3	EBEFD	(1-400µg/ml)	94.42 ± 11.30µg/ml
4	EBEFD	(1-600µg/ml)	89.46 ± 8.25µg/ml
5	ELEFD	(1-200µg/ml	97.14 ± 11.74µg/ml
6	ELEFD	(1-400µg/ml)	81.22 ± 7.29µg/ml
7	ELEFD	(1-600µg/ml)	63.99 ± 10.25µg/ml
8	WBEFD	(1-200µg/ml)	127.39 ± 4.56µg/ml
9	WBEFD	(1-400µg/ml)	113.24 ± 10.13µg/ml
10	WBEFD	(1-600µg/ml)	96.64 ± 11.13µg/ml
11	WLEFD	(1-200µg/ml	122.41 ± 7.47µg/ml
12	WLEFD	(1-400µg/ml)	110.23 ± 5.32µg/ml
13	WLEFD	(1-600µg/ml)	102.56 ± 12.52µg/ml

DPPH radical-scavenging activity of different concentration extracts and ascorbic acid. Data are represented as mean ± SD of three replicates

3.3.2. Determination of reducing power activity of ELEFD, WLEFD, EBEFD and WBEFD

The ability of reducing power was maximum in leaf extracts when compared to other extracts (table 4). The reducing power was moderate in bark exracts and the values were comparable

to that of ascorbic acid standard. This may be due to the biologically active compounds in the extract which possess potent donating abilities.

Table 4: Percentage of Scavenging Inhibition of ELEFD, WLEFD, EBEFD and WBEFD in reducing power activity.

S. No	Concen-tration (µg/mL)	Plants Extracts				Standard
		EBEFD	ELEFD	WELFD	WBEFD	Ascorbic Acid
01	20	10.14±1.16	18.45±0.51	26.92±0.42	15.29±0.97	20.94±1.92
02	40	23.14±0.85	26.64±0.55	32.98±0.24	24.06±1.00	39.18±1.26
03	80	39.05±0.75	48.28±1.09	49.06±0.89	39.11±0.67	44.04±1.08
04	160	48.71±1.27	61.00±0.40	61.49±0.60	52.84±1.90	70.56±1.45
05	320	61.58±1.72	78.17±0.96	78.80±1.06	64.81±0.56	96.93±1.21

Reducing power activity of different concentration extracts and ascorbic acid. Data are represented as mean ± SD of three replicates

3. 4. ASSESSMENT OF STZ induced Diabetes

3.4.1. Effect of ELEFD on body weight

The effects of STZ administration and ELEFD on body weight are summarized in table 5. No difference in the initial body weight was observed in any experimental group. Two-way ANOVA revealed that STZ subjected rats gained less body weight than normal rats. After a period of 28 days of STZ pronounced decrease in body weight was found as compared to normal rats. Higher dose of ELEFD and standard drugs significantly prevent the decreased in body weight at 14 and 28 days. Lower treatment did not produce pronounced difference in body weight as compared to diabetic control rats.

Table 5: Effect of oral administration of ELEFD on body weight (g) in normal and STZ-induced diabetic Rats.

Groups	Treatment	Mean body weight in gram			
		0^{th} day	7^{th} day	14^{th} day	28^{th} day
Group I normal control Rats)	Vehicle	227.37 ± 5.32	242.48±5.58	256.73±4.35	270.55±4.44
Group ll (Plant extracts per se)	500 mg/kg	228.62 ± 5.25	245.28±5.26	261.70±6.11	273.51±6.41
Group III (Diabetic control Rats)	STZ only	230.14 ± 5.94	214.71±5.17	180.46±5.55**	154.83±4.68**
Group lV (Plant extract	STZ+ plant extract (100 mg/kg)	229.70 ± 4.07	220.68±3.61	198.91±5.38	178.73±4.37
Group V (Plant extract	STZ + plant extract (200 mg/kg)	228.35 ± 0.37	226.98±4.23	210.91±4.38a	232.73±5.37a
Group VI (Plant extract	STZ+ plant extract (500 mg/kg)	230.50 ± 5.17	227.28±4.13	230.39±3.68a	242.13±3.37a

Effect of extract on body weight. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; *P<0.01 as compared to Vehicle control Group; aP<0.05 as compared to Diabetic control group.

3.5. Biochemical estimation

3.1.1. Blood Glucose level

The administration of extracts to normal rats did not produce any significant *per se* effects on various parameters assessed in the present study.

Fasting blood glucose levels of all animals before treatment were within the normal levels. Fasting blood glucose level was significantly elevated ($p < 0.05$) after 3 days of STZ treatment with respect to control level. The results showed that Rats in group I (normal control) showed blood glucose levels of 92.30 ± 5.21,94.80±1.55, 91.39±2.1, 90.41±6.21, 93.12±8.15 at 0, 7, 14, 21 and 28 day of experiment. In this normal control group, no significant change in blood glucose levels is observed at 7, 14, 21 and 28 day of experiment (table 6).

Rats in group II, showed blood glucose levels of 89.19 ± 3.71, 94.80±5.69, 90.65±3.76, 95.43±6.3, 91.22±3.56 at 0, 7, 14, 21 and 28 day of experiment respectively. In this group, oral administration of ELEFD (500 mg/kgbwt) was given to normal rats upto 28th day. No significant changes in blood glucose levels were observed at 28 day of experiment when compared to group I.

In group III, (STZ control) treatment with single dose of STZ at a dose of 180 mg/kgbwt after 3 days caused significant increase ($p < 0.05$) in blood glucose levels of rats. Rats showed blood glucose levels of 94.58 ± 7.70, 349.80±7.81, 330.45±8.16, 336.12±14.3, 312.21± 6.32, at 0, 7, 14, 21 and 28 day of experiment. This group showed significant increase in blood glucose levels at 7, 14, 21, and 28 day of experiment compared to group I. Nearly 4 time increase in blood glucose level was observed in STZ control animals.

Rats in group IV, showed blood glucose levels of 392.32±5.55, 345.32±12.43, 321.40±5.67, 290.45±11.56, 270.45±5.32 at 0, 7, 14, 21 and 28 day of experiment respectively. In this group, oral administration of ELEFD (100 mg/kgbwt) was given. Significant reduction in blood glucose levels was observed at 28 day of experiment when compared to group III.

Further, a significant decrease ($p<0.05$) in mean blood glucose level was observed in the hyperglycemic diabetic rats of the group V treated with ELEFD (200mg/kgbwt). Rats showed blood glucose levels of 93.55 ± 3.78, 320.15±8.98, 287.23±10.87, 262.18±13.67, 220.42± 15.98 at 0, 7, 14, 21 and 28 day of experiment respectively. In this group, significant reduction in blood glucose levels was observed at 14, 21, and 28 day of experiment when compared to group III.

Rats in group VI showed blood glucose levels of 91.80±7.94, 305.18±18.65, 213.98±10.34, 193.54±20.32, 153.31± 20.12, at 0, 7, 14, 21, and 28 day of experiment respectively. In this group, significant reduction in blood levels was observed at 14, 21, and 28 day of experiment when compared to group III.

Rats in group VII showed blood glucose levels of 88.72±6.14, 210.18±6.97, 177.43±4.56, 113.35±4.35, 99.63±7.43, at 0, 7, 14, 21, and 28 day of experiment respectively. Treatment with Glipizide (4 mg/kg body weight) significant decreased the glucose level at 7, 14, 21 and 28 days when compared with diabetic control rats.

Table 6: Effect of oral administration of ELEFD blood glucose in normal and STZ-induced diabetic Rats.

Groups	Treatment	Blood glucose level (mg/dl)				
		Day 0	Day 7	Day 14	Day 21	Day 28
(Group-I) Normal	Vehicle	92.30 ± 5.21	94.80±1.55	91.39±2.1	90.41±6.21	93.12±8.15
(Group-II) Plant per se	500 mg/kg	89.19 ± 3.71	94.80±5.69	90.65±3.76	95.43±6.32	91.22±3.56
(Group-III) Diabetic control	STZ only	94.58 ± 7.70	349.80±7.81**	330.45±8.16**	336.12±14.3**	312.21± 6.32**
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	392.32 ± 5.55	345.32±12.43	321.40±5.67	290.45±11.56	270.45± 5.32*
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	93.55 ± 3.78	320.15±8.98	287.23±10.87	262.18±13.67a	220.42± 15.98a
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	91.80 ± 7.94	305.18±18.65	213.98±10.34a	193.54±20.32a	153.31± 20.12a
(Group-VII) Standard Treatment	Glipizide (4 mg/kg)	88.72±6.14*	210.18±6.97*	177.43±4.56a	113.35±4.35a	99.63±7.43a

Effect of extract on serum glucose. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; *P<0.01 as compared to Vehicle control Group; aP<0.05 as compared to Diabetic control group.

3.1.2. Effect of ELEFD on serum lipid profile

3.1.2.1. Effect of ELEFD on Serum total cholesterol

The results showed that the total cholesterol levels in normal rats were 95.19 mg/dl (table 6). In STZ-induced diabetic rats, the serum TC levels increased to 167.39 mg/dl. Diabetic rats treated with standard drug glipizide decreased TC levels up to 113.68 mg/dl. Plant extracts of FD medium and high dose decreased the TC levels up to 131.67 mg/dl and 109.55 mg/dl in dose dependent manner. However, lower dose did not significantly decrease the TC in diabetic rats. Among the plant extract doses, high dose worked effectively and decreased the TC levels in diabetic rats.

3.1.2.2. Effect of ELEFD on Serum TG

The results (table 6) showed that the TG levels in normal mean of rats were 110.25 mg/dl. STZ-induced diabetic rats have increased level of triglycerides, 178.13 mg/dl. Diabetic rats treated with standard drug glipizide decreased the TG levels up to 123.18 mg/dl. Diabetic rats treated with Plant extracts of FD medium and high dose decreased the triglycerides levels up to, 151.46 mg/dl and 128.13 mg/dl in dose dependent manner. However, lower dose did not significantly decrease the TG in diabetic rats. Among the plant extract doses high dose worked effectively and decreased the TG levels in diabetic rats.

3.1.2.3. Effect of ELEFD on Serum HDL

The results showed that the HDL levels in normal mean of rats were 46.72 mg/dl. STZ-induced diabetic rats have decreased level of HDL, 28.22 mg/dl. Diabetic rats treated with standard drug glipizide increased the HDL levels up to 44.24 mg/dl. Diabetic rats treated with ELEFD medium and high dose increased the HDL levels up to, 36.75 mg/dl and 41.55 mg/dl in dose dependent manner. However, lower dose did not significantly increase the HDL in diabetic rats. Among the plant extract doses high dose worked effectively and increased the HDL levels in diabetic rats (table 6).

3.1.2.4. Effect of ELEFD on Serum LDL

The results showed that the LDL levels in normal mean of rats were 26.42 mg/dl. STZ-induced diabetic rats have increased level of LDL, 103.54 mg/dl. Diabetic rats treated with standard drug glipizide decreased the LDL levels up to 44.80 mg/dl. Diabetic rats treated with ELEFD medium and high dose decreased the LDL levels up to, 66.43 mg/dl and 42.37 mg/dl in dose dependent manner. However, lower dose did not significantly decrease the LDL in

diabetic rats. Among the plant extract doses high dose worked effectively and decreased the LDL levels in diabetic rats (table 6).

3.1.2.5 Effect of ELEFD on Serum VLDL

The results showed that the VLDL levels in normal mean of rats were 22.05 mg/dl. STZ-induced diabetic rats have increased level of VLDL, 35.62 mg/dl. Diabetic rats treated with standard drug glipizide decreased the VLDL levels up to 24.63 mg/dl. Diabetic rats treated with ELEFD medium and high dose decreased the VLDL levels up to, 30.29 mg/dl and 25.62 mg/dl in dose dependent manner. However, lower dose did not significantly decrease the VLDL in diabetic rats. Among the plant extract doses high dose worked effectively and decreased the VLDL levels in diabetic rats (table 6).

Table 6: Effect of ELEFD *on* blood lipid profile in normal and STZ-induced diabetic Rats.

Groups	Treatment	Total Cholesterol (mg/ dl)	Triglycerides (mg/dl)	HDL Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	VLDL (mg/dl)
(Group-I) Normal	Vehicle	95.19±4.20	110.25±4.72	46.72±4.32	26.42±2.46	22.05±1.36
(Group-II) Plant per se	500 mg/kg	91.14±9.48	107.24±6.55	44.29±6.11	25.4±5.44	21.45±4.60
(Group-III) Diabetic control	STZ only	167.39±6.55***	178.13±3.35***	28.22±5.55***	103.54±5.44***	35.62±1.55***
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	151.45±9.50	165.88±7.72	31.46±4.80a	86.81±6.15	33.17±2.31
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	131.67±8.88a	151.46±6.67aa	36.75±3.67a	66.43±6.12aa	30.29±1.48aa
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	109.55±6.67aaa	128.13±9.95aaa	41.55±4.62aa	42.37±4.56aaa	25.62±1.39aa
(GROUP-VII) Standard treatment	Glipizide (4 mg/kg)	113.68±4.22aa	123.18±5.97aaa	44.24±5.56aaa	44.80±4.35aaa	24.63±2.43aaa

Effect of extract on serum lipid. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; *P<0.01 as compared to Vehicle control Group; aP<0.05 as compared to Diabetic control group

3.1.3. Effect of ELEFD on serum oxidative/antioxidant parameters

3.1.3.1. Effect of ELEFD on serum TBARS

Table 7 shows the effect of plant extract on the serum TBARS level in serum of diabetic rats. Diabetic rats had significantly increased level of serum TBARS, an index of lipid peroxidation as compared to normal control rats (table 7). Chronic administration of higher doses of plant extracts significantly reduced elevated levels of TBARS in serum of diabetic rats in comparison to the levels observed in vehicle treated diabetic control rats. However, lower dose did not significantly decrease the TBARS level in diabetic rats. Diabetic rats treated with standard drug glipizide also significantly decreased the TBARS levels. Among the plant extract doses high dose worked effectively and decreased the TBARS levels in diabetic rats.

3.1.3.2. Effect of ELEFD on serum Glutathione level

Table 7 shows the effect of plant extract on the serum GSH level in serum of diabetic rats. STZ produced marked reduction in GSH activity in serum of diabetic rats as compared to normal control rats (table 7). Chronic administration of higher doses of plant extracts significantly restored decline levels of GSH in serum of diabetic rats in comparison to the levels observed in vehicle treated diabetic control rats. However, lower dose did not significantly increase the GSH level in diabetic rats. Diabetic rats treated with standard drug glipizide also significantly increased the GSH levels. Among the plant extract doses high dose worked effectively and increased the GSH levels in diabetic rats.

3.1.3.3. Effect of ELEFD on serum SOD level

Table 7 shows the effect of plant extract on the serum SOD level in serum of diabetic rats. STZ produced marked reduction in SOD activity in serum of diabetic rats as compared to normal control rats (table 7). Chronic administration of higher doses of plant extracts significantly restored decline levels of SOD in serum of diabetic rats in comparison to the levels observed in vehicle treated diabetic control rats. However, lower dose did not significantly increase the SOD level in diabetic rats. Diabetic rats treated with standard drug glipizide also significantly increased the SOD levels. Among the plant extract doses high dose worked effectively and increased the SOD levels in diabetic rats.

3.1.3.4. Effect of ELEFD on serum CAT level

Table shows the effect of plant extract on the serum CAT level in serum of diabetic rats. STZ produced marked reduction in CAT activity in serum of diabetic rats as compared to normal

control rats (table 7). Chronic administration of higher doses of plant extracts significantly restored decline levels of CAT in serum of diabetic rats in comparison to the levels observed in vehicle treated diabetic control rats. However, lower dose did not significantly increase the CAT level in diabetic rats. Diabetic rats treated with standard drug glipizide also significantly increased the CAT levels. Among the plant extract doses high dose worked effectively and increased the CAT levels in diabetic rats.

Table 7: Effect of ELEFD on oxidant/anti-oxidant profile in normal and STZ-induced diabetic Rats.

Groups	Treatment	MDA (nM/mg)	Glutathione (µg/mg)	Catalase (µM /min /mg)	SOD (Unit/min//mg)
(Group-I) Normal	Vehicle	10.50±0.80	25.77±3.10	19.13±1.80	14. 47±1.87
(Group-II) Plant per se	500 mg/kg	9.78±1.20	25.01±2.32	20.87±2.12	13. 99±2.20
(Group-III) Diabetic control	STZ only	21.30±1.86***	11.67±2.26***	11.70±0.75***	5.70±1.50***
(Group-IV) Plant extract 100mg/kg	STZ+ extract (100 mg/kg)	20.53±0.80	14.30±1.68	13.70±2.95	7.60±1.65
(Group-V) Plant extract 200mg/kg	STZ+ extract (200 mg/kg)	16.83±2.35aa	17.99±1.96aa	15.11±3.34aa	10.65±1.23aa
(Group-VI) Plant extract 500 mg/kg	STZ+ extract (500 mg/kg)	13.50±2.15aaa	20.20±1.70aaa	17.73±2.16aaa	12.69±1.92aaa
(GROUP-VII) Standard treatment	Glipizide (4 mg/kg)	11.11±1.67aaa	23.12±2.37aaa	18.99±2.55aaa	13.92±2.09aaa

Effect of extract on oxidant/anti-oxidant profile. Data are mean ± SEM; Data were analyzed using one-way ANOVA followed by Tukey's multiple test; *P<0.01 as compared to Vehicle control Group; aP<0.05 as compared to Diabetic control group.

4. DISCUSSION

Medicinal plants have a long-standing history in many indigenous communities, and continue to provide useful tools for treating various diseases. Researchers today examine folk and traditional uses of plants to find new drugs for cancer, AIDS, and many incurable diseases. The practices of traditional medicine are based on hundreds of years of belief and observations, which predate the development and spread of modern medicine.[27] This interest primarily stems from the believe that herbal medicines are safe, inexpensive and have no adverse effects.

4.1. Phytochemical Studies

Recently a number of studies have been reported on the phytochemicals of medicinal plants.[28-30] Preliminary phytochemical analysis was performed with different chemical

reagents to detect the nature of the phytoconstituents and their presence in the extracts of the leaves and bark of FD plant. The results of the phytochemical screening revealed that, all the bark and leaf extract of the FD plant showed the presence of flavonoids, Saponins, steroids, alkaloids, tannins, phenolic compounds, triterpenoids and carbohydrates whereas, glycosides, anthraquinones, and reducing sugars were absent. Moreover, ELEFD, WELFD, EBEFD and WEBFD found more rich in these phytoconstituents as compare to hexane and chloroform extracts.

4.2. Quantitative estimation of phytoconstituents

Moreover, quantitative estimation of total tannins, total phenolic and total flavonoids compounds were also performed in the leaf and bark of plant. The ELEFD, show the presence of higher amount of total phenol, total flavonoid and tannins components as compared to other extracts. This report eventually justifies the preliminary phytochemical studies and also helps in finding the suitable extracts for the pharmacological study.

4.3. *In-vitro* Anti oxidant activity

There has been increasing interest in natural antioxidants found in medicinal plants because of the carcinogenic effects of synthetic antioxidants. Oxidative stress causes collapse of the mitochondrial membrane potential, which is associated with many age-related diseases.[31] The mechanism of antioxidants includes the suppression of ROS formation, the hindrance of enzymes or chelating of elements engaged in free-radical production. Moreover, antioxidants scavenge reactive species, and unregulated antioxidant defences. Free radicals are persistently generated. The parts of medicinal plants which are generally rich in phenolic compounds, are flavonoids, phenolic acids, stilbenes, coumarins, tannins, lignans and lignins. These compounds have numerous biological effects as well as antioxidant activity.

DPPH scavenging activity has been widely used to assess the *in-vitro* antioxidant activity of crude plant extracts. The free-radical scavenging activity of DPPH radicals may be due to its hydrogen-donation ability. The antioxidant reacts with DPPH radical (purple colour) and converts it into a colourless 1-1-diphenyl-2-picryl hydrazine. The ELEFD, WBEFD, showed dose dependent increased in scavenging activity on free radicals in our present study. The results indicate that all the extracts have potent antioxidant activity.

Moreover, in reducing power assay, free radical is neutralized with antioxidant substance and conversion of $Fe^{3+}(CN-)_6$ to $Fe^{2+}(CN-)_6$ take place. In the present study, higher absorption at higher concentration indicates the strong reducing power potential of the extracts. It is suggested that the extracts have high redox potential and can act as reducing agents. This effect indicates that the extracts may consist of polyphenolic compounds that regularly illustrate immense reducing power. This has been justified by methanol extract being the most reducing agent with highest phenolic content.

The data obtained in this study is supported by previous studies, those reported the anti-oxidant activity of leave and bark extract of FD, in reducing power activity assays. Previous studies reported that flavonoid, alkaloids and other constituents present in plant extract prevent oxidant injury by several mechanisms, such as scavenging oxygen radicals.[32,33] Several reports have conclusively shown close relationship between total phenolic content and antioxidative activity of the plant extracts.[34,35] Thus, the antioxidant property of extracts may be due to present of the flavonoid, alkaloid and phenolic content.

Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activities and their effects on human nutrition and health are considerable.[35] Hence, the leaf and bark extracts of FD, could be a good source of antioxidant.

4.4. Antidiabetic activity

STZ is a N.-nitro derivative of glucosamine which has effective alkylating activity along with specifically cytotoxic to pancreatic β-cells in mammals. STZ [2-deoxy-2-(3-(methyl-3-nitrosoureido)-d-glucopyranose)] synthesized by *Streptomyces achromogenes*, impaired the glucose oxidation thereby inhibiting insulin synthesis and its secretion as a consequence of β-cells damage in pancreas.[36] The pancreatic β-cell preferentially uptake STZ resulting in formation of superoxide radicals and nitric oxide lead to β-cells death.[37,38]

The results, based on biochemical parameters, were compared with normal control, diabetic control and positive control rats treated with glipizide. The result of the present study showed significant changes in biochemical parameter of the experimentally induced diabetes. In support to earlier reports, increased fasting blood glucose (FBG) level was seen in STZ induced diabetes rats compared to control group.[39,40] FBG was significantly attenuated by treatment with plant extract ELEFD, which is consistent with our previous

report. Plant extracts normalizes the glucose level similar to standard drug and exhibited as a potent antidiabetic effect. The decrease in the level of FBG might be attributed to the insulin secretion from residual pancreatic cells or regeneration β-cell. Moreover, increased in insulin secretion from remnant β-cells and increase in the peripheral utilization of glucose may also contribute to the anti-hyperglycemic action of plants. So, this study divulged the association between glucose level, and insulin levels.

Along with hyperglycemia, induction of diabetes with STZ is associated with characteristic weight loss. Oral administration of ELEFD, improved body weight in diabetic rats. The decreased body weight was due to protein metabolism and muscle wasting. After treatment with plant extracts, diabetic animals showed improvement in body weight. This increased body weight might be linked to insulin secretion which improves glucose level in diabetic animals.

The correlation in hyperglycemia and dyslipidemia is well known.[41] Increase level of lipids leads to atherosclerosis which may cause the diabetes and complications of diabetic (290). Abnormalities in lipid profile (increased TC, TG, LDL and VLDL) during DM play a crucial role in the progression of diabetic complications.[42,43] This leads to mainly complication at level of microvascular and also manifested into cardiovascular disorders.

In our study, significant increase in TC, TG, VLDL and LDL level was observed along with a significant reduction in HDL level in diabetic rats. Administration of plant extracts effectively increased serum HDL and reduced the level of TC, TG, LDL and VLDL cholesterol. Interestingly, plant extracts at higher dose shown quite similar result as like to standard treated group in the reduction of TC, TG, LDL and VLDL indicating equivalent hypolipidemic activity similar to available standard drugs. Moreover, in case of HDL, the highest dose of both the extracts showed more pronounced effects as compared to standard drug.[44,45]

Moreover, etiology of diabetes involves various factors like increased oxygen free radical, alteration in antioxidant enzymes, nonenzymatic protein glycosylation, impaired glutathione metabolism and lipid peroxidation.[46] STZ-induced hyperglycemia elevates ROS generation and depresses antioxidant defense resulting in cellular disruption and increased lipid peroxidation.[47] Thus, oxidative stress can be diminished *via* diminution of free radical generation. Antioxidant enzyme such as SOD, CAT and GSH, provides defense against these

free radicals leading to reduced oxidative stress. Moreover, in oxidative stress malondialdehyde (MDA) is considered as a primary biomarker of lipid damage. TBARS (markers of lipid peroxidation) are elevated in oxidative stress conditions thereby causing destruction of lipid membrane.

In our study various antioxidant enzyme level like SOD, CAT, and GSH were significantly decreased in diabetic rats, whereas the level of TBARS increased significantly It is previously reported that the activity of antioxidant enzymes is reduced in serum and tissue homogenate of diabetic rats.[48] In this study, treatment with ethanolic leaf extract of plant reduced TBARS level and increased SOD, CAT as well as GSH levels, due to its potential antioxidant activity. Reduction in the level of the TBARS after treatment shows the effective antioxidant activity of ELEFD.

Flavonoids and other phenolic compounds are known to reduce oxidative stress, reduce necrosis and regenerate β-cells. Saponins present in the extract may be assigned and contributes for the protective effect on lipid peroxidation and moreover enhances the effect on antioxidant defense in STZ induced diabetes. Thus, the presence of these secondary metabolites may prove beneficial for counteracting the diabetes. Triterpenoids are known to stimulate insulin release from pancreas and reduces oxidative stress whereas phenolic compounds are potent radical scavengers which increase antioxidant enzyme level and reduced lipid peroxidation.

5. CONCLUSION

In the light of the above consideration, it can be concluded that with the support of the phytochemical studies and the in- vitro and in-vivo pharmacological studies were done and ELEFD showed promising antioxidant and anti-diabetic activities in a dose dependent manner. Further studies have to be focused on SAR of phytoconstituents isolated from the ethanolic leaves extracts. This scientific study revealed the efficacy of the drug and it would be definitely have an extensive reach in future.

6. AKNOWLEDGEMENT

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