Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF ETHANOLIC EXTRACT OF *ANJIR* LEAVES (*Ficus carica)* IN ALLOXAN INDUCED DIABETIC RAT

S. P. Jayakumar*[1], Malini Sen[1], M. Jagadeesan[1], R. Sundararajan[1]

[*1]Department Pharmacology, Mohamed Sathak A. J. College of Pharmacy, Sholinganallur, Chennai – 600119, Tamil Nadu, India.

ABSTRACT

Ficus is a pan-tropical genus of trees, shrubs and vines occupying a wide variety of ecological niches; most are evergreen, but some deciduous species are endemic to areas outside of the tropics and to higher elevations. Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. All successive extracts of *Anjir* leaves in 0.5% tween 80 was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the ethanolic extract of *Anjir* Leaves according to the procedure. The present study, diabetic rats had lower body weights, high blood glucose level as compared to the normal rats, which confirmed the induction of diabetic by alloxan. In spite of the increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. The treatment with ethanolic extract of *Anjir* leaves improved the average body weights of rats which indicate control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

KEYWORDS

Glycosides, Hyperglycemic, Ethanol, and Antioxidant.

Author for correspondence:

S. P. Jayakumar,
Department Pharmacology,
Mohamed Sathak A. J. College of Pharmacy, Sholinganallur, Chennai-600119, Tamil Nadu, India.

Email: spjayapharm@gmail.com.

INTRODUCTION

The therapeutic properties of medicinal plants are conditioned by the presence of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect humans and animals or which are biologically active in relation to the causative agents of various diseases[1]. A special group of medicinal plants are antibiotics. However, treatment with medicinal plants must be conducted under the supervision of a physician[2]. More than 30,000 tons

of raw materials from approximately 220 species of medicinal plants are used annually in the USSR. Of the plants collected, more than 75 percent of the species grow wild, accounting for 50 percent of the total weight. The rest are cultivated on 23 sovkhozes of the Ministry of Medicinal Industry[3]. The opium poppy and peppermint are also cultivated on kolkhozes. A few dozen species of medicinal plants are exported annually from the USSR, including several thousand tons of licorice roots[4]. Many medicinal plants are used in the food industry, in the perfume industry and in metallurgy. Diabetes is a metabolic disorder that is characterized by high blood glucose and either insufficient or ineffective insulin. 5.9% of the population in the United States has diabetes, and diabetes is the seventh leading cause of death in our country. Diabetes is a chronic disease without a cure; however, with proper management and treatment, diabetics can live normal, healthy lives. There are two main types of diabetes, Type I and Type II[5]. An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate. Antioxidants get their name because they combat oxidation. They are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation[6]. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. *Ficus* is a pan-tropical genus of trees, shrubs and vines occupying a wide variety of ecological niches; most are evergreen, but some deciduous species are endemic to areas outside of the tropics and to higher elevations[7]. Fig species are characterized by their unique inflorescence and distinctive pollination syndrome, which utilizes wasp species belonging to the Agaonidae family for pollination. Grind four fig leaves along with sugar candy. Regularly take this mixture along with a glass of water two times in a day. This fig home remedy is useful in the natural treatment of liver cirrhosis. Consuming one teaspoon of fig seeds along with a teaspoon of honey daily to control diabetes. Follow this therapy for about a week[8].

MATERIALS AND METHODS

Preparation of ethanolic extracts of *Anjir* leaves

The leaves was separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Anjir* leaves was extracted with 250ml ethanol in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of ethanol. The concentrated product was used as ethanolic extract of leaves of *Anjir*.

Phytochemical investigation on *Anjir* leaves [9-12]

Test for saponins

Take 2ml of drug solution in a test tube. To it add small amount of water, shake well, stable froth (foam) is formed.

Test for tannins

To the 5ml of aqueous extract add 0.5 gm of sodium acid phosphate. Then warm it and filter. To the filtrate add 2% Phenazone solution, precipitate is formed which is often coloured.

Test for amino acids

To the test solution add 2ml of millions reagent, white precipitate indicates presence of amino acid.

Test for proteins

The test solution take in a test tube and heat in boiling water bath, proteins get coagulated.

Xanthoproteic test

To the 5ml of test solution, add 1ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40 %sodium hydroxide solution, orange colour is formed.

Glycosides

Keller kiliani test

The test consists of boiling about 1 g finely powered sample with 10 ml 70 % alcohol for 2 to 3 minutes. The extract is filtered. To the filtrate is added, 5 ml water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with equal volume of chloroform and

evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml concentrated sulphuric acid. A reddish brown layer acquiring bluish-green colour after standing is observed.

Test for cardiac glycosides

Keddes test

Extract the drug with chloroform, evaporate to dryness. Add one drop of 90% alcohol and 2drops of 2% 3, 5 Di nitro benzoic acid in 90% alcohol. Make alkaline with 20% sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5 Di nitro benzoic acid depends on the presence of alpha, beta unsaturated lactones in the aglycone.

Test for alkaloids

Dragendorff's test

To 2-3 ml filtrate, add few drops of Dragendorff's reagent. Orange brown precipitate is formed.

Mayer's test

2-3 ml filtrate with few drops of Mayer's reagent gives precipitate.

Test for carbohydrates

Molisch's test

The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with alpha napthol and concentrated sulphuric acid which gives purple colour ring at the junctions of two layer.

Test for flavonoids

Shinoda test

To dry powder or extract, add 5 ml 95 % ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour observed.

To small quantity of extract, add lead acetate solution. Yellow colored precipitate is formed.

Experimental part

Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. All successive extracts of *Anjir* leaves in 0.5% tween 80 was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality[13,14]. Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee prior to the beginning of the project work from Institutional Animal Ethical Committee (IAEC) of SASTRA University, Thanjavur, and Tamilnadu.

Experimental Designs

Anti-diabetic and Anti- oxidant activity of *Anjir* leaves (Alloxan-induced diabetic model)

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg b. wt. intraperitonially. After 1 h of alloxan administration, the animals were given feed *ad libitum*, and 5% dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 48 h blood glucose was measured by glucometer[15,16]. The diabetic rats (glucose level >300 mg/dl) were separated and divided into five different groups for experimental study, with each group containing six animals.

Group I was served as Normal Control, **Group II** was served as Diabetic control, **Group III** was served as Diabetic + Glibenclamide (5 mg/kg), **Group IV** was served as Diabetic + *Anjir* leaf Extract 200 mg/kg, **Group V** served as Diabetic + *Anjir* leaf Extract 400 mg/kg.

For all the animals the blood glucose level is measured on Day 0, 5, 10 and 15. The results of group IV and group V (Anjir leaf extract treated groups) is compared with group I, II (control groups) and group III (standard drug treated group).

Blood samples collected from all the animals are further subjected to tests for determining Lipid profile, Hepatic glycogen level and tissue concentration of LPO, SOD and CAT. The results of the Anjir leaf extract treated groups are compared that with the control and standard drug treated groups.

RESULT AND DISCUSSION

The preliminary phytochemical screening like Saponin, Tanins, Amino Acid, Protein, Alkaloids, Carbohydrates, Glycosides and Flavonoids was done. The results of preliminary test of ethanolic extract of *Anjir* Leaves were shown in Table No.1.

Anti-diabetic and Anti- oxidant activity of *Anjir* leaves (Alloxan-induced diabetic model)

Phytochemical screening of all the extract of *Anjir* leaves showed the presence of various chemical constituents, mainly tannins, saponins and flavonoids which may be responsible for its antidiabetic and anti-oxidant properties was shown in Table 1. The results obtained were comparable and satisfied the standard literature. To ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by alloxan-induced model. As expected, in the diabetic control, there was severe hyperglycemia when compared with the normal animals. When compared with the Diabetic control, the ethanolic extract of Anjir leaves as shown in Table No.2 and Figure No.1, lowered the elevated blood glucose levels. It was observed that the standard drug Glibenclamide lowered the blood glucose level significantly bringing it nearly back to normal, whereas ethanolic extract of *Anjir* leaves significantly decreased blood serum glucose in the diabetic rats on fifth, tenth, fifteenth and twentieths days compared with the diabetic control rat's blood serum glucose levels.

In the present study, diabetic rats had lower body weights, high blood glucose level as compared to the normal rats, which confirmed the induction of diabetic by alloxan. In spite of the increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. The treatment with ethanolic extract of *Anjir* leaves improved the average body weights of rats which indicate control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

Alloxan causes massive reduction in insulin release, through the destruction of b-cells of the Islets of Langerhans. In our study, we have observed a significant increase in the plasma insulin level when alloxan diabetic rats were treated with *Anjir* leaves. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of *Anjir* leaves in alloxan diabetic rats may also be due to enhanced glucose utilization by peripheral tissues. The serum lipid profile in Table No.3 and Figure No.2 treated with *Anjir* leaves extract returned to values nearing that of the control group. This showed that treatment with *Anjir* leaves significantly improved the lipid profile in diabetic animals and reduces the glycogen level in liver shown in Table No.4 and Figure No.3. Table No.5 and Figure No.4 show the concentration lipid peroxidation and hydroperoxides in the liver of both control and experimental groups of rats. There was a significant elevation in tissue lipid peroxidation and hydroperoxides in diabetic rats. Administration of *Anjir* leaves to diabetic rats decreased the levels of tissue lipid peroxidation and hydroperoxides to normal levels. The concentration of tissues LPO, SOD and CAT were significantly decreased in diabetic rats when compared to the control group. Administrations of *Anjir* leaves extract to diabetic rats tend to bring the activities of these enzymes to near normal level.

DISCUSSION

The currently-available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs. This study was undertaken to assess the antidiabetic effect of *Anjir* leaves. In the present study, the oral treatment of *Anjir* leaves extract decreased the blood glucose levels in diabetic rats. It has been reported that using medicinal plant extract to treat alloxan-induced diabetic rats results in activation of *ß*-cells and insulinogenic effects. *Anjir* leaves may also have brought about hypoglycaemic action through stimulation of surviving *ß*-cells of islets of

Langerhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with *Anjir* leaves. Since the percentage fall in plasma glucose levels was different in models with varying intensity of hyperglycaemia, it implies that the anti hyperglycaemic effect of that plant is dependent on the dosage of diabetogenic agent, which in turn leads to *ß*-cell destruction. A number of other plants have also been observed to exert hypoglycaemic activity through insulin release stimulatory effects. The concentrations of lipids, such as serum cholesterol were significantly higher in diabetic rats than in the control group. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic rats treated with Glibenclamide shows normalised lipid levels. Thus, the results indicate that *an Anjir leaf shows* insulin-like action by virtue of its lipid lowering levels. Oxidative stress has been shown to play a role in the causation of diabetes mellitus. Antioxidants have been shown to have a role in the alleviation of diabetes mellitus. In diabetes mellitus, oxygen free radicals (OFRs) are generated by stimulating H_2O_2. In our study, concentrations of lipid peroxides and hydroperoxides were increased in liver of diabetic rats, indicating an increase in the generation of free radicals. Increased lipid peroxidation in diabetes mellitus can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. The present finding indicates significantly increased lipid peroxidation of rats exposed to alloxan and its attenuation by *Anjir* leaves *treatment*. This suggests that the protective role of *Anjir* leaves extracts could be due to the antioxidative effect of flavonoids present in the leaf, which in turn act as strong superoxide radicals and singlet oxygen quenchers. Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes mellitus. In the current study, the LPO, SOD and CAT activities were significantly reduced in the liver of diabetic rats. These observations emphasize the critical importance of maintaining the antioxidant potential of the pancreatic *ß*-cell in order to ensure both its survival and insulin secretion capacity during times of increased oxidative stress. The decreased activities of SOD and CAT in the liver during diabetes mellitus may be due to the production of reactive oxygen free-radical that can themselves reduce the activity of these enzymes.

Table No.1: Phytochemical screening results of *Anjir* leaves

S.NO	PHYTOCONSTUTIENT	RESULT
1.	SAPONINS	+
2.	TANNINS	+
3.	AMINO ACIDS	+
4.	PROTEINS	+
5.	GLYCOSIDES	–

6.	CARDIAC GLYCOSIDES	+
7.	ALKALOIDS	+
8.	CARBOHYDRATES	+
9.	FLAVONOIDS	+

PRESENT = (+), ABSENT = (-)

Table No.2: Anti Diabetic Activity of Ethanolic Extract of *Anjir* leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Day 0	Day 5	Day 10	Day 15	Day 20
I	Normal Control	98.67±3.45	92.56±6.23	85.87±8.53	91.23±5.89	87.48±6.74
II	Diabetic control	375.39±13.67	398.45±16.72	407.51±13.34	436.67±15.49	452.74±18.39
III	Diabetic + Glibenclamide	368.73±10.45	304.92±14.39	250.79±14.27	233.44±11.25	208.25±11.59
IV	Diabetic + Extract 200 mg/kg	372.21±12.34	364.32±06.53	336.12±04.98	343.46±12.22	295.54±06.74
V	Diabetic + Extract 400 mg/kg	377.63±12.53	352.38±15.64	316.37±16.83	284.79±10.64	253.70±12.92

Table No.3: Antioxidant Activity of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No,	Treatment group	LPO	SOD	CAT
		No. of moles MDA formed/ mg of protein	Amount of protein required for 50% inhibition	Moles of H2O2 decomposed/minute/mg of protein
I	Normal Control	2.86	7.27	93.64
II	Diabetic control	4.23	3.89	49.37
III	Diabetic +glibenclamide	3.12	6.53	81.35
IV	Diabetic + Extract 200 mg/kg	4.16	4.20	55.26
V	Diabetic + Extract 400 mg/kg	3.59	5.66	72.89

Table No.4: Hepatic Glycogen Level of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Liver glycogen (mg/g)
I	Normal Control	42
II	Diabetic control	15
III	Diabetic + Glibenclamide	36
IV	Diabetic + Extract 200 mg/kg	17
V	Diabetic + Extract 400 mg/kg	23

Table No.5: Serum Cholesterol Level of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Serum cholesterol (mg/dl)
I	Normal Control	97
II	Diabetic control	225
III	Diabetic + Glibenclamide	126
IV	Diabetic + Extract 200 mg/kg	194
V	Diabetic + Extract 400 mg/kg	152

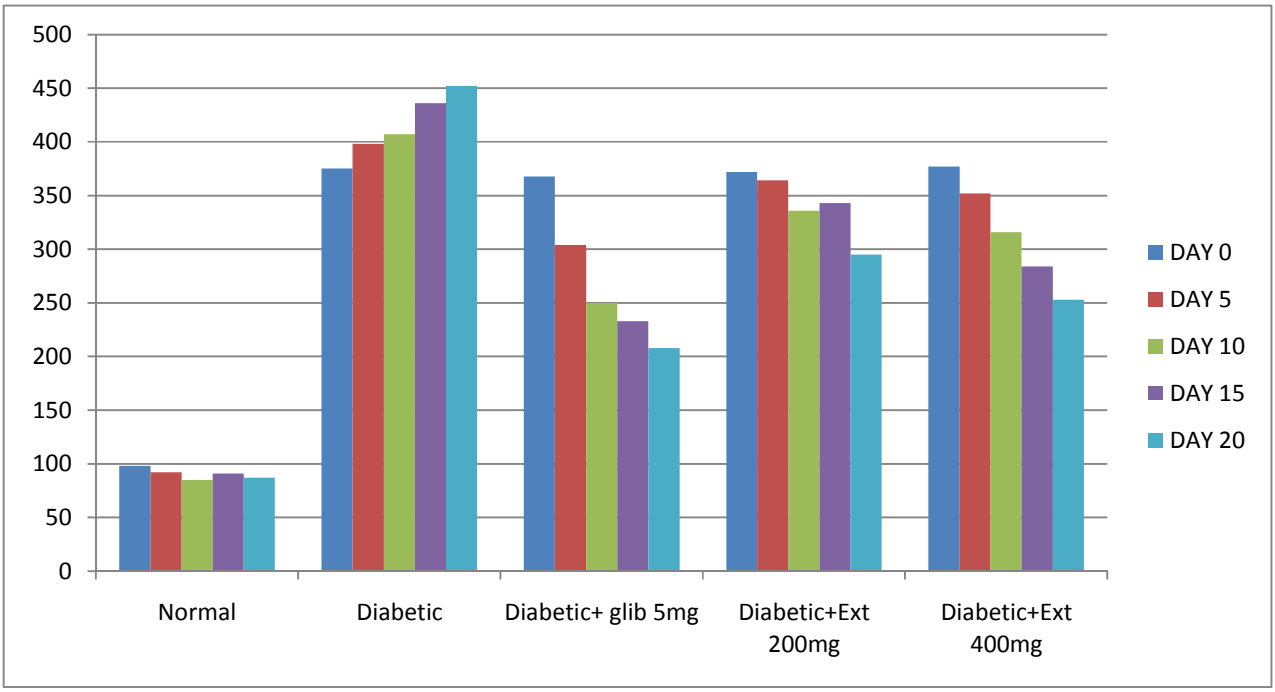

Figure No.1: Anti Diabetic Activity of Ethanolic Extract of *Anjir* leaves

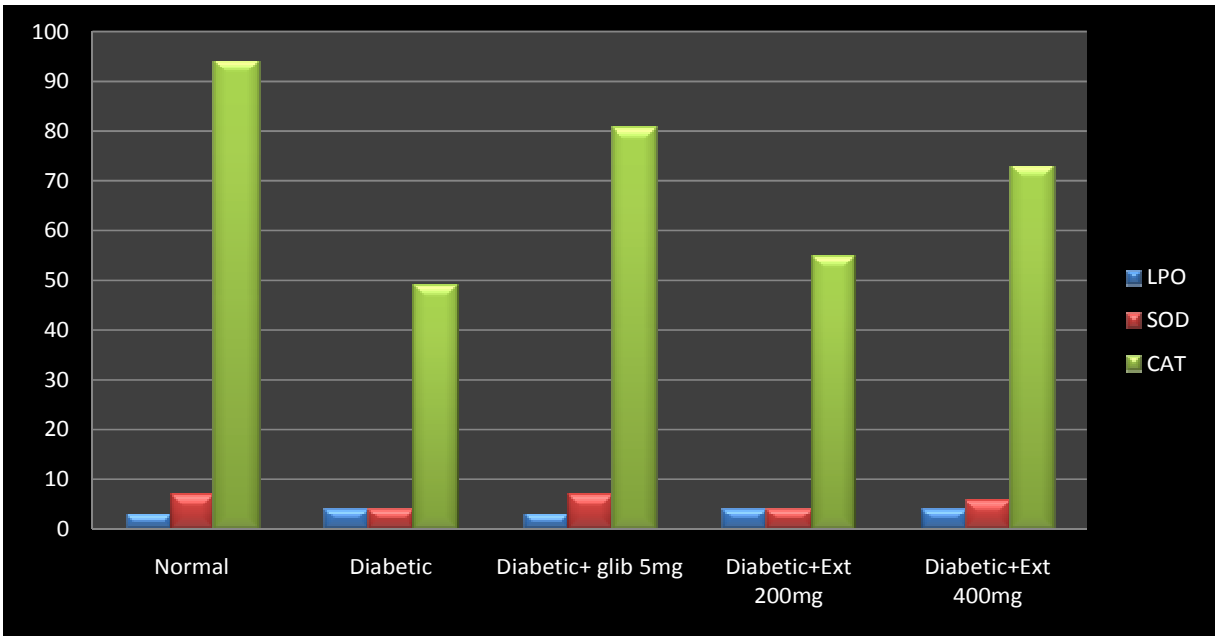

Figure No.2: Antioxidant Activity of Ethanolic Extract of Anjir leaves

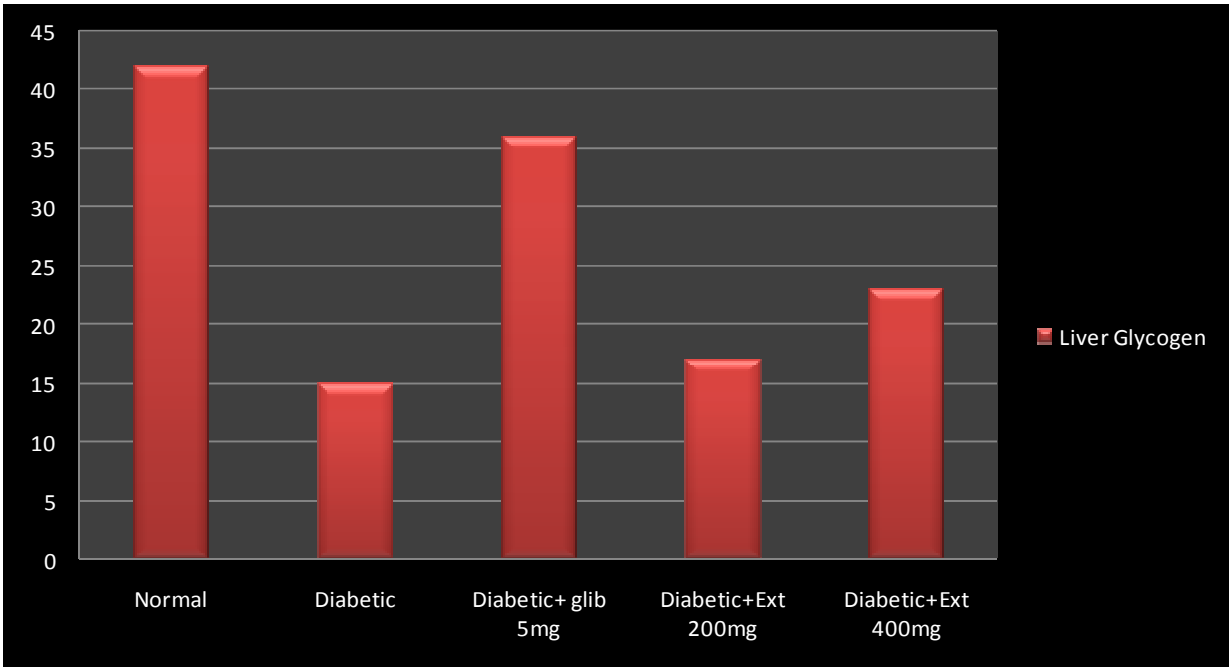

Figure No.3: Hepatic Glycogen Level of Ethanolic Extract of Anjir leaves

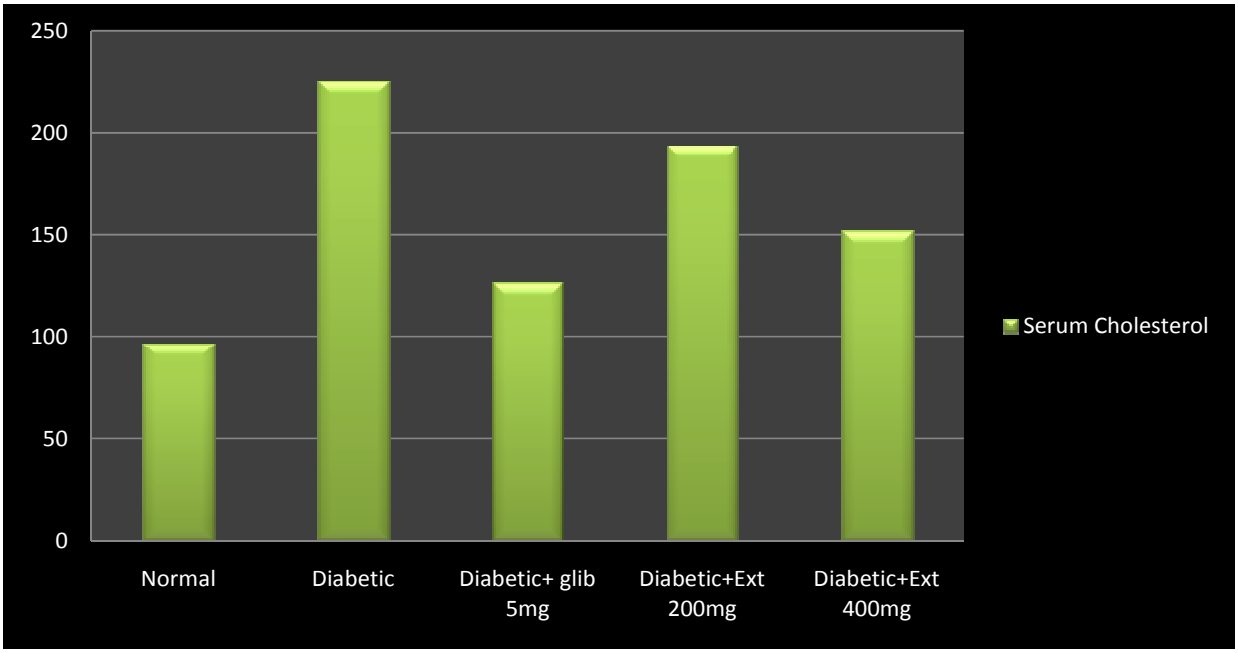

Figure No.4: Serum Cholesterol Level of Ethanolic Extract of Anjir leaves

SUMMARY AND CONCLUSION

1. Preliminary phytochemical analysis report data.
2. In this experimental model, the ethanolic extract *Anjir leaves* (200, 400 mg/kg) with reference standard Glibenclamide 5mg/kg significantly effective in abnormalities of enzyme profile in experimental rats.
3. The data that *Anjir leaves* extract is beneficial in controlling the blood glucose level, improves the lipid metabolism and prevents diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats.
4. This could be useful for prevention or early treatment of diabetic disorders.
5. We conclude that the *Anjir leaves* have potent anti-diabetic and anti-oxidant effects in alloxan induced diabetic rats.
6. The present investigation has also opened avenues for further research especially with reference to the development of potent formulation for diabetes mellitus from *Anjir leaves.*

ACKNOWLEDGEMENT

The authors are sincerely thanks to Mohamed Sathak A. J. College of Pharmacy, Sholinganallur, Chennai, Tamil Nadu, India for providing the facilities to complete this research work.

REFERENCE

1. Sofowora A. Medicinal Plant and Traditional Medicine in Africa, *John willey and sons*, 1, 1993, 50-58
2. Mashkovskii MD. *Lekarstvennye sredstva*, 7th edition, *Moscow*, 1972, 1–2.
3. Acworth IN and Bailey B. Reactive Oxygen Species. In: The handbook of oxidative metabolism. Massachusetts, *ESA Inc*, 1997, 1-1 to 4-4.
4. Alessio HM and Blasi ER. Physical activity as a natural antioxidant booster and its effect on a healthy lifestyle, *Res. Q. Exerc. Sport*, 68 (4), 1997, 292-302.
5. Rhett D. Figs and the diversity of tropical rain forests. *Bioscience*, 55(12), 2005, 1053–1064.
6. Jeong MR, Cha JD, Lee YE. Antibacterial activity of Korean Fig (*Ficus carica* L.) against food poisoning bacteria, *Korean J Food Cookery Sci*, 21, 2005, 84-93.
7. Gilani AH, Mehmood MH, Janbaz KH, Khan AU and Saeed SA. Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*. *J. Ethnopharmacol*, 1(5), 2008, 119.
8. Rahul Kumar Modi1, Manisha Kawadkar, Saima Sheikh, Ravindra Kastwar and GouravTiwari. A review on: Comparative studies on ethanolic extract of root and stem bark of Ficus carica for analgesic and antiinflammatoryactivities. *Int. J. of Pharm. & Life Sci*, 3(8), 2012, 120-127.
9. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan, 1999.
10. Wealth of India. Raw materials, New Delhi, *CSIR*, 1976, 100–104.
11. Wallis TE. Practical Pharmacognosy. J&A Churchill Ltd, *London Edition V*, 1984.
12. OCED 425 guidelines. OCED Guidelines for testing animals, 1(2), 2001, 1-26.
13. Bolton S. Analysis of variance. Pharmaceutical statistics: practical and clinical applications. Drugs and Pharmaceutical Sciences Series. Basel: Marcel Dekker, *In Swarbrick* J, 1997, 215-265
14. Pari L, Uma Maheswari J. Antihyperglycaemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan diabetic rats. *Phytother Res*, 14, 2000, 136-138.
15. Stanley P, Prince M, Menon VP. Hypoglycaemic and other related actions of Tinospora cordifolia in alloxan-induced diabetic rats. *J Ethno pharmacol*, 70, 2000, 9-15.
16. Rajalingam R, Srinivasan N, Govindarajulu P. Effect of alloxan induced diabeties on lipid profiles in renal cortex and medulla of mature albino rats. *Indian J Exp Biol*, 31, 1993, 577-579.