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ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF AN ETHANOL EXTRACT OBTAINED FROM THE LEAVES OF *FEIJOA SELLOWIANA*

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ABSTRACT

Diabetes mellitus has become a major health concern worldwide and development and progression of diabetic related complications are accelerated by reactive oxygen species which are generated and accumulated due to hyperglycemia. herbal remedies are convenient for the management of diabetes due to their traditional acceptability and availability, low costs and lesser side effects. the present study was carried out to determine qualitative analysis of various phytochemical constituents, the in vitro antioxidant activity, and invitro and in vivo antidiabetic activity ethanol extract of leaves of feijoa sellowiana. qualitative analysis of phytochemical constituents was performed by the well-known tests protocol available in the literature and antioxidant activity was studied through DPPH assay, ABTS+ radical scavenging assay, nitric oxide scavenging method using ascorbic acid as standards. Antidiabetic activity was determined by in-vitro alpha-amylase inhibition assay and invitro antidiabetic activity was determined by diabetes was induced in rats by streptozotocin (100mg/kg; *i.p.*). The phytochemical screening revealed the presence of saponin, phenols, glycosides, flavonoids, terpenoids and alkaloids. The activities of ethanolic leaves extract against DPPH assay, ABTS+ radical scavenging assay, nitric oxide scavenging method were concentration dependent. In in-vitro antidiabetic test, highest α-amylase inhibition was found in ethanolic extract of *feijoa sellowiana.* Oral treatment of ethanolic extract of *feijoa sellowiana* using rat oral needle at 200 and 400mg/kg doses significantly ($P < 0.001$) decreased blood glucose levels. In conclusion, the above actions might be responsible for the antidiabetic activity of extract due to presence of gallic acid and other biomarkers.

KEYWORDS: Diabetes mellitus, Phytochemical constituents, Antidiabetic activity, Antioxidant activity.

INTRODUCTION

The traditional medicine all over the world is nowadays revealed by an extensive activity of researches on different plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer activities. Currently, about 30% of the active component was identified from plants that are used as prescribed medicines.[1] Reactive oxygen species (ROS) exert oxidative damaging effects by reacting with nearly every molecule found in living cells including protein, lipid, amino acids and DNA, if excess ROS are not eliminated by antioxidant system. They play important roles in aging and in the pathogenesis of age-related disorders such as cancer, hypertension, atherogenesis, alzheimers disease and parkinsons disease. The most practical way to fight degenerative diseases is to increase antioxidant activity in our body and that could be achieved by consumption of vegetables, fruits or edible plants.[2] There is an increasing interest in natural antioxidants e.g. polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases.[3] Different parts such as seeds, leaves and bark of stem and root known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids, tannins having the ability to inhibit the free radicals that are excessively produced, hence can act as antioxidants.[4] The continued search among plant secondary metabolites for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal remedies as potential sources of phenolic oxidants.[5] Several synthetic antioxidant agents including butylated hydroxyanisole and butylated hydroxytoluene (BHT) are commercially available, however, are reported to be toxic to animals including human beings which have stimulated the interest of many investigators to search

natural antioxidant.[6] Diabetes mellitus (DM) is one of the most significant chronic metabolic disorders characterized by hyperglycemia. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response.[7] The overall prevalence of diabetes mellitus in the global population is approximately 6%, of which 90% is type 2 diabetes. India had 32 million diabetics in 2000, and this number is expected to increase to 80 million by 2030.[8,9] Characteristic of diabetes is associated with disturbances in the metabolism of carbohydrates, lipids and proteins due to defects in insulin secretion, insulin action or both.[10] Diabetic complications are nephropathy, retinopathy, neuropathy, atherosclerosis and fatty liver. In all these cases continual hyperglycemia plays a significant role in the induction of oxidative stress by increasing glucose autooxidation, nonenzymatic protein glycation and activation of polyol pathway.[11] Also hyperglycemia induced stress sensitive signaling pathways including nuclear factor (NF)-kB. Activation of NF-kB increased cytokine concentrations such as tumor necrosis factor-a (TNF-α). The renal cells are capable to synthesis TNF- α moreover the sensitive to changes of serum's TNF- α level. This process suggests a causal role for hyperglycemia in the immune activation of diabetes.[12] Since ancient times, consumption of medicinal herbs has considered in treatment of several diseases.[13] In recent years this kind of treatment has received growing attention because it is natural and has a few side effects.[14] Many medicinal plants extract such as Bougainvillea spectabilis, Moringa oleifera, Curcuma longa, Cynodon dactylon and Trichosanthes dioica were used for treatment of diabetes mellitus due to having hypoglycaemic effects.[15-19] *Feijoa sellowiana* (Myrtaceae) is native to southern of South America. Owing to its easy adaptability in subtropical regions; nowadays, it is extensively cultivated in many countries and also in Iran where its fruit are very popular. Although the chemical composition of Feijoa has been clearly reported[20], pharmaceutical studies of its constituents have barely been carried out. Its fruits are rich in vitamin C, polyphenols, terpenes, tannins and flavonoids. In the literature various biological activities have been described. Feijoa showed potent antimicrobial and antifungal activity and a sensible activity against Helicobacter pylori.[21] Moreover, its antioxidant activities have been reported.[20,21] It has good nephroprotective activity.[22] To the best of the author's knowledge, antidepressant activity of Feijoa has not been reported to date and nothing was found about mechanism or antidepressant activity of this plant. Therefore, the aim of this study was phytochemical screening and evaluation of antioxidant activity and antidiabetic activity of ethanolic leave extract of *Feijoa sellowiana.*

MATERIALS AND METHODS

Plant material

The leaves of *Feijoa sellowiana* were collected from the forests of Pachmari MP, and which was authenticated in the month of February. The plant material was identified and authenticated by Dr. Saba Naaz, Botanist, Safia Science College, Bhopal MP.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade. Streptozotocin from Loba Chemie. Standard Glibenclamide (Daonil) from Aventis Pharma. Ethanol (Analytical grade) and 5% Dextrose solution Glucose Estimation Kit from Gluco Dr Super sensor.

Extraction procedure

The leaves were shade dried at room temperature for 10 days. Then these were milled into powder by mechanical grinder. This powder was sequentially extracted to their increasing polarity with Petroleum ether, Ethyl acetate, Ethanol respectively. About 500gm of powdered leaves was uniformly packed into a thimble in a soxhlet apparatus and extracted with 1000ml petroleum ether, ethyl acetate and ethanol, respectively. Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 1-2 hours for each solvent. The excess solvent was evaporated and the dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis and pharmacological screenings.[23]

Qualitative phytochemical analysis of plant extract

The *Feijoa sellowiana* leaves extract obtained was subjected to the preliminary phytochemical analysis following standard methods.[23] The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

IN VITRO ANTI-DIABETIC ACTIVITY

α-amylase Inhibitory (AAI) Assay

A total of 500 µl of test samples and standard drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitro salicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were

conducted in similar way by replacing extract with vehicle.[25]

ANTIOXIDANT ACTIVITY

DPPH method

DPPH scavenging activity was measured by modified method. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (100-500 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

ABTS radical cation decolorization assay

ABTS+ was dissolved in water at a concentration of 7mM. The stock solution was mixed with .45mM potassium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours prior to use in incomplete oxidation of ABTS+. The radical remained stable in this form for more than two days (in the dark at room temperature). The reagents were added in the following order: The incubation mixture in a total volume of 5 ml contained 0.54 ml of ABTS+, 0.5 ml of phosphate buffer and different concentrations of (all extracts of *Feijoa sellowiana* leaves) (100-500µg/ml). The blank sample contained water instead of sample or the standard. Absorbance was measured spectra photometrically at 734 nm and compared with the standard (Ascorbic acid).[26]

Nitric oxide (NO^o) radical scavenging assay

The determination of NO^o radical scavenging ability of the extracts is based on the inhibition of NO^o radical generated from sodium nitroprusside in phosphate buffer saline solution by Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Scavengers of nitric oxide act against oxygen, prompting to lessened production of nitrite ions which can be monitored at 546 nm.[27] Briefly, sodium nitroprusside (0.6 ml, 5 mM) solution was mixed with and without varying the concentration of the extracts or Ascorbic acid (2 ml, 100-500µg/ml) and incubated at 25 ± 2°C for 5 h. Incubated solution (2 ml) was mixed with equal volume of Griess reagent and absorbance of the purple-colored azo dye chromophore was measured at λmax 546 nm using UV-Vis spectrophotometer. The NO^o radical scavenging ability was calculated using following formula:

$$\text{Scavenging activity } (\%) = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} x10$$

Acute toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen. In most case these are desired and useful many effects are not advantageous. Acute, subacute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD50(It is the lethal dose (causing death) to 50% of tested group animals)

(median lethal oral dose)

LD 50 (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of animal(mg/kg). In this study acute toxicity study was carried out in Wistar albino rats. The procedure was followed by using OECD 423(Acute toxic class method). The rats are fasted overnight prior to dosing. The three dose levels are administered by the help of oral feeding needle over the prior of 24 hours. After the drugs has been administered, food may be withheld for a further 3-4 hours in rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5,50,300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg). Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected In vitro antidiabetic activity of *Feijoa sellowiana* leaves extract in streptozotocin induced diabetic Wistar albino rats. Wistar albino rats (150- 200 grams) of both sexes were procured from Suras lab, Hyderabad, India. Prior to the experiment the rats were housed in a clean polypropylene cage (6 rats/ cages) for a period of 7 days under standard temperature (25-30^0 c), relative humidity (45 – 55%), dark / light cycle (12

/12 hrs). The studies were performed with the approval of Organizational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of wate.

Hypoglycemic Test

Groupings were done as follows: Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat),

Group II served as Positive control – Glibenclamide (2mg /kg),

Group III served as ethanolic extract of *Feijoa sellowiana* – (200mg/kg),

Group IV served as ethanolic extract *Feijoa sellowiana* – (400mg/kg). Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test

Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g rat),

Group II served as Positive control – Glibenclamide, (2 mg /kg),

Group III served as ethanolic extract of *Feijoa sellowiana* (200mg/kg),

Group IV served as ethanolic extract of *Feijoa sellowiana* (400mg/kg).

All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Induction of diabetes to animals

The single dose (100 mg/kg b.w., i.p.) of streptozotocin monohydrate dissolved in sodium citrate buffer was used for the induction of diabetes in rats after overnight fasting. After 1 hr of streptozotocin monohydrate administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycemics phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, and 14 days.

estimation of biochemical parameters Serum blood glucose

On 1, 7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0. All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

RESULTS AND DISCUSSION

The crude extracts so obtained after the soxhlation process; extract was further concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of methanolic extract was found to be 24.35%. Phytochemical analysis of ethanolic extract of *Feijoa sellowiana* levees showed the presence of saponin, phenols, glycosides, flavonoids, terpenoids and alkaloids Table 1. Antioxidant activity of the samples was calculated through DPPH assay, ABTS radical cation decolorization assay and nitric oxide radical scavenging. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 100µg/ml to 500µg/ml. A dose dependent activity with respect to concentration was observed Table 2-4. Percentage inhibition of ethanolic extract of *Feijoa sellowiana* leaves was found to be 63 µg/ml and percentage inhibition of acarbose (Positive control) was found to be 66 µg/ml. minimum percentage inhibition was found in ethanolic extract of *Feijoa sellowiana* leaves which resemblance to percentage inhibition of positive control, so ethanolic extract of *Feijoa sellowiana* contain active constituents of antidiabetic Table 5. The hypoglycemic test results have shown Table No: I, which indicated ethanolic extract of *Feijoa sellowiana* treated animals 200 & 400, significantly decreased in blood glucose ($P<0.05$)*, ($P<0.001$)**& ($P<0.0001$)*** when compared to control and positive control Table 6. The experimental results have indicated. The negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose

level for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased Glibenclamide 2mg/kg treated group (P<0.05)*, (P<0.001) **& (P<0.0001) *** when compared with control group at 7th and 14th days. The ethanolic leaves extract of *Feijoa sellowiana* treated groups 200 & 400 mg/kg were dose dependent manner decreased (P<0.001)**& (P<0.0001)*** when compared with control group but positive control have more anti diabetic activity at 7th day. The ethanolic leaves extract of *Feijoa sellowiana* at the dose level 400mg/kg have equipotent activity when compared with positive control at 7th day. The ethanolic leaves extract of *Feijoa sellowiana* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action (P<0.001)**& (P<0.0001) *** when compared to control and positive control. On day 14th, ethanolic leaves extract of *Feijoa sellowiana* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (P<0.001) **& (P<0.0001) *** when compared to control and positive control Table 7. The ethanolic leaves extract of *Feijoa sellowiana* at the dose level 400mg/kg have equipotent activity. when compared with positive control at 7th day. The ethanolic leaves extract of *Feijoa sellowiana* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action (P<0.001) **& (P<0.0001) *** when compared to control and positive control. On day 14th, ethanolic leaves extract of *Feijoa sellowiana* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level (P<0.001) **& (P<0.0001) *** when compared to control and positive control. The oral glucose tolerance test (OGTT) results have been expressed on Table 8. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased for, ethanolic extract of *Feijoa sellowiana* 200 & 400 mg/kg (P<0.001) **& (P<0.0001) ** when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels(200, 400 mg/kg (P<0.05)*, (P<0.001)**& (P<0.0001)*** were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the equipotent activity.

Table 1: Phytochemical analysis of ethanolic leaves extract of *Feijoa sellowiana.*

Class of compounds	Tests performed	Results
Carbohydrates	*Molisch's test Fehling's test*	- -
Phenols	*Phosphomolybdic acid test*	+++
Flavonoids	*Shinoda test Lead acetate test*	++ ++
Tannins	*Braemer's test*	–
Alkaloids	*Wagner's Mayer's Dragendorff's test*	+ + +
Glycosides	*Legal's test Brontranger's test*	+ +
Saponins	*Foam test*	+
Sterols	*Salkowski's test*	–
Amino acids	*Ninhydrin test*	–
Terpenoids	*Lieberman Burchardt test*	+

Table 2: *In vitro* antioxidant activity of 200 mg and 4000mg of ethanolic extract of *Feijoa sellowiana* using DPPH Assay.

Conc. (µg/ml)	100	200	300	400	500
Ascorbic acid	64.99±0.56	70.30±0.43	76.01±1.52	80.37±0.56	88.02±0.54
200 mg of EEFS	50.19±0.54	54.89±0.67	60.15±0.77	62.05±1.33	63.53±0.87
400 mg of EEFS	64.41±0.76	65.13±1.36	67.33±0.67	68.55±1.56	70.56±1.76

Conc.: Concentration; AC: Ascorbic Acid;

Table 3: In vitro antioxidant activity of 200 mg and 4000mg of ethanolic extract of *Feijoa sellowiana* using $ABTS^+$ scavenging method.

Conc. (µg/ml)	100	200	300	400	500
Ascorbic acid	80.33±1.76	87.33±1.54	89.04±1.91	94.20±1.87	97.27±0.22
200 mg of EEFS	35.26±0.57	45.08±0.91	57.05±1.28	63.28±3.29	75.25±0.56
400 mg of EEFS	37.20±1.05	47.50±1.70	57.26±1.15	64.86±1.03	77.58±1.53

Table 4: In vitro antioxidant activity of 200 mg and 4000mg of ethanolic extract of *Feijoa sellowiana* using Nitric Oxide scavenging method.

Conc. (µg/ml)	100	200	300	400	500
Ascorbic acid	44.78±0.47	55.13±0.98	68.89±0.33	80.44±2.70	91.67±0.24
200 mg of EEFS	20.41±1.93	34.74±1.10	37.12±0.67	45.68±0.43	56.03±0.46
400 mg of EEFS	31.03±0.56	40.27±0.68	48.59±0.91	56.95±0.12	65.29±0.62

Conc.: Concentration; AC: Ascorbic Acid;

Table 5: α-Amylase inhibition of acarbose and ethanolic extract of *Feijoa sellowiana.*

Concentration (µg/ml)	Percentage Inhibition (%) acarbose	Percentage Inhibition (%) ethanolic extract
0	0	0
25	26	31
50	46	36
75	54	52
100	57	56
125	66	63

Table 6 : hypoglycemic test.

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl)		
		0 min	0.5hr	1 hr
Control Carboxymethyl Cellulose	0.5 %	68.53±0.72	70.16±0.45	72.58±1.77
Positive Control Glibenclamide	2	69.51±0.65	55.93±0.17**	34.04±1.43***
Extract of *Feijoa sellowiana*	200	69.22±0.56	62.51±2.78*	61.28±0.90*
Extract of *Feijoa sellowiana*	400	69.17±0.75	55.15±0.63**	36.47±0.12***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals) ($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test.

Table 7: Results of the effects of ethanolic extract on blood Glucose levels.

S.No.	Treatment	Blood glucose level (mg/dl) day		
		Day 1	Day 7	Day 14
1	Normal control 10 ml/kg P.O	81.16±1.44	75.7±4.014	77.23± 0.98
2	Negative control 100 mg/kg I.P	266.19±0.46	270.1±2.9	275.32±0.51
3	Positive control (Glibenclamide 2mg/kg) P.O	256.49±0.86	135.63±3.8***	111.92±0.93***
4	EEFS 200 mg/kg P.O	259.75±0.57	251.53±2.65**	244.12±2.63**
5	EEFS 400 mg/kg P.O	262. 92±1.06	174.04±0.62***	162.56±0.58**

Table 8: Oral glucose tolerance test.

Treatment	Dose mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1 hr	1.5hr	2 hr	2.5hr	3hr
Control CMC	0.5 %	70.12± 0.56	141.91±0 .23	188.11±0.3 2	173.21±0.3 2	158.03±0.62	154.20±0.76	131.21±0.03
Positive Control Glibenclamide	2	68.90± 0.45	105.31±0 .64* *	111.54±0.4 3* **	94.01±0.43 ** *	82.41±0.71***	78.23±0.71** *	76.62±0.21***
EEFS	200	69.02± 0.23	128.42±6 .009	148.75±0.6 4*	138.32±0.6 0*	130.21±0.31*	117.21±0.14* *	109.3±1.12**
EEFS	400	68.53± 0.13	116.23±1 .32**	121.21±0.1 2* *	105.11±0.0 4* **a	94.11±0.41*** a	88.21±0.31** * a	85.30±1.92*** a

The glucose levels were analyzed by using glucometer and all values are expressed as Mean±SEM (n=6), Group 2 was compared with group 1, Groups —3,4 were compared with group 2; *p<0.05, **p<0.01,p<0.001*** evaluated by one way, ANOVA followed by Dunnet 't' test.

CONCLUSION

The *invitro* antidiabetic studies have been performed based on the α-amylase inhibition assay. Each extract was tested for α-amylase inhibition and the extract with minimum IC_{50} has been undergone phytochemical screening. The leaves extraction has been performed by sequential extraction method The leaves *of Feijoa sellowiana* using the solvent with increasing polarity order (petroleum ether, ethyl acetate and ethanol) and the active extract was tested by *invitro* antidiabetic screening method. The preliminary phytochemical tests were performed to identify the active phytochemicals present in the ethanolic extract of *Feijoa sellowiana* showed the presence of Phenols, Flavanoids, Alkaloids, Glycosides, Saponins and Terpenoids. Finally the *invivo* antidiabetic activity of ethanolic extract of *Feijoa sellowiana* leaf was tested by using STZ induced diabetic rat. Acute toxicity study was carried out in rats. The procedure was followed by OECD 423(Acute toxicity class method).$1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of the maximum safe dose (2000mg/kg) were selected for further study. The present study suggested that the isolation of active constituents from ethanolic extract of *Feijoa sellowiana* leaf and characterize the compounds by using preliminary phytochemical studies and LCMS instrument used to isolate the compounds like *Quercetin and Kaemferol* were confirmed by confirmatory chemical tests. Fasting blood sample were drawn from retino orbital puncture of rats at weekly intervals till the end of the study 1,7 and 14 days. On these days fasting blood glucose were collected and analyzed for glucose. At the end of the study (14^{th} day) the ethanolic extract of *Feijoa sellowiana* leaf (200mg/kg p.o and 400 mg/kg p.o) treated diabetic groups showed statistically significant decrease in blood glucose similar to the standard drug Glibenclamide (2mg/kg), which indicated block the alfa amyalase activity.

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