Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 1, 2011 ISSN - 0974-2441

Research Article

ANTIDIABETIC AND WOUND HEALING ACTIVITY OF VARIOUS BARK EXTRACTS OF *POLYALTHIA LONGIFOLIA*

APARNA LAKSHMI*[1], Y. MASTAN RAO[2], CH BHARGAVI[1] AND UMA SEELAM[3]

[1]Ratnam Institute of Pharmacy, Pidathapolur, Nellore, Andhrapradesh, India.
[2]SRM College of Pharmacy, SRM University, Kattankulathur, Kanchipuram, Tamilnadu, India.
[3]School of Pharmacy and chemistry, Kingston University, London, U.K.
E-mail: appu_007@yahoo.com

ABSTRACT

Aim: The present study was sought to investigate the antidiabetic and wound healing activity of bark extract of *Polyalthia longifolia* against alloxan induced diabetic rats.
Method: The antidiabetic activity is evaluated by estimating the blood glucose level, total protein, total cholesterol, creatinine and blood urea and nitrogen in alloxan induced diabetic rats. The wound healing activity property was studied by excision and incision methods.
Results: There is a significant decrease in the blood glucose levels from 1 week to 3 week in n-hexane extract, ethyl acetate and methanolic extract treated groups when compared to the diabetic control group. The n-hexane extract treated group has shown significant increase in the total cholesterol, creatinine and urea levels when compared to the other treated groups and is almost similar to the standard group. In contrast the methanolic extract has brought has brought the total protein level to the normal in diabetic induced rats.
Conclusion: In conclusion the present study indicated a significant antidiabetic effect of the methanolic extract of *Polyalthia longifolia* and supports its traditional usage in the control of diabetes. It was found to have strong wound healing property. Further studies are required for the detailed studies in isolation of the compounds and pharmacological investigations of the bark constituents, which possess its own traditional claim.

Key words: Antidiabetic, wound healing, polyalthia longifolia.

INTRODUCTION

Diabetes is becoming something of a pandemic and despite the recent surge in new drugs to treat and prevent the condition; its prevalence continues to soar. Perhaps the most worrying aspect of all is that the rise is even reflected in children. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major cause of morbidity and death [1].

Although several drugs targeted for carbohydrate hydrolysing enzymes (psuedosaccharides), release of insulin from pancreatic β-cells (sulphonyl ureas), glucose utilization (biguanides), insulin sensitizers, PPARg agonists (glitazones) are in clinical practise, the growing diabetes market observes a number of changes. Some of these drugs are linked to liver toxicity (troglitazone), including a number of deaths from hepatic failure and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone).

Therefore, as the long term of risk and effect on the complications of diabetes related with these drugs are not at clear. On the other hand, traditional medicinal plants with various active principles and properties have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer.

The beneficial multiple activities like manipulating carbohydrate mechanism by various mechanism, preventing and restoring integrity and function of β-cells, insulin-releasing activity , improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exiciting opportunity to develop them in to novel therapeutics. The multifactorial pathogenecity of diabetes demands multimodal therapeutic approach. Thus, future therapeutic stratagies require the combination of various types of multiple agents. Thus plant based herbal drugs and botanicals with free radical scavenging activity are emerging as the primary components of holistic approaches to diabetes management[2,3].

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin layer) is regenerated[(4)]. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialisation and wound contraction. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects[5].

Polyalthia longifolia is a large genus of shrub and trees distributed in tropics and subtropics. It belongs to the family Annonaceae, which comprises 120 genera and more than 2000 species. Polyalthia longifolia var. Pendular is a tall handsome, evergreen tree with a straight trunk and horizontal branches and is a native of Srilanka and cultivated all over Indo-Pakistan sub-continent. It is locally known as Seedha ashok.

The ethnopharmacological claims for *Polyalthia longifolia* include the use of its bark as a febrifuge. It depresses the heart, lowers blood pressure and stimulates respiration. The fungicidal effect of *Polyalthia longifolia* has been reported. It is useful in treating fever, skin diseases, diabetes, hypertension and helmenthiasis. It is also used in the treatment of wounds. The main objective of the present study was to evaluate the antidiabetic activity and effectiveness of bark extract of *Polyalthia longifolia* in wound healing.

MATERIALS AND METHODS

Plant material: The fresh plant bark of plant *Polyalthia longifolia* was collected from Tambaram, Tamilnadu, in the month of January. The bark was certified by Prof.P.Jayaraman, Director, Plant Anatomy Research centre (PARC), Medicinal Plant Research Unit, West Tambaram. Chennai.

Preparation of plant material

The powdered material was subjected to maceration by using different solvents. The plant material was filtered and further the powder was subjected to extraction with solvents like ethylacetate, methanol and n-hexane which was done for 7 days. The extract was filtered and concentrated to dryness under vacuum Tween-80 (5% v/v) was used as vehicle to suspend the extract. A crude powder was abtained and used to prepare suspensions of 300mg/kg.

Grouping of animals

Albino rats of Wistar strain (weighing 125 - 250 g) of either sex were used for the study. Rats used for the study were obtained from

King's Institute, Guindy, Chennai, India. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" (IAEC) (Proposal.No.IAEC 20/2007) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules. Animals were allowed to free access to water and standard chow diet up to the end of the experimental period and divided into following groups. Each group consists of 6 rats.

Group I: Normal group of rats received distilled water.

Group II: Diabetic control group of rats, diabetes was induced by alloxan at a dose of 120mg/kg for 2 days at intervals of 48 hrs and was administered intraperitoneally.

Group III: Standard group of rats received Glipizide at a dose of 0.5mg/kg through oral route along with alloxan.

Group IV: Test group of rats were received n-hexane bark extract of *Polyalthia longifolia* at a dose of 300mg/kg orally along with alloxan(120mg/kg, i.p).

Group V: Test group of rats were received ethylacetate bark extract of *Polyalthia longifolia* at a dose of 300mg/kg orally along with alloxan(120mg/kg, i.p).

Group VI: Test group of rats were received methanolic bark extract of *Polyalthia longifolia* at a dose of 300mg/kg orally along with alloxan(120mg/kg, i.p).

After 48 hrs of alloxan injection blood sample was withdrawn from animals fasted for over night. Serum glucose levels were estimated using commercially available GOD POD kit using auto analyser.

Biochemical parameters

At the end of experimental period of three weeks rats were fasted over night and sacrificed by cervical decapitation. Blood was collected in heparinized tubes. Plasma was separated by centrifugation, serum was obtained and used for estimation of glucose level by GOD/POD method (Mercks specialist Pvt Ltd)[6], total protein[7], cholesterol levels[8], Serum creatinine[9] and Blood Urea Nitrogen (BUN)[10]. Pancreas was dissected out and were washed in saline, fixed in Hollande-Bouin fixative for 48hrs and processed for paraffin embedding. The secretion stained in Ehlrich haemetoxylin, counter stained in eosin and mounted were observed.

Ultrastructural studies

Pancreas was dissected out after the experimental period and rinsed in physiological solution and used in 2% glutaraldehyde (primary fixative) over night and then post-fixed in 1% osmium tetroxide (secondary fixative) for 2-3 hrs. Subsequently the tissues were washed to remove excess osmium tetroxide. Sections were obtained using richer ultratome and stained in toludine blue 0 and processed for study.

Wound healing activity

This activity was performed as per the guidelines set by the Indian Science Academy, Newdelhi, India. Twelve week old healthy wistar rats (150-200g) of either sex were used for the study. All experimental procedures and protocols used in the study were reviewed by the "Institutional Animal Ethical Committee" (IAEC) (Proposal.No.IAEC 20/2007) and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules.

Study design

Animals were divided in to four groups, each group consists of 8 animals.

Group I: This group was considered as control group and were treated by topical application of vehicle.

Group II: This group received 5%w/w n-hexane bark extract of *Polyalthia longifolia* which was applied topically to the infected areas of both incision and excision wound healing. Four animals to study incision wound healing and remaining 4 for the study of excision wound design study. The extract was mixed using simple ointment base.

Group III: This group received 5%w/w ethylacetate bark extract of *Polyalthia longifolia* which was applied topically to the infected areas of both incision and excision wound study.

Group IV: This group received 5%w/w methanolic bark extract of *Polyalthia longifolia* which was applied topically to the infected areas of both incision and excision wound study.

Group V: This is standard group and received 5%w/w of Betadine ointment which was applied to the infected areas of both incision and excision wound study.

Wound models

Incision wound: On the depilated backs of the animals two paravertebral incisions of 6 cms length were made cutting through the full thickness of the skin. Interrupted sutures, 1cms apart were placed to approximate the cut edges of the skin. The sutures were removed on the 7th post wound day and the skin breaking strength was measured on the 10th day by continous water flow technique of lee[11].

Excision wound: An excision wound was infliucted by cutting away 500 mm^2 full thickness of a pre-determined area on the depilated back of the rat. Epithelialization period was noted as the number of days after wounding required for the eschar to fall off leaving no raw wound behind. Wound contraction rate was monitored by planimetric measurement of the wound area on alternate days. This was achieved by tracing the wound on a graph paper. Reduction in the wound area was expressed as percentage of the original size[12].

Statistical analysis

All data were expressed as mean±SEM. The groups were compared using one-way ANOVA followed by Dunnet`t test except wound contraction and breaking strength which were expressed by Turkey test.

RESULTS

There was a dramatic decrease in the body weight of diabetic control, standard and extract treated groups when compared to the normal control group.The blood glucose levels of H-Ext, EA-Ext and M-Ext treated groups were almost near to the normal group of rats. But methanolic bark extract of *Polyalthia longifolia* was found to be more effective than other treated group and the glucose levels were found to be almost near to standard group.

The total cholesterol, serum creatinine and BUN levels were gradually increased in diabetic control and H-Ext, EA-Ext and M-Ext treated groups these levels were decreased and are almost similar to normal group. But in contrast the total protein levels were decreased in diabetic control and in extract treated groups the levels were increased and almost similar to normal group. When compared to other extract treated groups methanolic extract treated group was found to be more effective.

DISCUSSION

Type 1 diabetes is one of the most common chronic childhood illness, affecting 18 to 20 per 1,00,000 children a year. No treatment has been shown to safely prevent type 1 diabetes in humans, although islet transplantation and new immunosuppressive regimens can cure [13].

In diabetes induced diabetic rats, increased food consumption and decreased body weight were observed. This indicated polyphagic condition and loss of weight due to excessive breakdown of tissue proteins [14]. It has been already stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.

It has been shown that *Polyalthia longifolia* bark extract markedly improved the glucose tolerance in alloxan induced diabetes in rats as compared to normal control. All the extracts at 300mg/kg dose show reduction in glucose level.

Table -1.1: Effect of methanolic, n-hexane and ethylacetate bark extract of *Polyalthia longifolia* on body weight (g) in alloxan induced diabetic rats

S. No.	Groups	Intial body weight (g)	Final bodyweight (g)
1.	Normal control	149.27 ± 1.92**	181 ± 3.59**
2.	Diabetic control	165.2 ± 2.3	115 ± 1.12
3.	Standard (Glipizide)	170 ± 2.76^{ns}	130 ± 2.30**
4.	H-Ext	155 ± 2.12*	129 ± 1.72**
5.	EA-Ext	150 ± 2.24**	125 ± 1.70*
6.	M-Ext	149 ± 1.97**	127 ± 1.65**

H-Ext: n-hexane extract 300mg/kg, EA-Ext: Ethylacetate extract 300mg/kg, M-Ext: Methanolic extract 300mg/kg, Values were expressed as Mean ± SEM; n=6. Statistical significance: (*p<0.01, **p<0.05 and NS- Not Significant) One way ANOVA followed by Dunnett test.

Table -1.2: Effect of methanolic, n-hexane and ethylacetate bark extract of *Polyalthia longifolia* on Blood Glucose level (mg/dl) in alloxan induced diabetic rats

S. No.	Groups	0week	1week	2 week	3week
1.	Normal control	126 ± 2.98**	128 ± 1.39**	127 ± 0.38**	127 ± 1.03**
2.	Diabetic control	345 ± 1.60	380.6 ± 3.01	367 ± 4.42	355 ± 4.17
3.	Standard	369.1 ± 5.98**	277 ± 2.30**	205.8 ± 5.65**	164 ± 4.04 **
4.	H-Ext	368.3 ± 5.87**	295 ± 3.01**	264.1 ± 4.16**	181.6 ± 4.94**
5.	EA- ext	366.6 ± 4.77**	290.3 ± 2.61**	265 ± 4.64**	180.6 ± 5.70**
6.	M-Ext	368.3 ± 3.33**	285.1 ± 1.74**	261.5 ± 4.16**	179.1 ± 4.74**

H-Ext: n-hexane extract 300mg/kg, EA-Ext: Ethylacetate extract 300mg/kg, M-Ext: Methanolic extract 300mg/kg,Values were expressed as Mean ± SEM; n=6. Statistical significance: (**p<0.05) One way ANOVA followed by Dunnett test.

Table -1.3: Effect of methanolic, n-hexane and ethylacetate bark extract of *Polyalthia longifolia* on total protein (g/dl), total cholesterol (mg/dl), serum creatinine (mg/dl) and BUN (mg/dl)

S. No.	Groups	Total protein (g/dl)	Total cholesterol (mg/dl)	Serum creatinine (mg/dl)	BUN (mg/dl)
1.	Normal control	7.35 ± 0.07**	62.6 ± 0.49**	0.52 ± 0.006**	47.6 ± 0.44**
2.	Diabetic control	69 ± 0.07	80.1 ± 0.62	0.78 ± 0.008	75 ± 0.46
3.	Standard	6.18 ± 0.12**	65.8 ± 0.90**	0.58 ± 0.006**	53.3 ± 0.76**
4.	H-Ext	5.35 ± 0.08*	70.2 ± 0.50**	0.68 ± 0.008**	65.1 ± 0.80**
5.	EA-Ext	5.55 ± 0.95*	66.8 ± 0.54**	0.67 ± 0.009**	60.8 ± 0.60**
6.	M-Ext	5.65 ± 0.09**	66.1 ± 0.47**	0.63 ± 0.009**	58.83 ± 0.7**

H-Ext: n-hexane extract 300mg/kg, EA-Ext: Ethylacetate extract 300mg/kg, M-Ext: Methanolic extract 300mg/kg, Values were expressed as Mean ± SEM; n=6. Statistical significance: (*p<0.01, **p<0.05) One way ANOVA followed by Dunnett test.

Table -1.4: Effect of methanolic, n-hexane and ethylacetate bark extract of *Polyalthia longifolia* on wound healing in alloxan induced rats

S. No.	Groups	Breaking strength
1.	Control	286.8 ± 8.67
2.	Standard (Betadiene)	412.03 ± 6.423*
3.	H-Ext	342.78 ± 8.75*
4.	EA-Ext	346.27 ± 7.81*
5.	M-Ext	320.42 ± 6.87*

H-Ext: n-hexane extract 300mg/kg, EA-Ext: Ethylacetate extract 300mg/kg, M-Ext: Methanolic extract 300mg/kg
Values were expressed as Mean ± SEM; n=8. Statistical significance: (*p<0.001) One way ANOVA followed by Turkey test.

Table -1.5: Effect of methanolic, n-hexane and ethylacetate bark extract of *Polyalthia longifolia* on wound contraction in alloxan induced rats

S. No. Parameter	Control	Standard	H-Ext	EA- Ext	M-Ext
a) wound contraction(%)					
1. Day 2	21.6±0.34	35.3±2.32***	24.02±0.08*	28.81±0.87***	29.73±0.74***
2. Day 4	35.27±0.87	51.21±3.58***	47.82±2.12**	48.71±2.14**	48.01±1.79**
3. Day 6	58.13±0.37	78.2±2.39***	60.03±3.32^{ns}	65.24±2.93***	65.35± 2.37***
4. Day 8	65.2±0.84	92.13±3.14***	78.27±3.45*	83.56±3.58**	82.48±3.03**
5. Day 10	85.3±0.24	98.03±1.28**	89.67±0.86**	91.23±0.56***	92.35±0.73***
b)Period of epithelialisation (in days)	14.1±0.27	9.12±0.47***	10.75±1.21***	11.6±1.77*	11.9±1.47*

H-Ext: n-hexane extract 300mg/kg, EA-Ext: Ethylacetate extract 300mg/kg, M-Ext: Methanolic extract 300mg/kg, Values were expressed as Mean ± SEM; n=6. Statistical significance: (*p<0.01, **p<0.05, ***p<0.001 and NS- Not Significant) One way ANOVA followed by Turkey test.

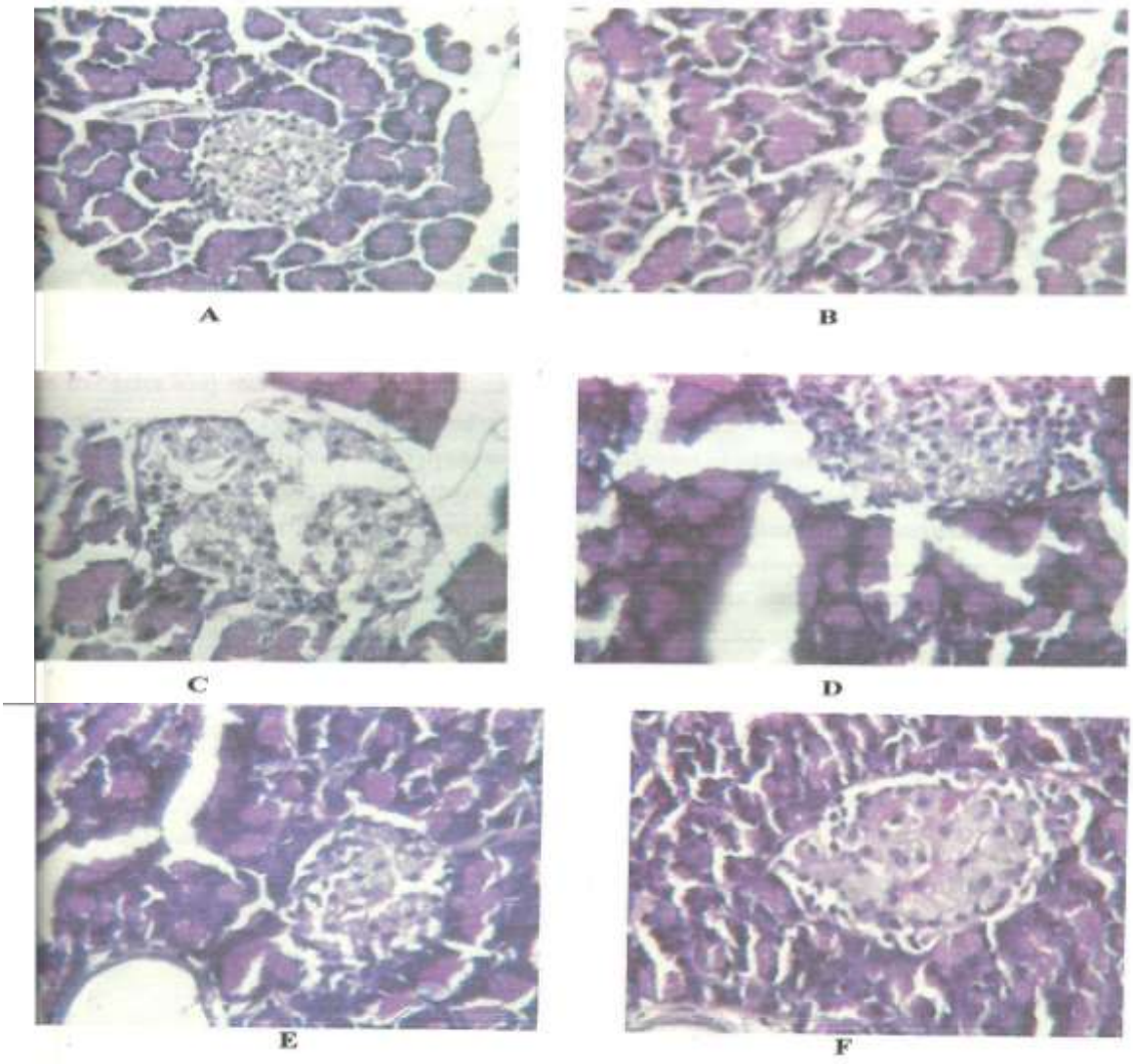

Figure -1: A) Islets of normal control rats with its acinar tissue, B)Diabetic control, C) Diabetic rats after standard therapy (Glipizide), D) Diabetic rat after treatment with n-hexane bark extract of *Polyalthia longifolia*, E) Diabetic rat after treatment with Ethyl acatate bark extract of *Polyalthia longifolia*, F) Diabetic rat after treatment with methanolic bark extract of *Polyalthia longifolia*.

DISCUSSION

Type 1 diabetes is one of the most common chronic childhood illness, affecting 18 to 20 per 1,00,000 children a year. No treatment has been shown to safely prevent type 1 diabetes in humans, although islet transplantation and new immunosuppressive regimens can cure [13]

In diabetes induced diabetic rats, increased food consumption and decreased body weight were observed. This indicated polyphagic condition and loss of weight due to excessive breakdown of tissue proteins [14]. It has been already stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins.

It has been shown that *Polyalthia longifolia* bark extract markedly improved the glucose tolerance in alloxan induced diabetes in rats as compared to normal control. All the extracts at 300mg/kg dose show reduction in glucose level.

In histopathological, study the light microscopic photograph orf islets from control rat appeared circular with the granulated beta cells appearing darker. Small, shrinken islets and destruction of beta cells were observed in diabetic condition. Well formed islets and increased cell number were observed in diabetic rats extract treated groups. The data presented in electron micrograph of the beta cell of normal and treated rats showed evidence for increased secretory granule synthesis and there by increased insulin secretion after the administration of bark extract of *Polyalthia longifolia* suggesting possible regeneration /repair of the islets of langerhans in alloxan treated rats.

We found that a 21 day administration of *Polyalthia longifolia* bark shows equal effectiveness in controlling diabetes as that of standard drug (Glipizide). Methanolic, ethylacetate and n-hexane extract of *Polyalthia longifolia* bark prooved to have hypoglycemic effect on alloxan induced diabetic rats. as a result there was a increase in insulin level, which brought a homeostasis in the above mentioned biochemical parameters such as cholesterol, urea, creatinine total protein and in the enzyme activities.

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal stage and wound contraction is a process of shrinkage of area of the wound .it depends upon the reparative abilities of the tissue, type and extent of the damage and general state of health of the tissue. The granulation tissue of the wound is generally composed of fibroblasts, collagen, edema and small new blood vessels [15].

Since *Polyalthia longifolia* enhanced wound contraction it would have either enhanced contractile property of myofibroblastsor increased the number of myofibroblasts recruited in the wound area. In exicision wound model *Polyalthia longifolia* fasten the

period of epithelization significantly by 10th day. It appears that *Polyalthia longifolia* was able to promote epithelization either by proliferation of epithelial cells or by increasing the viability of epithelial cells.

CONCLUSION

In conclusion the present studies indicated a significant anti-diabetic effect of the various extracts of bark extracts of Polyalthia longifolia and support its traditional usage in the control of diabetes. And also concluded that the bark extracts of Polyalthia longifolia has strong effect on wound healing in albino rats. Further studies is required for the detailed studies in isolation of the compounds and pharmacological investigations of the bark constitiuents, which have many pharmacological activity reported traditionally and its exact mechanism of action.

ACKNOWLEDGEMENTS

Authors are grateful to Prof. M. Gopinath, Principal, Ratnam college of pharmacy, for providing necessary facilities to carry out this work. Dr.K.S.Lakshmi, Dean, College of Pharmacy, SRM University, and Dr.K.Ilango Vice-Principal, College of Pharmacy, SRM University, Kattankulathur, for their support in carrying this work.

REFERENCES

1. Thiruvenkatasubramaniam R, Jayakar B. Anti-hyperglycemic and anti-hyperlipidemic activities of *Premna corymbosa* (Burm.F.)Rottl on Streptozotocin induced diabetic rats. Der Pharmacia Lettre 2010; 2: 505-509.
2. Aynur B, Sedef NE. Determination of In Vitro Antidiabetic Effects, Antioxidant Activities and Phenol Contents of Some Herbal Teas. Plant Foods for Human Nutrition, 2008;1: 27.
3. MC Sabu, R Kuttan. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol 2002; 2: 155.
4. Nayak BS, Lexley M Pinto Pereira. *Catharanthus roseus* flower extract has wound healing activity in Sprague dawley rats. BMC Complementary and Alternative Medicine 2006;6:41.
5. Shivananda Nayak B, Steve S, Anderson M. evaluation of the wound healing activity of ethanolic extract of *Morinda citrifolia* leaf. Evidence based complementary and alternative medicine 2009;3:351-356.
6. Fiodaliso F, Cuccovillo I, Bianchi R, Bbi A, Doni M, Salio M, De Angelis N, Ghezzi P, Latin R, Masson S. Cardiovascular Oxidative stress is reduced by an ACE inhibitor in a rat model of streptozocin-induced diabetes. Life sciences 2006; 79:121-129.
7. Gornall AG, Bardawill CJ, David MM. Determination of serum protein by means of biuret reaction. J Biol Chem 1949; 177: 751-766.
8. Ireland JT. The colorimetric estimationof total cholesterol in whole blood, serum, plasma and other biological material. J Biol Chem 1941; 35:283-293.
9. Roscoe MH. The estimation of creatinine in serum. J Clin Path 1953:6:201-207.
10. William EN, Milissa T, Kathlen DC. Mary G. Artifactually elevated BUN values on the Boehringer Mannheim-Hitachi 737 and 705 automated chemistry analysers and the development of a kinetic BUN method. J Clin Path Aut 1987; 9: 41-43.
11. Ehrlich HP, Hunt TK. The effect of cortisone and anabolic steroids on the tensile strength of healing wounds. Ann Surg 1968; 167: 117.
12. Mankani KL, Krishna U, Singh JSD. Evaluation of wound healing activity of stem bark of *Diospyros cordifolia*. Indian drugs 2004; 41: 628.
13. Devasenan D, Edwin liu, George S. Type 1 Diabetes: Recent developments. BMJ 2004; 328: 750-754.
14. Hakim ZS, Patel BK & Goyal RK . Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. Indian J Physiol Pharmacol 1997; 41: 353-360.
15. Singh JSD, Krishna U, Mankani KL. Wound healing activity of leaf extracts and deoxyelephantopin isolated from *Elephantopus scaber* linn. Indian J Pharmacol 2005; 37: 238-242.