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PHARMACOLOGICAL EVALUATION OF *CATHARANTHUS ROSEUS*

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ABSTRACT:

The present work is aimed at evaluating antibacterial and antidiabetic activities of whole plant extract of *Catharanthus roseus.* Dichloromethane: methanol (1:1) extract was used for carrying out *in-vitro* antibacterial and *in-vivo* antidiabetic activity. Antibacterial activity was carried out using seven different gram positive and gram negative bacterial strains. The extract was found to have considerable antibacterial action against all bacterial strains. The study on antidiabetic activity involves induction of diabetes to all male Wister albino rats using alloxan monohydrate (80mg/kg body/weight) except control group followed by treatment of diabetic rats with extract (500mg/kg body weight) daily for 14 days. The results were compared with standard drug glibenclamide (5 mg/kg body weight) by measuring glucose levels and body weights of all animals. Glucose level was found to decrease and body weight was increased in extract treated and standard treated groups when compared to diabetic group. The influence of extract on biochemical parameters like total cholesterol, urea, creatinine and alkaline phosphatase were also measured. The results obtained confirms antibacterial and antidiabetic activity of extract and therefore can be used not only against treating infections but also be used in treating diabetes.

Key words: antibacterial, antidiabetic, *Catharanthus roseus,* alloxan monohydrate, glibenclamide

INTRODUCTION:

In recent years, many drugs have been isolated from natural source as the modern medicine system treats the symptoms and suppresses the disease but does little to ascertain the real cause. Toxic drugs which may suppress or relieve some ailments usually have harmful side-effects. Drugs usually hinder the self-healing efforts of the body and make recovery more difficult. Therefore, the current scenario is to isolate the active constituents present in the plant material to develop medicinally drugs which are having rare chances of adverse effects.

Medicinally important plants are also rich source of antibacterial agents. There are number of plants investigated for their antibacterial activity, to cite few examples are: St. John's wort against *S. aureus ,S. mutans, P. vulgaris, E. coli, P. aeruginosa,* Liquorice root against *S. aureus, S. mutans,* Uva ursi against *E. coli, S. aureus,* Garlic against *S. typhimurium, B. cereus, E. coli, S. aureus* and Sage against *B. cereus, S. aureus*[1-9].

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia (high blood glucose concentration) caused by insulin deficiency, often combined with insulin resistance.[10] Insulin is hormone released from β cells of pancreas and convert glucose (source of energy in body) to glycogen thus maintaining the glucose levels in the body. The deficiency of insulin leads to increase of glucose levels in blood and urine. Diabetes can also gives rise to other diseases like cataracts, cardiac problems, etc., There also occurs changes in biochemical parameters like cholesterol, urea, creatinine etc.

The present study involves *Catharanthus roseus* (Apocynaceae) also known as medagascar

periwinkle, is a perennial subshrub with green color simple, entire, petiolate leaves and violet pink-white or carmine red color flowers.[11] It contains 150 alkaloids including vincristine vinblastine, ajmalicine, etc.[12] The plant has been considered due to its wide range of pharmacological activity like anti-inflammatory, antimalarial, antimitotic, antihypertensive, antifertility, antihypercholesterolemic, antimutagenic, antidiuretic, antifungal, antispasmodic, antiviral, cardio tonic, CNS depressant, antitumor, cytotoxic, antispermatogenic, anticancer activities.The study involves whole plant of *Catharanthus roseus* for evaluating both antibacterial and antidiabetic activity.[13]

MATERIAL AND METHODS:

Plant Collection and Identification:

The whole plant material of *Catharanthus roseus* were collected from Medicinal Garden, Nizam Institute of Pharmacy and Research Centre, Deshmukhi, Nalgonda Dist, Andhra Pradesh. The plant was sent for identification. The plant was authenticated by the Department of Botany, Osmania University, Hyderabad. (Voucher no. 0359)

Preparation of Extract:

The whole plants of *Catharanthus roseus* were collected, washed with tap water and then with distilled water and kept for shadow drying in a clean place. The dried material was then subjected to chopping and electric grinding for obtaining coarse powder. About 100gms of coarse powder was weighed and subjected for extraction with dichloromethane: methanol (1:1)[14] using soxhlet apparatus for 11 hr. The extract so obtained was concentrated to obtain the dried form. The extract yield obtained was about 10 gm.

The dried extract obtained was subjected to phytochemical screening to know the presence of alkaloids and further for carrying out *in-vitro* antibacterial and *in-vivo* antidiabetic studies.

Phytochemical screening:

The extracted was subjected to chemical analysis for the presence of Alkaloids, Glycosides, Carbohydrates, Flavonoids, Phenols, Tannins, Proteins and amino acids and Fats and Oils[15]. TLC was also performed for qualitative analysis to know the presence of alkaloids.

TLC was performed using precoated plates on which the standard vinblastine sulphate (1 mg/ml in methanol) solution and extract (10 mg/ml in methanol) were spotted and then placed in chamber equilibrated with the mobile phase choloroform**:**methanol (19:1). After running ¾th of chromatogram R_f values were calculated by observing in UV light.[16]

IN-VITRO ANTIBACTERIAL STUDY:

Test organisms:

Pure cultures of seven bacterial isolates (*K. pneumoniae, E. coli, P. aeruginosa, P. vulgaris, B. cereus, B. subtilis, and S. aureus)* were collected from the M.T.C.C., Chandigarh, India.

Preparation of extract solutions:

10, 25, 50, 75 and 100 mg of the dried whole plant extract were dissolved separately in each 1 ml DMSO (dimethylsulphoxide).

Procedure:

The method used for the determination of antibacterial activity is, agar diffusion method[17, 18] In this method, nutrient agar medium was prepared and sterilized along with washed and dried petriplates. After sterilization the nutrient agar medium approximately 20 ml was poured in all the petriplates under aseptic conditions and allowed to solidify. After solidification, all the petriplates were marked and were inoculated with 100µl of respective bacterial culture from the nutrient broth medium and incubated for 5 min in inverted position. Then bores were made using cork borer and 100 µl of extract of different concentrations were poured in the wells. Similarly standard Streptomycin was also placed in seven different bacterial strains. All the plates were incubated at 37ºC for 24 hrs and zone of inhibitions were

measured in mm. The experiment was repeated thrice.

***IN-VIVO* ANTIDIABETIC ACTIVITY:**

Animals:

Healthy, adult female albino rats of Wistar strain, weighing 180-220 g and having normal glucose levels were obtained from the animal house of Nizam Institute of Pharmacy and Research Centre, Deshmukhi, Nalgonda, Andhra Pradesh, India. The animal house was well ventilated and the animals were exposed to 12 h day and night cycles at a temperature of 20 ± 2^0C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and standard rat pellet obtained from M/s Hindustan Lever Ltd., Bangalore, India

Animal Grouping:

Group I: Control (Normal rats)

Group II: Alloxan induced Diabetic rats (80mg/kg body weight)

Group III: Diabetes induced rats+ whole plant extract (500mg/kg body weight)

Group IV: Diabetes induced rats+ Glibenclamide (5mg/kg body weight)

Diabetes induction:

Alloxan monohydrate was used for the induction of diabetes and the solution (80 mg/kg body wt) was prepared in normal saline and was given intra-peritoneally.

Preparation of standard solution:

The weighed amount of powered standard glibenclamide (5mg/ kg body wt.) was dissolved in normal saline and was given p.o.

Preparation of extract solution:

The weighed amount of powdered extract was suspended in normal saline solution using few drops of tween 80 and was given the dose 500 mg/ kg body wt., p.o.[19]

Diagnostic kits:

Diagnostic kits used for the estimation of Urea, Cholesterol, Creatinine, Alkaline Phosphatase were obtained from Hychem Laboratories, Hyderabad, India.

Procedure:

Animals having normal glucose level were considered and diabetes was induced, using 0.5ml of Alloxan monohydrate (80mg/kg body weight) i.p to group II, III and IV keeping group I as normal control. After 24 hrs, the body weights and glucose levels were measured. Daily dose of whole plant extract (500mg/kg body weight) and standard (glibenclamide, 5mg/kg body weight) was given orally (0.5ml p.o) to group III and IV for 14 days respectively. Glucose levels and body weights were measured on seventh and fourteenth day.[19]Animals were sacrificed by decapitation on 14th day, the Pancreas were isolated and send for histopathology studies in A-Z Pathology laboratory, Malakpet, Hyderabad. The blood samples (2ml) were also collected and subjected to centrifugation at 5000rpm for 20 minutes. The serum after centrifugation was analyzed for Urea (urease, Berthelot, Endpoint method) Creatinine, (Alkaline picrate method), Alkaline Phosphatase (Kind and King's method) and Total Cholesterol level using diagnostic kits.

STATISTICAL ANALYSIS:

Data obtained for antibacterial and antidiabetic activity were written as mean± SEM . Statistical treatment applied is ANOVA two way classifications. The results are significant if $p<0.01$ and $p<0.05$.

RESULTS AND DISCUSSION:

Phytochemical Screening:

Chemical analysis showed the presence of alkaloids and carbohydrate (Table 1). TLC performed indicates that R_f value for the extract was same as that of standard vinblastine sulphate indicating its presence in the extract (figure 1).

Phytoconstituents	**Test**	**DME**
Carbohydrates	Molish's Test	+
Alkaloids	Dragandorff's	+
	Mayer's	+
	Wagner's	+
	Hager's	+
Glycosides	Glycoside test	-
Flavonoids	Flavonoid test(lead acetate solution)	-
Tannins	Tannins test(5% $FeCl_3$ solution)	-
Saponins	Saponin test	-
Protein	Biuret test	-
Amino acids	Ninhydrin solution	-
Fats and Oils	Staining paper	-

Table 1: Phytochemical profile of *Catharanthus roseus*

DME: dichloromethane: methanol whole plants extract

Thin layer chromatography:

Figure 1: Thin layer chromatogram for extract (ext 1) and standard (VB)

R_f value of standard:

Distance travelled by standard solution = 0.6 cm

Distance travelled by solvent front= 4.5 cm

R_f = 0.13

R_f value for whole plant extract:

Distance travelled by solvent front = 4.5 cm

Distance travelled by test sample = 0.65 cm

R_f = 0.14

***IN-VITRO* ANTIBACTERIAL ACTIVITY:**

In *in-vitro* study, it is found that dichloromethane: methanol (1:1) whole plant extract of *C. roseus* exert inhibitory activity against all the bacterial strains in the concentration range of 10- 100 mg/ml and showed increased zone of inhibition with increase in concentration (dose dependent). As shown in Table 4, the extract showed significantly more activity against *E. coli* with the zone of inhibition of 21mm for 100mg/ml when compared to standard with a zone of inhibition 18.70mm ($p<0.01$). *P. vulgaris, B. cereus, B. subtilis and S. aureus* also showed more activity with zone of inhibitions of 11.75,13.25, 11.50 and 13.00mm respectively for 100mg/ml concentration when compared to standard with the zone of inhibition 8.50.11.33,9.50 and 12.66mm respectively ($p<0.01$).*K. pneumoniae* and *P. aeruginosa* also showed to have nearly same activity as that of standard.(Table 2 and chart 1).

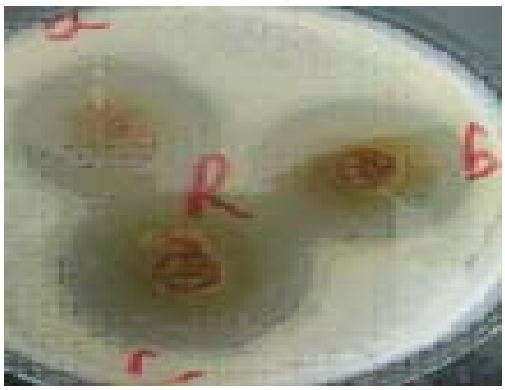
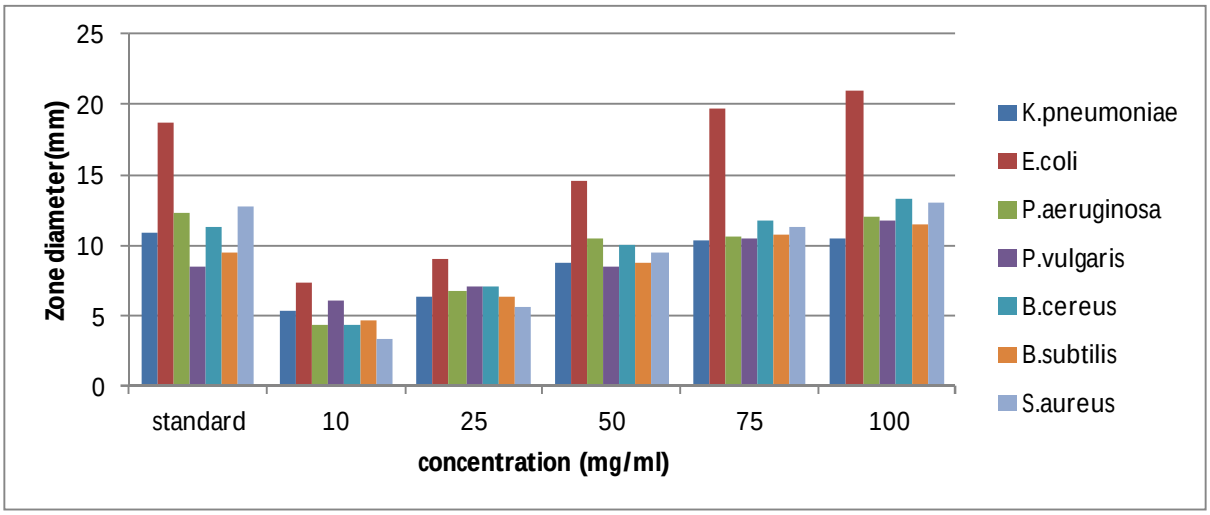


Figure 2: Zone of inhibition at different concentrations against *E. coli* strain in whole plant extract of *C. roseus*

S.No.	Bacterial strain used	Zone of inhibition in mm (Excluding the bore diameter =8mm)					
		Streptomycin (500 µg/ ml)	Whole plant dichloromethane methanol extract				
			10mg/ml	25mg/ml	50mg/ml	75 mg/ml	100 mg/ml
1	*K.pneumoniae*	10.83±0.47	5.33±0.33	6.33±0.67	8.8±0.80	10.25±0.63	10.50±1.32
2	*E. coli*	18.70±0.58	7.33±1.33	9.00±0.00	14.6±2.50	19.75±1.93	21.00±3.08
3	*P.aeruginosa*	12.33±0.33	4.33±0.33	6.67±0.33	10.5±1.19	10.6±1.03	12.00±1.08
4	*P. vulgaris*	8.50±0.29	6.00±0.58	7.00±1.00	8.40±0.72	10.5±0.50	11.75±1.11
5	*B. cereus*	11.33±0.88	4.33±0.33	7.0±0.00	10.0±0.63	11.75±0.73	13.25±1.31
6	*B. subtilis*	9.50±0.29	4.67±0.33	6.33±0.33	8.75±0.25	10.75±0.48	11.5±0.29
7	*S. aureus*	12.66±0.34	3.33±0.33	5.60±0.34	9.40±1.17	11.25±1.25	13.00±0.48

Table 2: Zone of inhibition of whole plant extract of *Catharanthus roseus* against different bacterial strains
Values are mean± SEM

Chart 1: Chart showing zone of inhibition diameter against different bacterial strains of different concentrations of whole plant extract and standard



IN-VIVO ANTIDIABETIC STUDIES:

In-vivo study showed that alloxan induced diabetic rats when treated with dichloromethane: methanol (1:1) whole plant extract (500 mg/kg body wt.) have significant decrease in the glucose levels(Table 3, Chart 2) and increase in the body weights(Table 4, chart 3) when compared to normal and standard treated diabetic rats ($p<0.05$).

S. No.	0th day	1st day	7th day	14th day
Group I	73.33±1.20	75.00±5.00	76.33±3.76	80.00±5.29
Group II	85.33±7.42	228.67±18.70	192.00±6.11	119.00±6.08
Group III	88.33±12.75	228.33±20.48	179.33±19.68	103.67±1.86
Group IV	78.33±1.20	185.33±7.86	133.00±2.65	82.00±3.33

Table 3: Glucose levels for different groups on different days
Values are given as mean±SEM

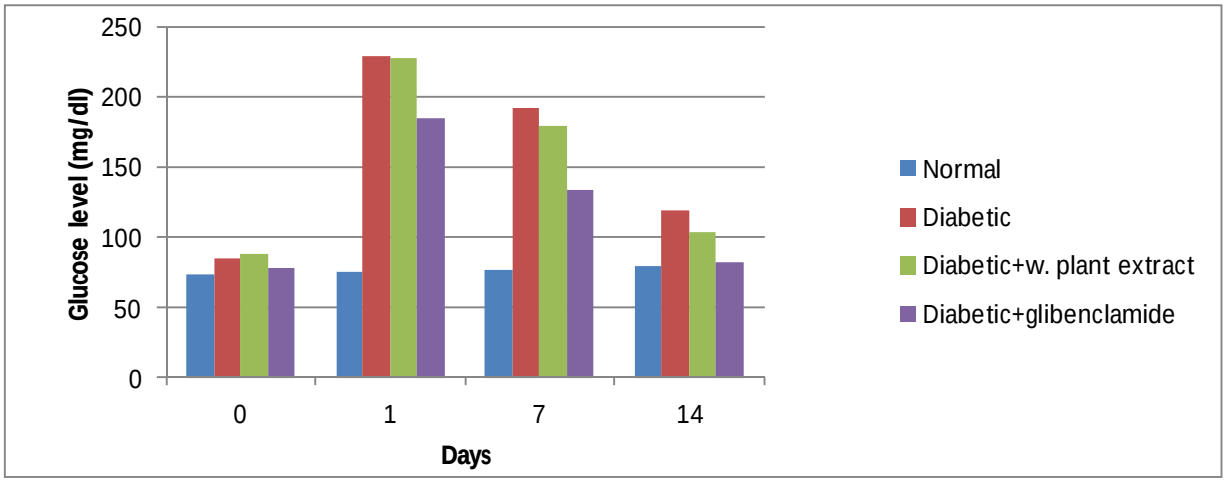


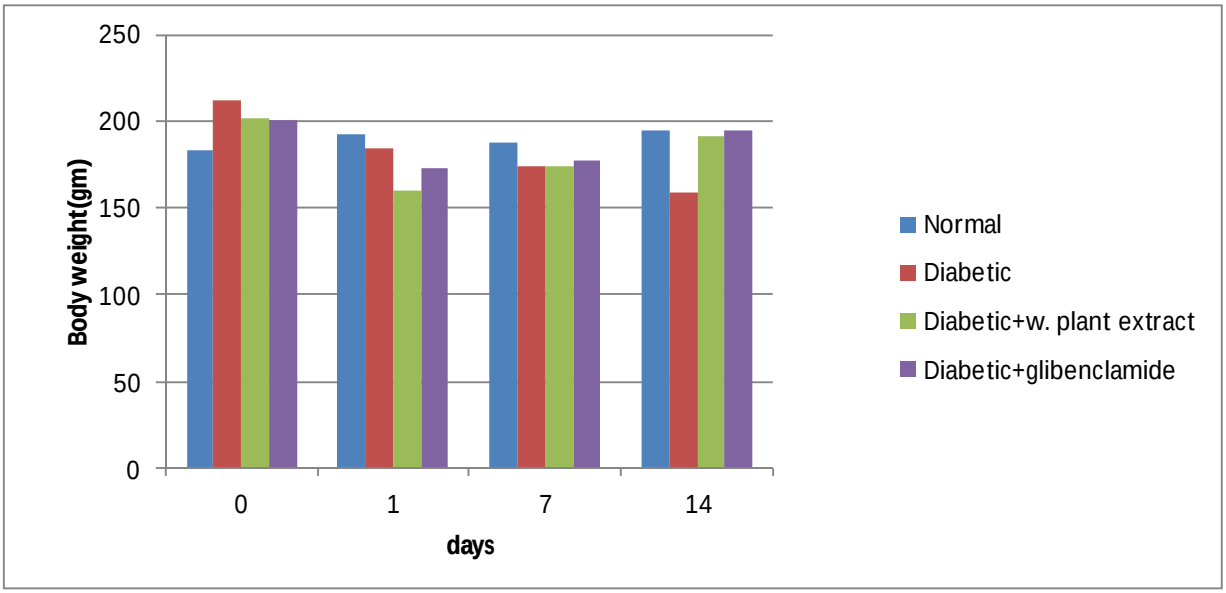
Chart 2: Chart showing glucose level profile for normal, diabetic and diabetic rats treated with standard and extract

Table 4: Body weights measured for various group of nimals on different days

S. No.	0th day	1st day	7th day	14th day
Group I	183.33±8.08	192.33±6.49	187.30±6.23	195.00±7.64
Group II	211.67±1.67	184.00±2.31	174.00±5.03	159.00±14.44
Group III	201.67±7.27	160.33±12.33	173.33±14.53	190.67±6.36
Group IV	200.00±5.77	172.67±7.33	177.30±7.51	195.00±1.20

Values are given as mean±SEM

Chart 3: Chart showing the variation in body weights on normal, diabetic and diabetes treated with standard and extract



Biochemical parameters:

It is also observed that biochemical parameters like Urea, Creatinine, Total Cholesterol and Alkaline Phosphatase levels increased considerably in diabetes induced rats but are nearer to normal for standard and extract treated animals. From Table 5 and chart 4 & 5, it is observed that Urea, Creatinine, Cholesterol and Alkaline Phosphatase values for dichloromethane: methanol (1:1) whole plant extract were found to be 43.33, 0.36, 120 and 85 mg/dl respectively which is nearer to standard and normal group values.

Table 5: Biochemical parameters values measured for all groups from the blood serum using diagnostic kits

S. No.	Urea (mg/dl)	Total cholesterol (mg/dl)	Alkaline Phosphatase (mg/dl)	Creatinine (mg/dl)
Group I	42.50±0.29	116.00±0.58	81.83±0.73	0.36±0.01
Group II	63.33±0.34	183.67±0.34	136.00±1.53	1.35±2.15
Group III	45.83±0.34	99.33±0.33	90.43±3.65	0.41±3.65
Group IV	43.33±0.60	119.67±0.88	85.33±1.76	0.37±2.31

Values are given as mean±SEM

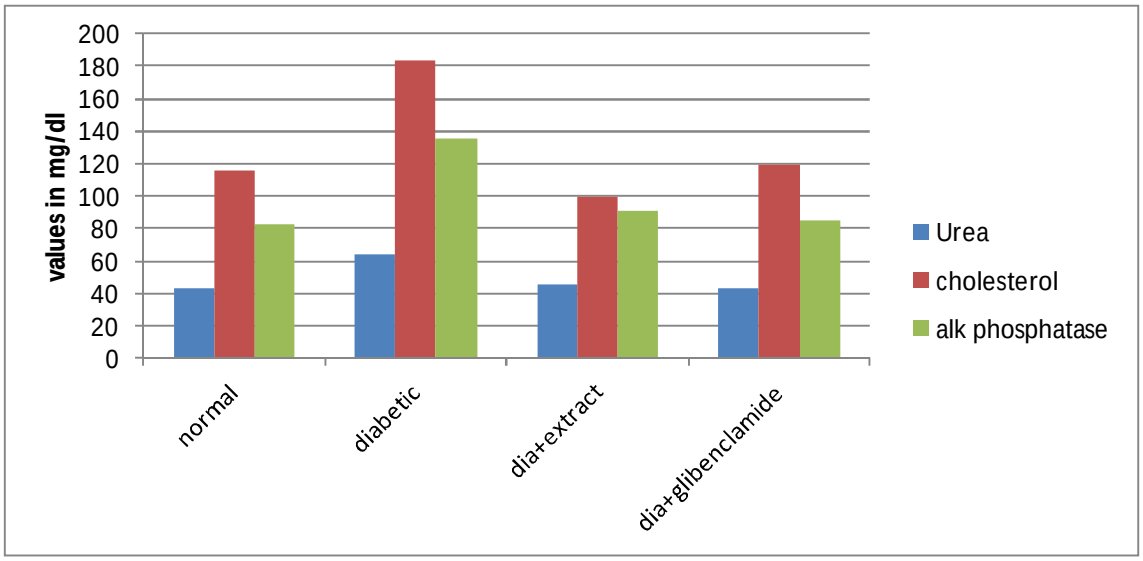


Chart 4: Biochemical parameters plotted for normal, diabetic and diabetes treated rats

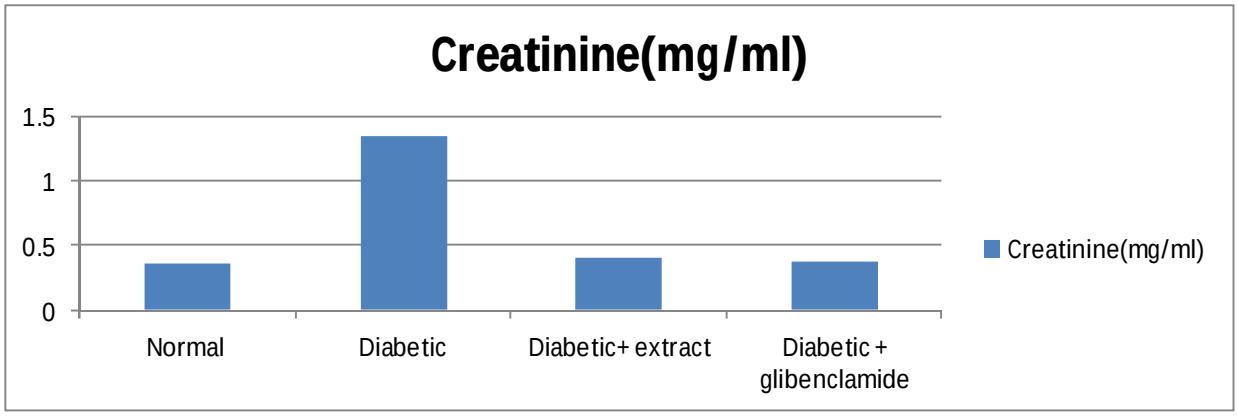


Chart 5: Creatinine levels plotted for normal, diabetic and diabetes treated rats

Histopathology:

Histopathology of pancreas showed damage of islets of langerhans in diabetic rats when compared to normal but shows healing of pancreas in diabetic rats treated with whole plant extract of *Catharanthus roseus* and standard glibenclamide drug (5mg/kg body wt.) (figure 3,4,5,6).

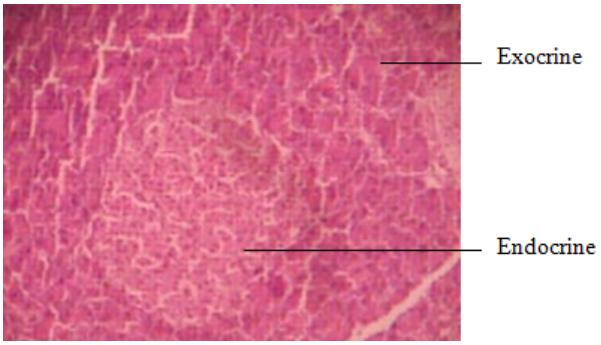


Figure 3: Photograph showing endocrine and exocrine parts in normal Pancreas

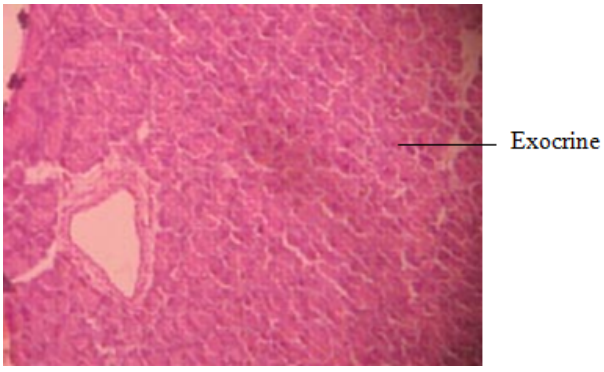


Figure 4: Photograph showing only exocrine and blood vessel in diabetic rat

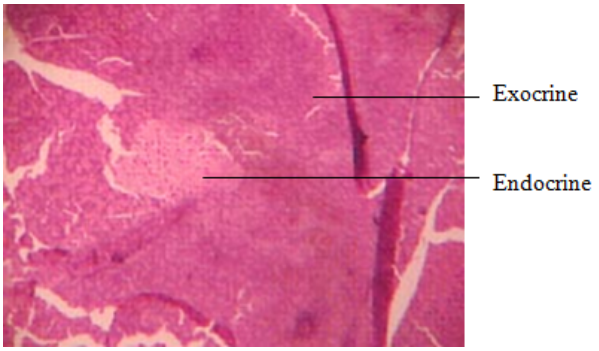


Figure 5: Photograph showing exocrine and endocrine part of Pancreas in whole plant treated rat

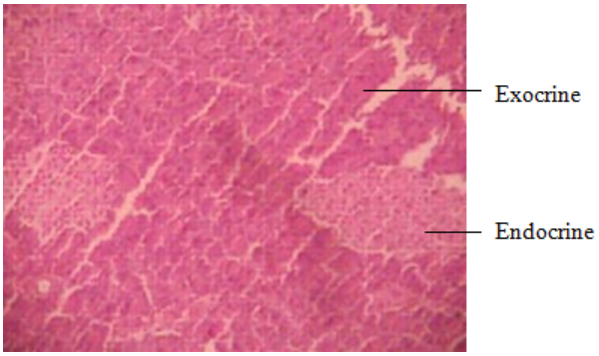


Figure 6: Photograph showing endocrine and exocrine part of Pancreas in standard treated rat

CONCLUSION:

The present study showed that, *Catharanthus roseus* whole plant dichloromethane methanol extract is found to exhibit a significant antibacterial activity against, almost all the bacterial strains and antidiabetic activity in alloxan induced diabetic rats. Therefore, further work can be carried out for the isolation and molecular

characterization of active constituents responsible for antibacterial and antidiabetic activity, can be assessed for the bioactivity of the *Catharanthus roseus* plant extract and also be explored for its activity against wide spectrum of microbes to develop it into a powerful antibiotic.

REFERENCES:

1. Hahn F E and Ciak J. Berberine Antibiotics *1976;*3: 577-588.
2. Amin A H, subbaiah T V and Abbasi KM. Berberine sulfate, Antimicrobial activity, bioassay, and mode of action. *Can J Microbiol 1969; 15: 1067-1076.*
3. Kaneda Y, *et al*. In vitro effects of berberine sulfate on the growth of Entamoeba histolytica, giardia lamblia, and Trichomonas vaginalis. *Annals Trop Med Parasitol 1991; 85:417-425.*
4. Coeugniet EG and Kuhnast R. Recurrent candidiasis.Adjuvant immunotherapy with different formulations of Echinacin. *Therapiewoche* 1986; 36: 3352-3358.
5. Barbagallo G and Chisari G. Antimicrobial activity of three Hypericum species. *Fitoterapia* 1987; 58: 175-77.
6. Cooper WC and James J. An observational study of the safety and efficacy of hypericin in HIV + subjects. *Int Conf. AIDS* 1990; 6: 369 (abstract no. 2063).
7. Klose S and Wernet AP. successful long-term treatment over 40 months of HIV patients with intravenous hypericin. *Int. Conf AIDS* 1993; 9 :470 (abstract no. PO-B26-2012).
8. Furner V and Bek Gold M. A Phase I/II unblended dose ranging study of hypericin in HIV positive subjects. *Int Conf AIDS* 1991; 7:199 (abstract no. W.B. 2071).
9. Mitscher L, Park Y and Clark D, Antimicrobial agents from higher plants. *Antimicrobial isoflavonoids from Glycyrrhiza glabra. J Nat Products* 1980;43:259-269.
10. Rang HP and Dale MM .Text Book of Pharmacology. 5th edition; 385-8*6*
11. Kokate CK and Purohit AP.Text Book of Pharmacognosy. 37th edition; 2007: 484-87.
12. Treas and Evans WC. Text book of Pharmacognosy. 2002; 15th edition: 402-403.
13. Huxley A., ed. (1992). *New RHS Dictionary of Gardening*. Macmillan ISBN 0-333-47494-5.
14. Jayanthi M, Sowbala N,*et al.* Study of Antihyperglycemic effect of *catharanthus roseus* in alloxan induced diabetic rats. *International J. of Pharmacy and Pharmaceutical Sciences*;2010:2(4)
15. Khandelwal KR. Practical Pharmacognosy. 2007: 18th edition: 149-153.
16. Ashutosh kar. Pharmaceutical Drug Analysis. 2005; 2nd edition: 409-13.
17. Gunakkunru A, Padmanaban K, Thirumal P, *et al. J. Ethnopharmacol.*2005;98: 241-244.
18. Pulok K. Mukherjee, Kakali Saha, Murugesan T, *et al. J. Ethnopharmacol.*1998;60: 85-89.
19. Mohammed Fazil Ahmed, Syed Mohammed Kazim, *et al.* Antidiabetic activity of *Vinca rosea* Extracts in Alloxan Induced Diabetic Rats. *International Journal of Endocrinology*,2010;6 pages