ORIGINAL ARTICLE

Anti-Diabetic and Anti-Oxidative Role of a Local Medicinal Plant *Justicia Adhatoda* L in Diabetes Mellitus

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ABSTRACT

Background: Diabetes mellitus is one of the common metabolic and endocrinal disorder. This disorder is associated with many complications which may lead to morbidity and death of patient. There are many available synthetic antidiabetic agents that are used worldwide. Most of these drugs are expensive, and have undesirable pathological effects. Thus it is essential to look for cheap, effective, natural and safe antidiabetic agents.
Aim: To evaluate antidiabetic, and antioxidant effects of a local plant *Justicia adhatoda* L commonly known as, Bhaiker. It is a shrub, up to 100cm tall, abundantly found in sub-tropical zone, including Pakistan.
Method: By using methanolic extracts of leaves and flowers of *J.adhatoda* in –vivo on mice, antidiabetic and reducing power assays were performed including blood glucose, glycosylated hemoglobin, Ferric chloride (FeCl3)„2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and2,2'-azino-bis3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay.
Results: The results showed significant reduction in blood glucose level of mice by leaves (2.32 and 2.0 mg/ ml), glycosylated hemoglobin (65 and 85 mg/ ml) in male and female mice respectively. $FeCl_3$ Reducing power, DPPH, Reducing power assay and ABTS assay have shown significant results. Maximum antioxidant values has been represented by leaves of same plant.
Conclusion: *J. adhatoda* possesses numerous bioactive compounds that include phenolic compounds, flavonoids and alkaloids and this plant has great potential to be developed as natural antidiabetic and antioxidant drugs.
Keywords*: Justicia adhatoda*, Blood glucose, Glycosylated Hemoglobin, DPPH scavenging assay,

INTRODUCTION

DM is predominantly metabolic disorder worldwide [1].Almost 387 million people are living with diabetes globally. Out of this about 77% diabetic mass belongs to low and middle income countries. Major complications which diabetes are stroke, heart diseases, kidney failure, blindness and lower limb amputation [2].These circumstances are also linked to the oxidative stress and hence demands the evaluation of antidiabetic and antioxidant potential of indigenous plant extracts [3].Complications of diabetes all highlight the urgent need of effective treatment of patients. Nowadays different kind of therapies are available worldwide like insulin, diet and pharmacotherapy. These all therapies exert antidiabetic effects through many mechanism .These mechanism may include stimulation of pancreatic beta cells or increase peripheral absorption of glucose. The drug treatment which are usually used have some undesirable effects including drug resistance and even toxicity.

Now a day's use of medicinal plant is recommended even by WHO to cure many diseases including diabetes mellitus[4]. Most of the medicinal plants have antidiabetic and anti-stress activity due to available phytochemical[5].

J. adhatoda commonly known as Malbar Nut or Bhiker have been used in medicine for more than 2000 years. It is commonly found in our country and well recognized in the native systems of medicine for its useful properties, predominantly in bronchitis and its all parts are used widely for handling cold, whooping-cough, cough, asthma and chronic bronchitis[6, 7].

Present work has been done by keeping in view the following objectives:

1. Assessment of *in vivo* antidiabetic activity of flowers and leaves of *J. adhatoda*
2. Assessment of antioxidant activity of flowers and leaves of *J. adhatoda* by using different methods.

The probable mechanism through which plant extracts shows anti-hyperglycemia activity maybe from beta cell of pancreas or by increased movement of blood glucose to peripheral tissue potential, pancreatic secretion of insulin and was prominent by high amount of insulin in diabetic rats by treating with plants extracts, while antioxidant activity is may be due to available phytochemicals of this our native plant[8,9].

Ethics approval & sample collection: The study was conducted at Applied Microbiology and Biotechnology Lab of the DBB, IIUI while antidiabetic activity was conducted at NVL. All work has been done under the ethical committee of *International Islamic University, Islamabad, Pakistan* and National Veterinary Laboratories, Chak Shahzad, Islamabad. Intact plants of *J. adhatoda* (label) were uprooted during rainy season of July 2015 without damaging the roots and collected aseptically in fine plastic bags from Margalla Hills, Islamabad, Pakistan (33.73.46N, 73.05.45 E). Plant was identified by an expert (Dr. Rehmatullah Qureshi, Associate Professor) at the Department of Botany, PMAS AAUR. Fresh leaves and flowers of the plant were used to examine with antidiabetic and anti-oxidative activity. The specimen has been

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submitted with voucher no. 227 to the Herbarium of PMAS AAUR for further use.

MATERIAL AND METHOD

Fresh leaves and flowers (free from disease) of healthy *J. adhatoda* plant were washed and dried in shade then drying in hot air oven at 40°C. Flowers and leaves were detached, milled into residue by using a chopper and filtered by 80 mesh strainer. Later at temperature it was kept in air tight bottles with labeling tag of date and area of collection for further use. A highly concentrated ethanol extracts of the plant flowers and leaves dissolving 5g samples (80 mesh) in 100ml ethanol (70%) at room temperature followed by filtration and evaporation. Finally, a highly concentrated ethanol extracts of the plant flowers and leaves was saved at 4ºC for further assay. Residue was discarded.

For the experiment 30-40 g weight of albino mice Balb- C were used. All animals were healthy and in good condition. All work has been done by Animal Use Protocol (AUP) which was reviewed and approved by the Animal Care and Use Committee (ACUC) in an Office of Laboratory Animal Care (OLAC) veterinarian in planning of the procedures at National Veterinary Laboratories (NVL), Islamabad. All animal were divided in five groups control, control diabetic, diabetes with oral antidiabetic effect, and diabetic with extracts of leaves and diabetic with effect of flower respectively. Deprivation of diet of animals was performed by fasting, for at least1/3 part of day.

Details of five groups are as below:

Group Name	Dose
negative control/ vehicle	400 µl of 0.1 Molar Citrate buffer at pH= 4.5) per kg b.w
positive control for diabetes/ Diabetic control	400 µl of newly primed STZ / kg b.w of mice.(7mg STZ/ ml of citrate buffer were used)
positive control for antidiabetic drug/ Antidiabetic control	Inoculated with Glucophage at 1mg/ kg of dose b.w of animal. 1mg Glucophage/ ml of citrate buffer were prepared as antidiabetic drug (Glucophage) was prepared
Experimental groups (for flower extract)	Introduced with flower extracts at 1mg/ kg a dose of b.w of animal
Experimental groups (For leave extract)	Inserted with leave extracts (1mg/ kg b.w)

Group 2 - 5 were injected with standard diabetic drug (STZ) before one hour of experiment. Diabetes was established by the measuring the concentration of glucose during hours between 8-12 of fasting and repeatedly (following 120 minutes of feed) condition. The amount of blood glucose was evaluated for three successive days after giving of STZ for the validation of diabetes by intraperitoneal injection and blood was taken from heart and tail of the mice. After inducing diabetes, Oral glucose tolerance test (OGTT) is achieved to monitor, which animal can develop diabetes more quickly as equated to all mice.

Prior to experiment, 10% glucose solution in water was prepared. Each and every mouse was weighed to resolute quantity of glucose to inject and their abdomen was labeled. After keeping mice in fasting for measurement of glucose level in blood was performed and 1 unit glucose solution/ 1 gram of weight was injected. Glucose solutions were also injected to animals after Thirty, Sixty, Ninety and two hours monitored and blood glucose level record was maintained by Glucometer (EUSURE, SN: T044040130029). Glycosylated hemoglobin level was also measured by Kit method. The antioxidant effect can be simply assessed by the reduction of UV absorption at 517 nm. Assay was performed rendering to method of[10] Free radical scavenging plant sample activity was determined by ABTS radical cation de colorization assay. ABTS and cation radical results by the reaction between 7 mM ABTS in H_2O and 2.45 mM $K_2S_2O_8$ (1:1).

ANTIOXIDANT ACTIVITY

DPPH Scavenging Assay: This is most commonly used method as it is simple and accurate. DPPH shows a absolute absorption which is highest at 517 nm (purple). Assay was performed rendering to method of [11].The antioxidant effect .of DPPH can be simply assessed by the reduction of UV absorption at 517 nm by using formula % DPPH scavenging effect = $[A_o-(A-A_b)]/A_o \times 100$

$FeCl_3$ Reducing Antioxidant Assay: The methanolic extract at different concentrations (10-50µg/mL) of *J. adhatoda* leaves and fruit were mixed to 2.5mL of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution and 2.5mL of 0.2 M sodium phosphate buffer (pH 6.6). Reaction mixture was vortexed evenly and at 50°C for half hour and incubated by using vortex shaker. At the final stage of incubation, 2.5mL of 10% $C_2HCl_3O_2$ was mixed to the mixture and centrifuged at three thousand rpm for ten min. 2.5mL of supernatant was added with 2.5mL of deionised H_2O and 0.5mL of 0.1% $FeCl_3$. Colored solution was checked at 700 nm against blank with ascorbic acid as a reference standard to compare reducing power of samples on UV Spectrophotometer[12].

ABTS Radical Scavenging Assay: Free radical scavenging plant sample activity was determined by ABTS radical cation decolorization assay. ABTS·and cation radical results by the reaction between 7 mM ABTS in H_2O and 2.45 mM $K_2S_2O_8$ (1:1), stored at RT for twelve to sixteen hours in dark before use. Then diluted ABTS solution CH_3OH to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to diluted ABTS solution, the absorbance was measured at half hour after the initial mixing. An appropriate solvent blank was run in each assay. The measurements were carried out at least three times. Ascorbic acid as standard substance was used and percent inhibition of absorbance at 734 nm was calculated using the formula,

ABTS· scavenging effect (%) = ((AB–AA)/ AB) ×100

Where, AB is absorbance of ABTS radical + CH_3OH; AA is absorbance of ABTS radical + sample extract/standard.

RESULTS

Before, during and after the experiments no side effects were seen like weight loss, diarrhea, anorexia etc. OGTT was performed to assess which animal's become diabetic more firstly. Amongst tested mice, those animals were selected to make diabetic whose blood $C_6H_{12}O_6$ was high than 130 and 220 (mg/dl) in male and female mice respectively (Table 1). In males during fasting condition, *J.*

adhatoda leaves lessened glucose level more rapidly than its flowers (2.32 mg/ml and 2 mg/ml in male and female mice, respectively) while in random conditions it was 1.9867 mg/ml and 1.9467 mg/ml in male and female mice, respectively. The lowering of blood glucose is done as do the Glucophage i.e. in fasting it was 0.8467 and 0.85 while 1.04 and 0.9295 (mg/ml) in random conditions in male and female mice, respectively. Glycosylated hemoglobin was more strongly reduced by its leaves (65 mg/ml in male mice) than flowers. Moreover statistical analysis showed that *J. adhatoda* flower has much capability to reduce hemoglobin that is glycosylated. Amount of total $C_{27}H_{46}O$ was more strongly reduced by its leaves (1.25 mg/ml) in female mice than male mice and by flowers (1.6134 mg/ml) in male mice as compared to female mice. Quantity of triglycerides was most strongly reduced by *J. adhatoda* leaves in female mice (0.8934 mg/ml) then in male mice (1.0667 mg/ml). In male mice triglycerides amount was heavily decreased by its flowers (1.043 mg/ml). Level of total urea in blood was equally affected by both *J. adhatoda* leaves and flowers in both male and female mice. *J. adhatoda* leaves showed best results in male mice (Table 2).

Table 1: Level of glucose (mg/dl) in blood of selected mice before diabetes (Values are mean of 3 replications).M=Male,F=Female

Groups		Blood glucose levels (mg/dl)				
		0 min	**30 min**	**60 min**	**90 min**	**120min**
Group 1 (Negative control)	M	110.34±56.88	136±78.9	117±60.00	130.34±54.81	91±21.74
	F	186.67±59.09	212.34±21.36	203.67±14.46	195.67±29.02	166.67±32.25
Group 2 (Diabetic control)	M	151.67±14.64	313.34±63.50	236.34±68.38	245.67±87.37	120.67±51.29
	F	259.67±53.45	234±17.22	237.34±14.21	249±76.39	185.67±24.54
Group 3 (Antidiabetic control)	M	218±40.58	183±35.38	223.34±67.30	110.34±57.735	152.67±40.69
	F	198.67±57.07	244.67±37.93	233.34±14.20	218±44.69	233.67±84.60
Group 4 (*J. adhatoda* flower)	M	218±40.58	183±35.38	190±17.22	110.34±57.73	119.34±23.58
	F	198.67±57.07	244.67±37.93	233.34±14.20	218±44.69	233.67±83.60
Group 5 (*J. adhatoda* Leaf)	M	195.67±84.07	348.67±2.31	195±12.58	196.34±37.31	154.34±53.07
	F	159.67±23.45	192.34±72.45	177.34±29.56	142.67±46.45	129±39.88

Table 2: Glucose concentration, Glycated Hemoglobin level (HBA1c, %), Cholesterol level, Triglycerides level and Total urea level of selected mice after the induction of diabetes.

Groups	Gender	Glucose concentration (mg/ml)		Total cholesterol (mg/ml)	Triglycerides (mg/ml)	Total urea (mg/ml)	HbAlc (mg/ml)
		Random	**Fasting**				
Group 1 (Negative control)	M	1.5634	1.8133	1.4788	1.3531	0.27	60
	F	1.1767	1.4866	1.2589	1.4644	0.3334	61
Group 2 (Diabetic control)	M	3.22	3.5833	2.6134	1.8185	0.4067	108.4
	F	2.1034	2.3834	2.9089	2.3404	0.5934	102.7
Group 3 ((Antidiabetic control)	M	1.0467	0.8467	1.0713	1.4315	0.3267	61
	F	0.9295	0.85	1.3431	1.2893	0.35	61.7
Group 4 (*J. adhatoda* Flower)	M	2.0334	2.08	1.6134	1.043	0.3084	82
	F	1.66	2.37	2.0267	1.026	0.3467	76
Group 5 (*J. adhatoda* Leaves)	M	1.9867	2.32	2.08	1.0667	0.3334	65
	F	1.9467	2	1.25	0.8934	0.34	85

±Values are mean of 3 replications M= Male F= Female

Fig. 1: $FeCl_3$ Assay of Extract with Standard

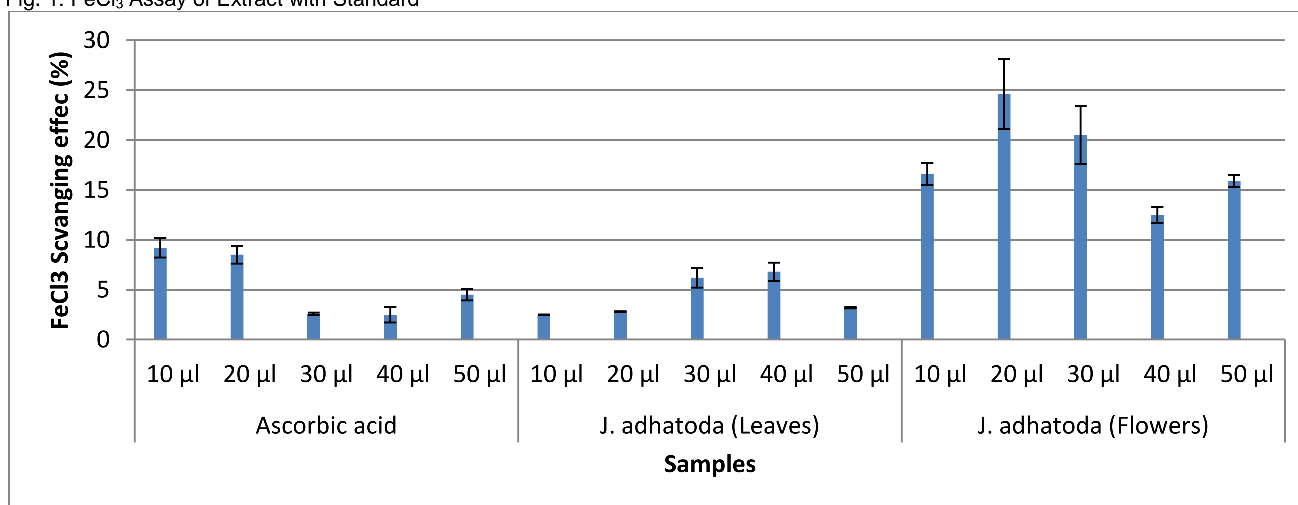


Fig. 2: DPPH Assay of Extract with Standard

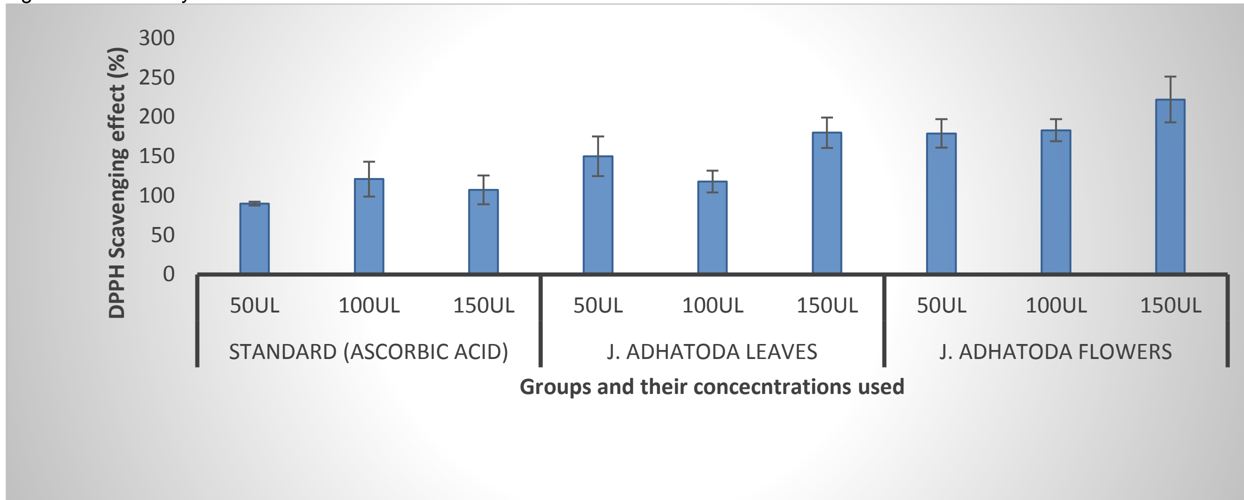
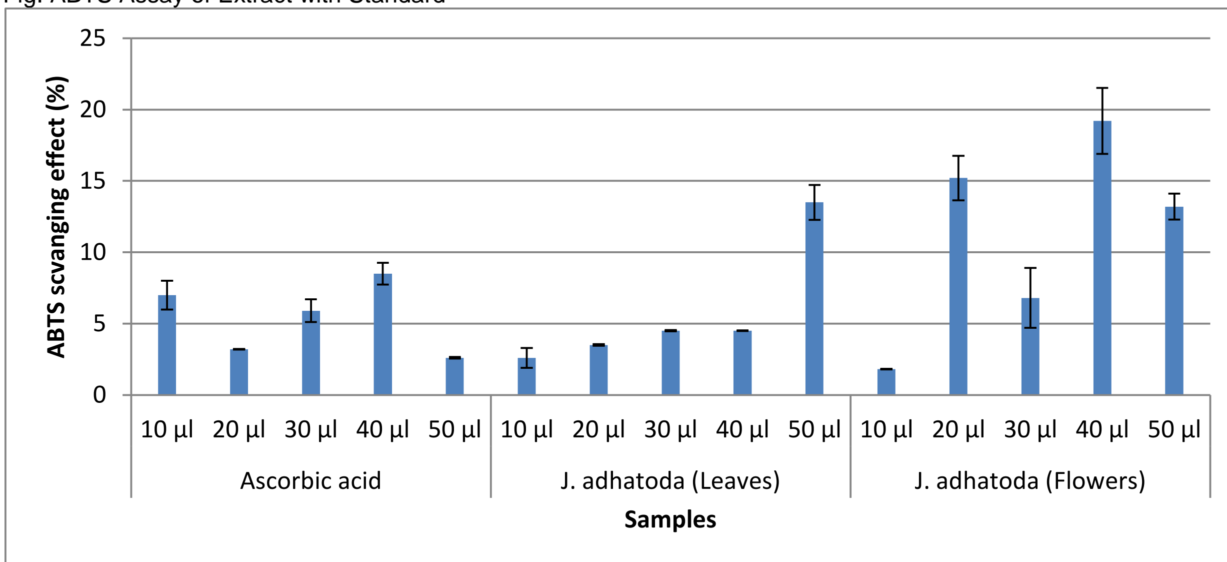


Fig. ABTS Assay of Extract with Standard



Antioxidant activity results: DPPH is frequently used as a substrate and is a fast, inexpensive and easy method to measure capacity of antioxidant natural compounds. Owing to the stability of DPPH it shows supreme absorption at 517 nm in ethanol/methanol Effect of antioxidants on DPPH radical scavenging is because of their proton-donating ability. In DPPH scavenging assay, the antioxidants are able to reduce the stable radical DPPH to the yellow-colored diphenylpicryl hydrazine. Based on this principle, the antioxidative activity of a substance can be expressed as its ability in scavenging the DPPH free radical. In this experiment, the DPPH free radical scavenging effect of *J. adhatoda* leaves and flowers was measured, and the results are shown in Figure 1 and it is clear that flowers have shown maximum antioxidant value against 150µl (222%) almost two folds higher then Ascorbic acid (used as standard) and leaves have shown a close values against 50µl and 150µl concentration (150% and 180% respectively). So it is obvious that *J. adhatoda* leaves do exhibit antioxidant activity and best activity is shown at 50 µl as the maximum value has come against 150µl but it could be due the increased concentration of the extract. Moreover flowers have shown minimum activity at 50µl (179%) which is equal to the antioxidant potential at 150µl by leaves (180%) (Fig. 1). It means flowers were more strongly scavenging H_2O_2 than leaves. According to previous literature available, the phenolic compounds, flavonoids, and alkaloids directly participate towards the antioxidant activity. While comparing the results of *J. adhatoda* leaves and flowers it became evident that *J. adhatoda* flowers possess strong antioxidant activity then leaves.

ABTS free radical scavenging effect of *J. adhatoda* leaves and flowers was measured, and the results are shown in Figure 2 and it is clear that flowers have shown maximum antioxidant value but almost two folds lower than standard and leaves have shown even less antioxidant potential than flowers. So it is obvious that *J. adhatoda*

leaves do exhibit antioxidant activity but it could be due the increased concentration of the extract. These results are similar to the results of DPPH assay as in that assay flowers showed more potential to scavenge free radicals.

$FeCl_3$ free radical scavenging effect of *J. adhatoda* leaves and flowers was measured, and the results are shown in Figure 3 and it is clear that flowers have shown maximum antioxidant value against 30µl (53.41%) and least at maximum plant concentration (53.6%). So it is obvious that *J. adhatoda* leaves do exhibit antioxidant activity and best activity at lower concentration. Moreover flowers have shown minimum activity at 10 µl (56.88%) which is even more than antioxidant potential of leave. It means flowers were more strongly scavenging H_2O_2 than leaves. According to previous literature available, the phenolic compounds, flavonoids, and alkaloids may participate directly to the antioxidant activity. While comparing the results of *J. adhatoda* leaves and flowers it became evident that *J. adhatoda* flowers possess higher amount of secondary metabolites which scavenged free radicals from reaction mixture.

DISCUSSION

The results of antidiabetic activity are compatible with the study of [13]who found a greatly remarkable reduction in blood glucose and cholesterol levels in diabetic and non-diabetic control animals and in animals which were induced with methanolic extract of *J. adhatoda* leaves. More over the methanolic extract of leaves have 67.85% antioxidant activity which showed that our plant had more antioxidant potential than old ones. Result verifies antidiabetic and antioxidant effects of this plant thus keeping the extracts more beneficial in curing not only diabetes but in addition to the associated secondary disorders.

Present results are also compatible with the findings that *A. zeylanica* Medic. has long time frequently used in Ayurveda system of medicine as it owns important activities like antibacterial, abortifacient, antitussive, anti-inflammatory, radio modulation, hypoglycemic, antiulcer, cardiovascular protection, ant tubercular, antiviral, hepatoprotective, ant mutagenic and antioxidant. These reports are designate and very hopeful that herb should be studied more precisely for its therapeutic benefits and clinical trials using this plant for a variety of combinations in different formulations should also be conducted.

CONCLUSION

In this study an experiment was lead to uncover the antidiabetic and antioxidant activity of *J. adhatoda*. On the basis of above results it is obvious that *J. adhatoda* possess these activities. The results showed that *J. adhatoda* leaves can effectively reduce blood glucose level, Glycosylated hemoglobin, Leaves and flowers of this plant have maximum antioxidant values comes against 50µl concentration of extracts. *J. adhatoda* possesses numerous bioactive compounds and this plant has great potential to be developed as natural antidiabetic and antioxidant agent. There are still some loopholes in whole process that can be improved by focusing some research and also there is a need to conduct clinical use in these conditions and to conduct clinical trials to prove its clinical utility.

REFRENCES

1. Hossain, T. M. and O. M. Hoq (2016). Therapeutic use of *Adhatoda vasica*. Asian Journal of Medicine and Biological Research, 2 (2), 156-163.
2. Luo, A., Y. Fan and A. Luo (2011). In vitro free radicals scavenging activities of polysaccharide from *Polygonum Multiflorum Thunb*. Journal of Medicinal Plants Research, 5(6), 966-972.
3. Orhan, N., U. G. İçöz., L. Altun and M. Aslan (2016). Anti-hyperglycaemic and antioxidant effects of *Bidens tripartita* and quantitative analysis on its active principles. Iranian Journal of Basic Medical Sciences, 19 (10), 1114– 1124.
4. Kooti W, Moradi M, Akbari SA, Sharafi-Ahvazi N, AsadiSamani M, Ashtary-Larky D. Therapeutic and pharmacological potential of Foeniculum vulgare Mill: A review. J HerbMed Pharmacol. 2015;4:1–9
5. Hui H, Zhao X, Perfetti R. Structure and function studies of glucagon-like peptide-1 (GLP1): the designing of a novel pharmacological agent for the treatment of diabetes. Diabetes Metab Res Rev. 2005;21:313–31. doi: 10.1002/dmrr.553. [PubMed]
6. Singh, N. B., R. B. Singh., L. R. Singh., D. Prakash., P. D. Singh. , K. B. Sarma and B. H. Singh (2009). Polyphenolics from various extracts/fractions of red onion (*Allium cepa*) peel with potent antioxidant and antimutagenic activities. Food and Chemical Toxicology, 47(6), 1161-1167.
7. Ilango, V., P. Chitra., P. Kanimozhi and G. Balaji (2009). Antidiabetic, antioxidant and antibacterial Activities of Leaf extracts of *Adhatoda zeylanica*. Medic (Acanthaceae). J. Pharm. Sci. and Res. , 1 (2): 67-73.
8. Ozturk, Y. , V. M. Altan and A. Yildizoglu (1996). Effect of experimental diabetes and insulin on smooth muscle functions. Pharmacol. Rev. 48: 69- 112.
9. Guyatt. G. H. , D. Osobe. , K.W. Wu. , Wyrwich and G. R. Noman (2002). Methods to explain the clinical significance of health status measures. Mayo Clin. Proc. 77: 371-383.
10. Szkudelski, T. (2001). The mechanism of alloxan and Streptozotocin action in beta cells of rat pancreas. Physiol. Res. 50: 536- 546.
11. Luo, A., Y. Fan and A. Luo (2011). In vitro free radicals scavenging activities of polysaccharide from *Polygonum Multiflorum Thunb*. Journal of Medicinal Plants Research, 5 (6), 966- 972.
12. Maruthamuthu Vijayalakshmi and Kandasamy Ruckmani. 2016. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh J Pharmacol. 2016; 11: 570-572
13. International diabetes federation. key findings 2014. 2014. [Last access date: 26.11.2016].
14. Ahmad, S. , G. Madhuka., A. Maksood., S. Mhaveer., A. Tanwir and A. S. Hussain (2009). A phyto-pharmacological overview on *Adhatoda zeylanica* Medic. syn. *A. vasica* (Linn.) Nees. Indian J. of Nat. Prod. And Resources; 8 (5): 549- 554.