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ANTIDIABETIC ACTIVITY OF *KALANCHOE PINNATA* (LAM.) PERS INALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The main objective of present work is find out good pharmacological activities in herbal source with their preliminary phytochemical study, and also it is aimed to investigate Anti-Diabetic activity of ethanolic and aqueous extracts of dried stem of plant *Kalanchoe pinnata (*LAM.)PERS against alloxan induced diabetes in rats. The Ethanolic and aqueous extract of dried stem of *Kalanchoe pinnata* (LAM.) PERS. shows the good hypoglycemic activity on normal fasted rats. The Ethanolic and aqueous extract of dried stem of *Kalanchoe pinnata* (LAM.) PERS. exhibits the significant Anti-hyperglycemic activity.The alcoholic extract of dried stem of kalanchoe pinnata (LAM.)PERS. shows the significant inhibition activity of α- amylase enzyme as compared to aqueous extract.

1. INTRODUCTION

The World Health Organization (WHO) defined health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity." So during the past decade, traditional systems of medicine have become a topic of global importance. Current estimate suggest that, in many developing countries a large proportion of the population relies heavily on traditional practioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

2. MATERIALS AND METHODS

Collection and authentication of plant material: The specimen copy (Herbarium) of selected plant collected in month of july-2007 from ABS Botanical garden, Karripatty, Distt. - Salem, Tamil Nadu, Mr. A .Balsubramnian. (Consultant-central siddha research)Executive Director, ABS botanical garden, Salem, authenticated the plant as *Kalanchoe pinnata* **(LAM.) PERS.** (Family- Crassulaceae).

Preparation of extract: The stem of ***Kalanchoe Pinnata*** **(LAM.)PERS**. Were dried under shade and than powdered with a mechanical grinder. The powder was passed through sieve No. 30 and stored in an airtight container for further use.

Solvent for extraction: Petroleum Ether (60-80^{o} C), Alcohol (95% v/v) and Distilled water with chloroform (0.25%)

Extraction procedure: The dried powders of stem of *Kalanchoe pinnata* were defatted with petroleum ether (60-80ºc) in a Soxhlet Apparatus by continuous hot- percolation. The defatted powder material (marc) thus obtained was Further extracted with ethanol (95% v/v) with same method and fresh powder used for aqueous extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Procurement of experimental animals: Swiss albino mice (20-25 g) and albino Wister rats (150-200 g) of either sex and of approximate same age are used in the present studies were procured from listed suppliers of Sri Venkateswara Enterprises, Bangalore, India. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-P.Col. / /2007) after scrutinization. The animals received the drug treatments by oral gavage tube.

Hypoglycemic activity: in present study, the hypoglycemic activity of plant extract of dried stem of *Kalanchoe pinnata* (LAM.)PERS. was studied for the decrease in blood glucose level (BGL) in normal fasted rats. 300 and 600 mg/kg were screened for hypoglycemic activity on normal rats up to 3 hrs. It produced significant hypoglycemic activity in a dose dependent manner. Significant reduction in blood glucose level was seen at 2nd hrs and maximum reduction occoured at 3rd hrs by treatment with ethanolic and aqueous extracts which was compared with the

control animal group and standard treated group. The alcoholic and aqueous extract treated group shows the significant reduction in blood glucose level during the study hour.

Antihyperglycemic activity: The plant extract of *Kalanchoe pinnata* (LAM.)PERS. exhibited significant Anti-Hyperglycemic activity.As in the alloxan treated group the BGL was high due to cytotoxic effect of alloxan on the β-cell of islet of langarhans. Present study has confirmed that the repeated treatment of ethanolic and aqueous extract for a period of 12 days caused a significant decrease in Blood glucose of diabetic rats. 300mg/kg and 600 mg/kg of both Ethanolic and Aqueous extract of stem of plant *Kalanchoe pinnata* (LAM.)PERS were screened for Anti-Hyperglycemic activity against alloxan induced diabetes. It produced significant anti-hyperglycemic activity in a dose dependant manner. The Aqueous extract of plant *Kalanchoe pinnata* (LAM.)PERS. at 600 mg/kg shows more significant Anti-hyperglycemic activity than ethanolic extract.The Anti-hyperglycemic activity exhibited by the ethanolic extract and aqueous extract was compared with that of the standard drug (Metformin).

***In–vitro* αlpha-amylase inhibition activity:** α- amylase enzyme present in different part of GIT which was responsible for digestion of starch and carbohydrate. The present study deals with the inhibition of α- amylase by plant extract of dried stem of *Kalanchoe pinnata* (LAM.)PERS. Both alcoholic and aqueous extract of stem having α- amylase inhibition activity which is shown by increase in reaction time i.e. the time taken by α- amylase to digest the starch. As the concentration of extract increases the time of reaction was also increases. Time taken for starch disappearance by Aqueous and Ethanolic extract were compared with that of standard drug. It has been found that ethanolic extract having significant inhibition as compared to aqueous extract.

Treatment design:

Group I: Normal control (5% CMC Solution 10ml/kg)
Group II: Positive control (Metformin HCL 250 mg/kg)
Group III: Alcoholic extract (300 mg / kg p.o.)
Group IV: Alcoholic extract (600 mg / kg p.o.)
Group V: Aqueous extract (300 mg / kg p.o.)
Group VI: Aqueous extract (600 mg / kg p.o.)

Evaluation of Hypoglycemic activity of extract of stem of plant *Kalanchoe pinnata* on normal fasted rats:

1. Male albino wistar rats weighing between 150-200 gm was purchased from Sri Venkateshwara Enterprises, Bangalore and marked with picric acid.
2. The rats were fasted for 18 hrs and water was given ad libitum.
3. After 18hrs the fasting blood sample were collected by retro orbital puncture (ROP) or tail pricking.
4. The initial fasting BGL was estimated by using Glucometer.
5. The animal showing very high > 150 mg/dl or low < 75 mg/dl were discarded and the animal showing optimum BGL 75-120 mg/dl were selected.
6. The drug solution was prepared and was administered orally to the body weight of the animal.
7. Blood sample was withdrawn from all animal at 0, 1, 2, 3 hrs by retro orbital puncture or tail pricking method and BGL was estimated by Glucometer and change in BGL, average and SD were calculated and tabulated.

Hypoglycemic study:

Table.1. Evaluation of Hypoglycemic activity of extract of stem of plant *Kalanchoe pinnata* on normal fasted rats

Group	Treatment Design	Dose mg/kg	Blood glucose level [mg/dl] at different time-interrval			
			0" hour	1 hour	2 hour	3 hour
I	Normal control	5%CMC 10 ml/kg	76.5 ±1.22	75.5 ± 1.64	76.16 ± 1.60	76.16 ± 1.16
II	Positive control	Metformin HCL 250 mg/kg	78.16 ±2.71	69.00* ±1.67	54.66** ± 2.42	42.16** ±2.36
III	Ethanolic extract	300 mg/kg	77.5 ± 1.76	66.83* ± 2.71	62.66** ± 1.50	52.66** ±3.71
IV	Ethanolic extract	600 mg/kg	77.33 ± 1.63	65.00* ± 2.09	59.83** ±1.60	50.33** ± 2.25
V	Aqueous extract	300 mg/kg	76.66 ± 1.50	65.33*± 3.61	59.66* ± 2.42	50.33* ± 3.44
VI	Aqueous extract	600 mg/kg	78.33± 2.42	65.66* ± 1.03	57.00**± 2.28	43.20**± 2.40

P values: * * P< 0.01; * P <0.05, Values are expressed in mean ±SD, n=6 animals in each group. One way ANOVA followed by DUNNETT'S, multiple comparison tests, Group I, was compared with groups II, III, IV, IV, V, VI

Anti-hyperglycemic study

Treatment design:

Group I: Normal control (5% CMC Solution 10ml/kg)
Group II: Diabetic control (Alloxan 150 mg / kg i.p.)
Group III: Positive control (Metformin HCL 250 mg / kg p.o.)
Group IV: Alcoholic extract (300 mg / kg p.o.)
Group V: Alcoholic extract (600 mg / kg p.o.)
Group VI: Aqueous extract (300 mg / kg p.o.)
Group VII: Aqueous extract (600 mg / kg p.o.)

Evaluation of Anti-Hyperglycemic activity of extract of stem of plant kalanchoe pinnata on Normal and Experimental animals during 12 day treatment:

1. Male albino wistar rats weighing between 150-200 gm was purchased from Venkateshwara Enterprises, Bangalore and marked with picric acid.
2. The rats were fasted for 18 hrs and water was given ad libitum.
3. After 18hrs the fasting blood sample were collected by retro orbital puncture (ROP) or tail pricking as per SOP No. 2 or 3.
4. The initial fasting BGL was estimated by using Glucometer.The animal showing very high > 150 mg/dl or low < 75 mg/dl were discarded and the animal showing optimum BGL 75-120 mg/dl were selected.
5. They are injected with Alloxan (150mg/kg) i.p.
6. After 72 hrs of alloxan injection the blood sample was withdrawn by retro orbital puncture or tail pricking method as per SOP. No. 2 or 3 and BGL were estimated by glucometer.
7. The animals that show BGL above 200 mg/dl and below 450 mg/dl were selected for study.
8. The drug solution was prepared as per SOP. No.4 and was administrated orally according to the body weight of animal as per SOP. No. 5.
9. Blood sample were withdrawn from all animal at 0, 1, 3, 6, 12, days by retro orbital puncture or tail pricking method and BGL was estimated by Glucometer.
10. Change n BGL, average and SD were calculated and tabulated.

Anti-Hyperglycemic Study:

Table.2. Evaluation of Anti-Hyperglycemic activity of extract of stem of plant kalanchoe pinnata on Normal and Experimental animals during 12 day treatment

Group	Treatment Design	Dose mg/kg	Blood glucose level mg/dl				
			Initial	1st day	3rd day	6^{th} day	12^{th} day
I	Normal control		80 ± 5.25	83 ± 5.58	79.5 ± 2.88	79.16 ± 3.6	80.16 ± 5.15
II	Diabetic control Alloxan only	150	284 ± 8.61	289.16**± 9.74	290.66 ± 8.74	291** ± 5.29	300**± 0.25
III	Standard treated (Metformin)	250	289.16± 8.96	265.33**± 6.34	190.33**± 6.80	134.5**± 7.18	93.33*± 13.39
IV	Ethanolic extract	300	291± 9.01	243.83** ± 9.10	158.5**± 4.59	126.66**± 5.16	119.00*± 7.09
V	Ethanolic extract	600	290.83 ± 5.07	241.66** ± 4.84	158.83**± 4.91	124.00**± 5.32	114.5 ± 3.39
VI	Aqueous Extract	300	290.33 ± 6.08	248**± 11.13	174.5* ± 7.79	134.5** ± 10.07	115.33* ± 7.17
VII	Aqueous extract	600	293.5 ± 9.15	243.5**± 7.25	166.83**± 8.10	127.83**± 8.95	110.66*± 8.38

P values: * * P< 0.01; * P <0.05, Values are expressed in mean ±SD, n=6 animals in each group. One way ANOVA followed by DUNNETT'S, multiple comparison tests,Group II, was compared with groups I, III, IV, V, VI, VII.

3. HISTOPATHOLOGY REPORT

Pancreas specimens:

GROUP 1:Normal Control [5% CMC solution, 10ml/kg p.o]: The islets are normal. The architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

GROUP 2:Diabetic control (alloxan monohydrate 150 mg/kg, i.p) : The islets are normal. The architecture is preserved. There is a diffuse infiltrate of lymphocyte and a few plasma cells throughout stroma, the acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

GROUP 3: Standard treated [metformin HCL 250 mg/kg]: The islets are normal. There are areas of focal hemorrhage with many small congested blood vessels. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.
GROUP 4: Ethanolic extract (300 mg/kg): There is a dense granulomatous infiltrate of lymphocytes within the stroma. The acinar cells are normal.
GROUP 5:Ethanolic extract (600 mg/kg): There are focal aggregates of lymphocytes within the stroma.the acinar cells are normal.
GROUPS 6: Aqueous extract (300 mg/kg): The islets are normal. The architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.
GROUP 7: Aqueous extract (600 mg/kg): The islets are normal. The architecture is preserved. The acini are lined by round to oval cells with moderate cytoplasm and small round to oval nuclei.

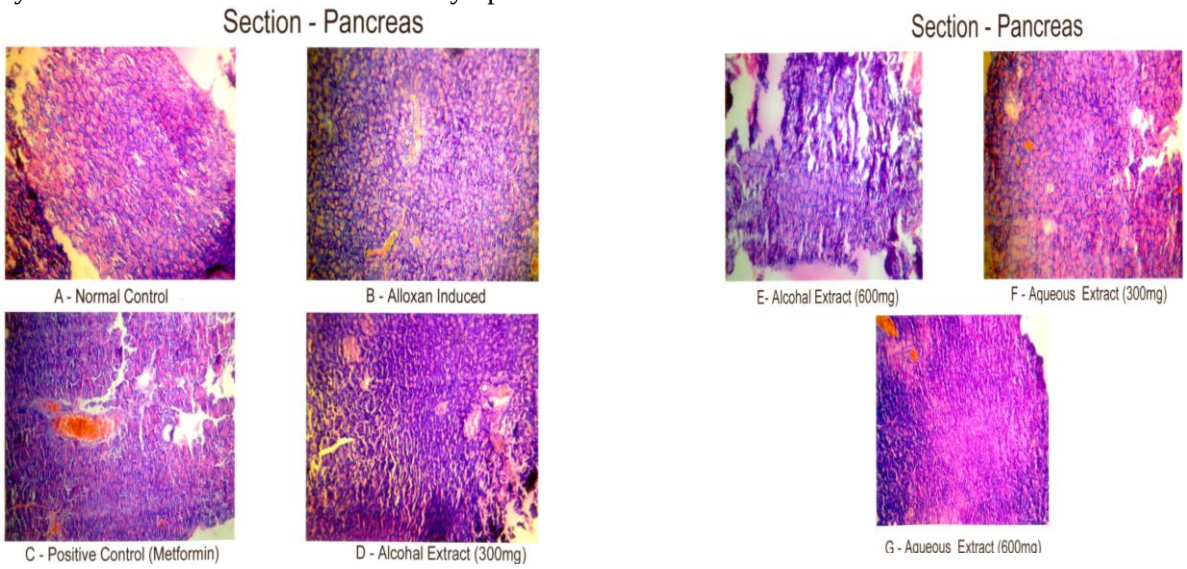

Figure.1. Pancreas specimens

Kidney specimens:
GROUP 1 normal control: - [5% CMC solution, 10ml/kg p.o]
The glomeruli appear normal. The tubules are normal and lined by a single layer of cuboidal cells.
GROUP 2diabetic control: - (alloxan monohydrate 150 mg/kg, i.p)
Glomeruli shows hypercellularity.the tubules are normal. Stroma shows small focal hemorrhages and a diffusive infiltrate of lymphocyte and plasma cells.
GROUP 3– standard treated: - [metformin HCL 250 mg/kg]
The Glomeruli shows mesangial hypercellularity with focal hemorrhage. The tubules show mild cloudy swelling of the cells lining them. The stroma is normal.
GROUP 4– Ethanolic extract [300 mg/kg]
The Glomerulus shows mesangial hypercellularity. Tubules are normal. Stroma shows a diffuse infiltrate of lymphocyte and plasma cells.
GROUP 5– Ethanolic extract [600 mg/k:]
The glomeruli show mesangial hypercellularity. Tubules are normal. Stroma shows a diffuse infiltrate of lymphocytes and plasma cells.
GROUP 6- aqueous extract [300 mg/kg]
The glomeruli show mesangial hypercellularity and focal glomerulosclereosis. Tubules are normal. Stroma is normal.
GROUP 7– aqueous extract [600 mg/kg]
The glomeruli appear normal. The tubules are normal and lined by a single layer of cuboidal cells. The stroma is normal.

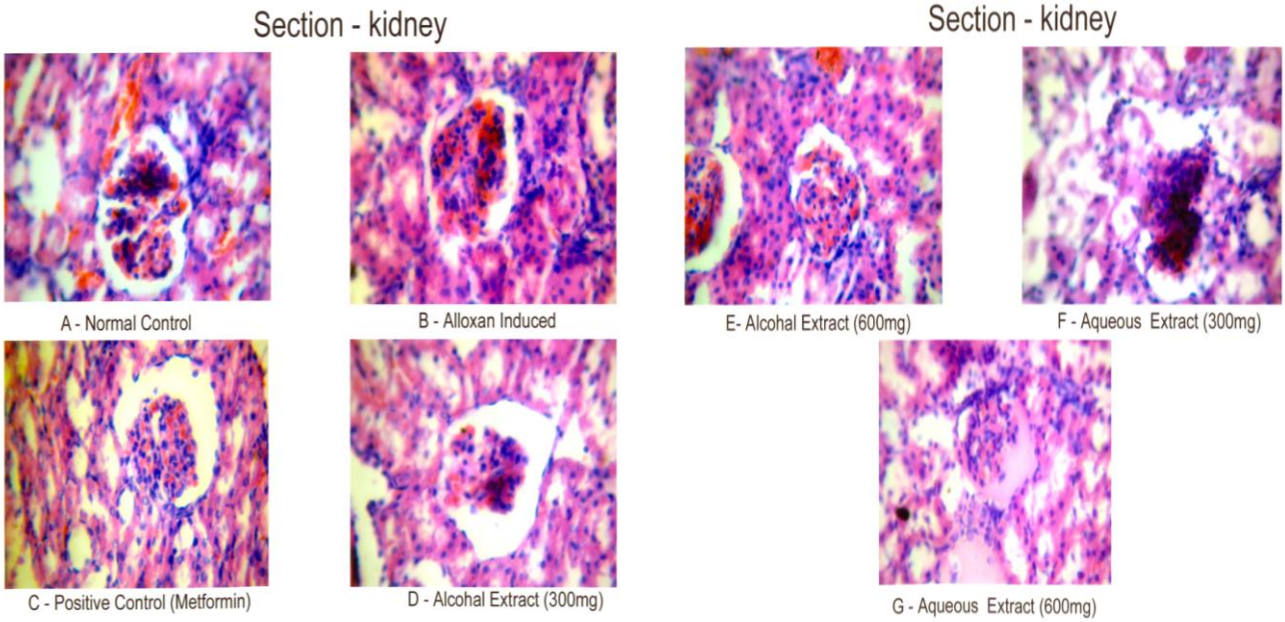

Figure.2. Kidney specimens

4. *IN-VITRO* α- AMYLASE INHIBITION STUDY:

α –Amylase inhibition activity: Alpha amylase enzyme is responsible for the metabolism of polysaccharide such as starch carbohydrate etc, the aim behind present experiment is to study the effect of α-amylase concentration on the rate of reaction and inhibition activity of alcoholic and aqueous extract of dried stem of *Kalanchoe pinnata* (LAM.)PERS.

Requirements: 1%starch solution, Buffer solution 6.8 PH, 8 wells spot plate, Iodine solution, α-amylase

Procedure:

- Prepare a 1:1 series of dilutions of the α-amylase solution of different conc.
- α-amylase solution kept in four test tube and from them 1 ml withdrawn and kept in another test tube for test.
- In spot plate put two drops of iodine solution in four row one row for each tube.
- Add 0.5 ml of 1% starch solution to each tube, mix it.
- Immediately take out one drop of solution and place it in the first well.
- After 1 min. Take out another drop and put it in second well.
- Continue taking a sample every 1 minute until all the starch has been digested and the colour of the well is light yellow brown or disappear.

Table.3. Normal Control of α-amylase inhibition activity

Tube	Amylase solution	Buffer solution Ph 6.8	Time until starch disappear
1	1ml tube +0.5 ml starch solution	20 drops	06
2	1ml tube +0.25 ml starch solution	20 drops	07
3	1ml tube +0.125 ml starch solution	20 drops	10
4	1ml tube +0.63 ml starch solution	20 drops	12

As the concentration of α-amylase increases the rate of reaction is also increases but the time of reaction decreases because high conc. Of α-amylase will digest the starch rapidly.

Table.4. **Standard drug [Riboflavin] of α amylase inhibition activity**

Tube	Amylase solution	Buffer solution Ph 6.8	Time until starch disappear
5	1ml tube +0.5 ml starch solution+ 0.063% standard drug solution	20 drops	10
6	1ml tube +0.5 ml starch solution+ 0.125% standard drug solution	20 drops	12
7	1ml tube +0.5 ml starch solution+ 0.25% standard drug solution	20 drops	15
8	1ml tube +0.5 ml starch solution+ 0.5% standard drug solution	20 drops	16

Riboflavin is a α-amylase inhibitory agent as the concentration of riboflavin increases the time of reaction is also increases because the number of enzyme required for digestion of starch is not in sufficient.

Table.5. **Alcoholic Extract of α- amylase inhibition activity**

Tube	Amylase solution	Buffer solution Ph 6.8	Time until starch disappear
5	1ml tube +0.5 ml starch solution+0.063% Alcoholic extract solution	20 drops	09
6	1ml tube +0.5 ml starch solution+0.125% Alcoholic extract solution	20 drops	11
7	1ml tube +0.5 ml starch solution+ 0.25% Alcoholic extract solution	20 drops	14
8	1ml tube +0.5 ml starch solution +0.5% Alcoholic extract solution	20 drops	15

Alcoholic extract of dried stem of kalanchoe pinnata having the α-amylase inhibition activity. From observation it was found that as the concentration of extract increases the time of reaction is also increases but as compare to standard drug they have little activity.

Table.6. **Aqueous Extract of α-amylase inhibition activity**

Tube	Amylase solution	Buffer solution Ph 6.8	Time until starch disappear
5	1ml tube +0.5 ml starch solution +0.063% Aqueous extract solution	20 drops	08
6	1ml tube +0.5 ml starch solution +0.125% Aqueous extract solution	20 drops	10
7	1ml tube + 0.5 ml starch solution + 0.25% Aqueous extract solution	20 drops	12
8	1ml tube +0.5 ml starch solution + 0.5% Aqueous extract solution	20 drops	14

From observation it was found that the aqueous extract of dried stem of kalanchoe pinnata having the α-amylase inhibition activity. But as compare to standard drug and alcoholic extract it has less activity**.**

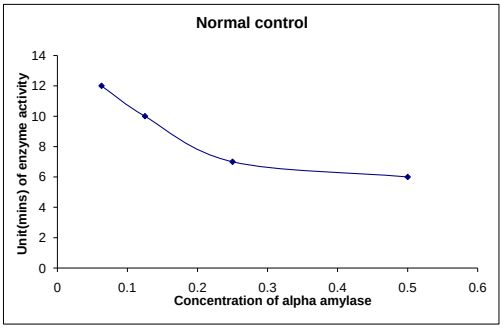

Figure.3.Normal control of α-amylase inhibition activity

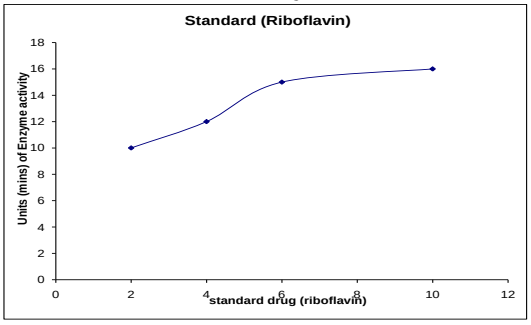

Figure.4.Standard (riboflavin) of α amylase inhibition activity

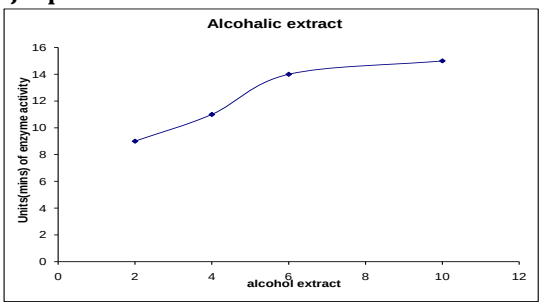

Figure.5.Alcoholic extract of α- amylase inhibition activity

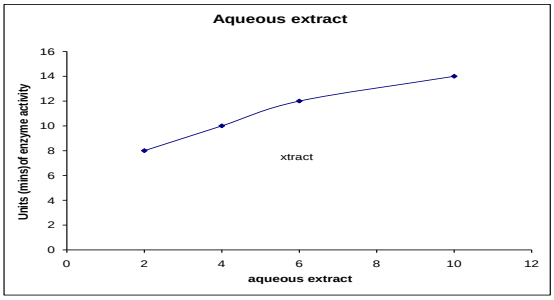

Figure.6.Aqueous extract of α-amylase inhibition activity

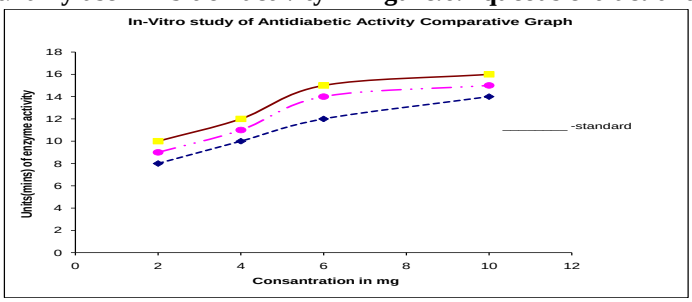

Figure.7.Comparative graph of ά-amylase inhibition activity

5. CONCLUSION

The Ethanolic and aqueous extract of dried stem of kalanchoe pinnata (LAM.)PERS. exhibits the significant Anti-hyperglycemic activity. The alcoholic extract of dried stem of kalanchoe pinnata (LAM.)PERS. shows the significant inhibition activity of α- amylase enzyme as compared to aqueous extract.

REFERENCES

Ghosh MN, Fundamentals of Experimental Pharmacology, 3rd edition, 177.

John A.O. Ojewole, Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract, Journal of Ethnopharmacology, 99, 1, 13-19.

Joshi Shashank R, Shah Siddhart N, Rising global burden of Diabetes, The Asian J. of Diabetology, 1999, vol-1, No.3, 13-15.

Kirthikar K.R et al, Indian Medicinal Plants, 2nd edition, 999.

Kulkarni S.K, Handbook of Experimental Pharmacology, 2003, 125-127.

Lenzen S, and Munday R, thiol-group reactivity, hydrophilicity,and stability of alloxan,its reduction products and its N-methyl derivatives and a comparison with ninhydrin, Biochemical Pharmacology, 1991,42, 1385-1391.

Lenzen S, Panten U, Alloxan: history and mechanism of action, Diabetologia 1988, 31, 337-342.

Lipnick R.L, Cotruvo J A, Hill R N, Bruce RD, comparison of the up and down method and the fixed dose procedure acute toxicity procedures, Fd Chen, Toxic, 33, 1995, 223-231.

Obeley L.W, Free radicals and diabetes, Free radical biology and medicine, 1998, 5, 113-124

Pincus I J, Hurwitz, Scott M E, effect of rate of injection of alloxan on development of diabetes in Rabbits. J Am Physio Soc, 86, 1954, 553-555.

Tripathi K D, Text book of Medical Pharmacology, 4th edition, 264-283.