World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Antidiabetic activity of stem bark of *Careya arborea* Roxb.

Wadkar Kiran A[1]*, Magdum Chandrakant S [2]

[1]Appasaheb Birnale College of Pharmacy, Sangli, South- Shivajinagar, Sangli 416416 (M.S.), India
[2]Rajarambapu College of Pharmacy, Kasegaon, Tal- Walwa, Dist- Sangli (M.S.), India

Received: 31-10-2014 / Revised: 09-11-2014 / Accepted: 16-11-2014

ABSTRACT

World Health Organization (WHO) Projects a 170% growth in the number of people with Diabetes in developing countries by 2025. Between 1995 and 2025 the number of the adult population affect by DM in developing countries is projected to grow by 170%, from 84 to 228 million people. By 2025, these countries will be home to 76% of all persons with diabetes, as compared with 62% in 1995. In the same period, the developed world will see a 41% increase, from 51 to 72 million people. Since ancient times, a number of herbal medicines have been used in the treatment of this disease and many studies have been carried out in the search of a suitable plant drug that would be effective in Diabetes mellitus. The associated disadvantages with insulin and oral hypoglycaemic agents have led to stimulation in the research for locating natural resources showing antidiabetic activity. There is increasing demand by patients to use the natural products with antidiabetic activity. A survey of the literature reveals that not much scientific evaluation has been conducted to check the antidiabetic potential of *Careya arborea* Roxb. So the present study was carried out to evaluate antidiabetic activity of alcoholic extract of bark of *Careya arborea* Roxb.

Key words: *Careya arborea* Roxb, Streptozotocin, diabetic rats, antidiabetic activity.

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide. Diabetes has been a clinical model for general medicine. It has been said that to know diabetes is to know medicine and health care. Although from a clinical standpoint this may be true, our increasing knowledge of the pathophysiology of the syndrome, together with the mechanisms of long- term complications, has placed diabetes research at the frontier of immunology and molecular biology [1].

Diabetes mellitus has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. Its pharmacotherapy however is over 80 years old. The word diabetes was coined by the Greek physician Aeretaeus in the first century A.D. In the 17th century, Willis observed that the urine of diabetics as wonderfully sweet as if imbued with honey or sugar. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755 [2].

Synthetic hypoglycaemic agents can produce serious side effects including haematological effects, coma and disturbances of the liver and kidney. In addition, they are not suitable for use during pregnancy [3]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been renewed interest in the plant remedies [4].

Traditional healers are using *Careya arborea* Roxb. in the treatment of hyperglycemia. A survey of the literature reveals that no scientific evaluation has been conducted to check the antidiabetic potential of *Careya arborea* Roxb. Keeping in view the medicinal importance of the genus and varied compounds reported, it is worthwhile to evaluate this drug pharmacologically for its antidiabetic property.

***Corresponding Author Address:** Wadkar K. A, Department of Pharmacognosy, Appasaheb Birnale College of pharmacy, Sangli, Maharashtra, India, E-mail: kiranwadkar@rediffmail.com*

Careya arborea (Lecythidaceae) is a medium sized deciduous tree; bark is dark grey exfoliating in thin strips, alternate leaves 15-30 by 7.5- 15 cm., broadly obovate or obovate-oblong, rounded or shortly acuminate, crenate-denticular, rather membranous, glabrous, lateral nerves 10-12 pairs; petiole 0-1.8 cm. long , stout, margined, flowers 6.3-9 cm. across, white, ill-smelling, sessile [5]. Methanolic extract of bark is potential source of natural antimicrobial and antioxidant agents [6]. Bark of *Careya arborea* showed analgesic [7] and antidiarrhoeal [8] activities. When it was tested against carbon tetrachloride induced liver damage in rats, it showed hepatoprotective and in vivo antioxidant effects [9].

MATERIALS AND METHODS

Collection and authentication of plant: The bark of the *Careya arborea*. Roxb were collected from Dajipur jungle (Radhanagari wild life sanctuary), Kolhapur, Maharashtra. The Plants were authenticated by the botanist in the botany department, Willingdon College, Sangli and also by Dr. S. S. Sathe, Padmabhushan Dr. Vasantdada Patil Mahavidyalaya, Tasgaon. The voucher specimen has been preserved in our laboratory for future reference.

Preparation of extract: Weighted quantity (500 g) of air-dried powder of the stem bark of *Careya arborea*. Roxb was extracted with ethanol using soxhlet apparatus. The extract was concentrated with rotary-vaccum (evator) evaporator.

Acute toxicity study: The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD), received draft guidelines, received from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India [10].

Evaluation of Anti-diabetic Activity: The acclimatized animals were kept fasting for 24 hrs with water ad libitum, Animals were separated according to their body weight. Diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (60 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia. After one week of the streptozotocin injection, the animals were tested for the evidence of diabetes by estimating their blood glucose level by using Glucometer (Accu-chek active, Roche Diagnostics GmbH, Germany). The blood glucose level more than 200 mg/dl of blood was the criteria.

The animals were segregated into four groups of six rats each, one group was normal control and others were diabetic control, alcoholic extract and standard glibenclamide group. To the animals, the test extract and standard drug glibenclamide were administered. The blood samples were obtained through the tail vein puncturing with Lancet. A 0.2 ml of blood was withdrawn at interval of initial (0 hr), 2, 4, 6 hrs of administration of single dose (for acute study).

Experimental Procedure: The animals were divided into four groups (n = 6).

Grouping Scheme for Antidiabetic Study

Group I	Control, rats given normal saline.
Group II	Diabetics rats.
Group III	Diabetic rats given with Alcoholic extract orally 300 mg/kg b.w.
Group IV	Diabetic rats given with std. Glibenclamide 10 mg/kg b.w.

Estimation of Glucose: The blood samples were obtained through tail vein by puncturing with Lancet. A drop of blood so obtained was placed on glucostrip, which was kept in the glucometer. The glucometer was kept on, then after 5 sec glucomonitor reading was recorded.

Chronic Study: The extracts were administered daily for a period of 21 days and blood sugar level of individual animals were observed at 1, 7, 14 and 21 days. Other biochemical parameter readings were taken in biochemical laboratory. The statistical analysis was done by one way ANOVA followed by Dunnett's test. [11,12].

Other biochemical Parameters: At the end of the treatment blood was collected by direct cardiac puncture and serum was separated by centrifugation at 2500 rpm. The rats were sacrificed by cervical dislocation and Pancreas were excised immediately and thoroughly washed with ice cold physiological saline. The serum collected was used for biochemical estimations.

Estimation of total cholesterol and HDL cholesterol (Wybenga and Pileggi Method): Total cholesterol and HDL cholesterol were estimated by using standard kit obtained from Biolab Diagnostics (I) Pvt. Ltd. Tarapur, Maharashtra.

Principle: In hot acidic medium, cholesterol oxidises ferric ions to a brown coloured complex which absorbs at 530 nm and is directly proportional to cholesterol concentration.

Procedure: The kit contents were brought to room temperature. The test tubes were labelled as B (Blank), S (Standard) and T (Test). Serum of test sample was added to test tube labelled T. These were mixed well by gently shaking the test tube and kept in boiling water bath for 90 seconds. Cooled for 5 minutes under running tap water. Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 530 nm.

Estimation of HDL cholesterol: This procedure consists of two steps:

Step 1: In a glass tube 0.2 ml serum was added with HDL reagent No. 3. After mixing, the tubes were incubated for 10 minutes at room temperature and then centrifuged. The clear supernatant obtained was taken for the HDL cholesterol estimation.

Step 2: Five ml of cholesterol reagent No. 1 was put in test tubes labelled B, S and T. 0.2 ml of HDL reagent No. 3 was added to test tube labelled B and S. Then 0.2 ml of clear supernatant obtained by step 1 was added to test tube labelled T, while cholesterol standard was added to standard tube (S). These were mixed well by gently shaking the test tube and kept in boiling water bath for 90 seconds, cooled for 5 minutes under running tap water. Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 530 nm.

Estimation of triglycerides (GPO-PAP Method): Triglycerides estimation kit consists of enzyme reagents, triglyceride standard and diluent buffer, was obtained from Biolab Diagnostics (I) Pvt.Ltd. Tarapur, Maharashtra.

Principle: Triglycerides are split into glycerol and fatty acids in the presence of lipoprotein lipase. In the presence of ATP and glycerol-kinase, glycerol is converted into glycerol-3-phosphate and ADP. Glycerol-3-phosphate oxidase dissociates glycerol-3-phosphate into dihydro-acetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and ESPAS (N-Ethyl-N-Sulfopyropyl-n-methoxyaniline) to form a red coloured quinoneimine as indicator.

Preparation of working reagent: The lyophilized material is dissolved with 1.5 ml buffer. A uniform solution takes place after 5 minutes which is ready to use.

Procedure: One ml of working reagent was added to test tubes labelled B, S and T. Blank test tube was added with 0.05 ml distilled water, while 0.05 ml of standard was added to test tube labelled S and 0.05 ml of sample (serum) was added to test tube labelled T. These were mixed well by gently shaking the test tube and incubated for 10 minutes at 37^0C. Absorbance of the standard (S) and test sample (T) were measured against the reagent blank (B) with the help of colorimeter at 500 nm within 30 minutes. Each test performed 3 times and the mean value used for the inhibitory activity of plant extracts.

α- glucosidase inhibitory activity: Normal healthy rats fasting for 20 hrs were sacrificed by cervical dislocation. The small intestine obtained was flushed several times with ice-cold NaCl, and 50 mM (pH 7.0) sodium phosphate buffer. The mucosa was scraped with glass slide on ice-cold glass surface. The obtained material was centrifuged and pellet homogenized in phosphate buffer containing 1% Triton X 100, further cold butanol was added to remove Triton and sample subjected to overnight dialysis. The enzyme thus obtained was used after proper dilution [13]. 5µmol P- Nitrophenyl-α-D- Glucopyranoside(PNPG), enzyme solution (0.1 µl), in 900µl of sodium phosphate buffer (50 mM), pH 6.8 in the final volume of 1ml. Each extract 100 µg was dissolved in 20µL of distilled water and added to the test mixture before adding the substrate. Blank sample contained whole test mixture and the extract without enzyme solution. Distilled water added to the control sample (20 µl) and in the positive control 20 µL acarbose (100 µl) was enhanced. The mixture incubated at 37^0C for 30 mins, the reaction terminated by adding 3 volumes of NH_4OH solution (0.05 M). The absorbance at 405 nm was determined by Spectrophotometer. [14].

RESULTS AND DISCUSSION

In present study the barks of *Careya arborea* Roxb. Were selected for pharmacological evaluation for possible antidiabetic activity. The authenticated barks were subjected to size reduction to get coarse

powder. The powdered bark was extracted with alcohol using soxhlet apparatus. Ethanolic extract of *Careya arborea* Roxb. showed LD_{50} at a dose of 3000 mg/kg. 1/10th of this LD_{50} was taken as effective dose (Therapeutic dose). The Antidiabetic activity was performed using wistar albino rats. The animals were divided into four groups, each group with six animals.

Acute Antidiabetic activity study: In group I control animals were given normal saline. There was no such difference in blood sugar level of normal animals at the end of 0 hrs, 2 hrs, 4 hrs and 6 hrs intervals (Table 1). There was marked increase in blood sugar level in the diabetic control animals in group II (Table 2). In group III alcoholic extract was given to diabetes animals. There was significant difference in blood glucose level at the end of 0 hrs, 2 hrs, 4 hrs and 6 hrs intervals. The results indicated that the alcoholic extract showed significant antidiabetic activity ($P<0.01$) (252.6±2.61) at the end of six hours (Table 3). The standard glibenclamide also showed significant antidiabetic activity (216±3.13) at the end of six hours (Table 4).

Chronic Antidiabetic activity study: The chronic Antidiabetic study was carried out for 21 days. The blood sugar levels were observed at 1^{st} , 7^{th} , 14^{th} and 21^{st} day. In the present study there was marked increase in blood glucose level in the diabetic control group as analysis was done on 1^{st} 7^{th} ,14^{th} and 21^{st} day of study (292.00±2.33, 291.0±2.12, 289.16±2.08, 288.8±2.25) as compared with normal group (86.66±2.31, 84.83±2.26, 81.33±2.97, 81.33±2.16) (Table5,6). The administration of alcoholic extract, exhibited better ($P<0.01$) antidiabetic activity & decreases blood glucose level on 1,7,14,21^{st} day of study (293.16 ±4.06, 225.16 ±3.10, 199.5±3.09, 171.0±2.86) (Table 7). Glibenclamide 10mg/kg significantly decreases blood glucose level of diabetic animals on 1,7,14,21^{st} day of study (295.0±2.43, 204.33±3.49, 156.33±3.45, 114.33±4.21) as compared with diabetic control (Table 8).

Other biochemical Parameters: Total cholesterol and triglyceride level were found to be significantly increased in diabetic control group as compared with normal control. Treatment with Std. Glibenclamide, alcoholic extract significantly attenuated ($P<0.01$) the elevated total cholesterol and triglyceride levels as compared with diabetic control. HDL level decreases in diabetic control group, treatment with alcoholic extract and standard significantly increases ($P<0.05$) the HDL level as compared with diabetic control (Table 9).

α- glucosidase inhibitory activity: One of the therapeutic approaches for reducing postprandial hyperglycemia in patients with diabetes mellitus is to prevent absorption of carbohydrates after food intake. Alcoholic extract showed (43.48±2.85%) α -glucosidase inhibitory activity which is comparable with reported standard Acarbose (51%).

CONCLUSION

In the present study, the barks of *Careya arborea* Roxb. family Lecythidaceae were selected for pharmacological evaluation for possible antidiabetic activity. By observing the results of acute antidiabetic study of *Careya arborea* Roxb., it can be concluded that the alcoholic extract had shown prominent antidiabetic activity at the end of 6^{th} hour. The chronic antidiabetic study for alcoholic extract had also shown more prominent antidiabetic activity at the end of 21^{st} day. The total cholesterol and triglycerides were found to be significantly increased in diabetic control group as compared with normal control. Treatment with alcoholic extract of *Careya arborea* Roxb. significantly attenuated the elevated total cholesterol and triglyceride levels as compared with diabetic controls. HDL cholesterol level decreases in diabetic control group while alcoholic extract significantly increases the HDL cholesterol level as compared with diabetic control. Alcoholic extracts of *Careya arborea* Roxb. showed α-glucosidase inhibitory activity. The present study revealed that alcoholic extract of this plant can be successfully utilized for the management of diabetes.

Acknowledgement

We gratefully acknowledge the financial support for this work from Central Council for Research in Ayurveda and Siddha, department of AYUSH, New Delhi under the scheme for Extra Mural Research in AYUSH, vide sanction No. Z-31014/1/2008/EMR/CCRAS.

Table 1: Effect of control group on blood sugar (mg/dl) of normal animals at 0hrs, 2hrs, 4hrs and 6hrs intervals.

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	212	93	94	93	94
2	187	79	79	78	79
3	179	81	80	81	82
4	195	87	88	86	87
5	176	88	90	88	89
6	185	92	91	92	91
Average ± SEM		86.66±2.31	87±2.50	86.33±2.43	87±2.29

Table 2: Effect of diabetic control on blood sugar (mg/dl) of diabetic animals at 0hrs, 2hrs, 4hrs and 6hrs intervals.

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	201	285	286	284	285
2	187	292	290	290	288
3	192	287	287	285	285
4	197	291	289	287	285
5	200	297	294	291	291
6	185	300	300	298	299
Average ± SEM		292.0 ± 2.33	291.0± 2.12	289.3 ± 2.08	288.8 ± 2.25

Table 3: Effect of alcoholic extract of *Careya arborea* Roxb on blood sugar (mg/dl) of diabetic animals at 0hrs, 2hrs, 4hrs and 6hrs intervals.

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	187	303	292	261	260
2	193	289	286	269	259
3	201	295	279	253	243
4	189	306	287	259	251
5	200	281	269	258	249
6	185	285	271	258	254
Average ± SEM		293.1 ± 4.06	280.6± 3.77	259.5± 2.15	252.6 ± 2.61

Table 4: Effect of glibenclamide on blood sugar (mg/dl) of diabetic animals at 0hrs, 2hrs, 4hrs and 6hrs intervals.

Animal Number	Animal Weight (in grams)	Blood Glucose Levels in mg/dl at the end of			
		0 hrs.	*2 hrs.*	*4 hrs.*	*6 hrs.*
1	200	304	284	251	230
2	193	287	263	242	215
3	187	294	279	245	216
4	180	291	284	247	209
5	202	295	281	241	217
6	193	299	279	239	209
Average ± SEM		295 ± 2.43	278 ± 3.2	244.1 ± 1.79	216 ± 3.13

Table 5: Effect of control group on blood sugar (mg/dl) of normal animals at 1st day, 7th day, 14th day and 21st day.

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day.*	*21st day*
1	212	93	91	89	87
2	187	79	77	73	75
3	179	81	80	74	76
4	195	87	84	78	79
5	176	88	87	89	85
6	185	92	90	85	86
Mean ± SEM		86.66±2.31	84.83±2.26	81.33±2.97	81.33±2.16

Table 6: Effect of diabetic control on blood sugar (mg/dl) of diabetic animals at 1st day, 7th day, 14th day and 21st day.

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day*	*21st day*
	201	285	286	284	285
2	187	292	290	290	288
3	192	287	287	285	285
4	197	291	289	287	285
5	200	297	294	291	291
6	185	300	300	298	299
Mean ± SEM		292.0±2.33	291.0 ±2.12	289.16±2.08	288.83±2.25

Table 7: Effect of alcoholic extract of *Careya arborea* Roxb. on blood sugar (mg/dl) of diabetic animals at 1st day, 7th day, 14th day and 21st day.

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day.*	*21st day.*
1	187	303	227	209	169
2	193	289	221	197	172
3	201	295	219	201	181
4	189	306	237	190	160
5	200	281	217	193	175
6	185	285	230	207	169
Mean± SEM		293.16±4.06	225.16±3.10	199.5±3.09	171.0 ±2.86

Table 8: Effect of Glibenclamide on blood sugar (mg/dl) of diabetic animals at 1st day, 7th day, 14th day and 21st day.

Animal Number	Animal Weight (in grams)	Blood sugar levels in mg/dl on			
		1st day.	*7th day.*	*14th day.*	*21st day.*
1	200	304	207	149	123
2	193	287	197	152	107
3	187	294	210	162	97
4	180	291	209	171	123
5	202	295	191	151	119
6	193	299	213	153	117
Mean± SEM		295±2.43	204.33±3.49	156.33±3.4	114.33±4.2

Table 9: Effect of *Careya arborea* Roxb. bark extracts on other biochemical parameters.

Group	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)
Normal control	90.66±2.90	43.33±1.64	123.33±2.02
Diabetes control	173.66±7.45	33.00±1.22	194.66±3.93
Std. Glibenclamide	102.66±2.60	42.66±1.52	135.33±3.28
Alcoholic extract	107.33±4.70	41.33±1.69	132.00±3.60

REFERENCE

1. Debra-Haire-Joshu. Management of diabetes mellitus. 2nd Edn. Perspective of care across the life span, 1991**:** 3.
2. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotheraputics; 16th Edn . Mumbai: Popular prakashan,. 1999**:** 874.
3. Pari L et al. Antihyperglycaemic effect of Diamed, herbal formulation, in experimental diabetes in rats. J. Pharm and Pharmacol 2001; 53:1139-43.
4. Davidson MB. Diabetes mellitus: Diagnosis and treatment. 2nd Edn. New York, A wiley medical publication, John wiley & sons, 1986: 1.
5. Kirtikar KR, Basu BD, Indian medicinal plants, Vol. II, 2nd Edn. Periodical exports book agency, Delhi., **1991**: 1061-2.
6. Kumar RS et al, Antimicrobial and Antioxidant activities of Careya arborea Roxb. stem bark , Indian journal of pharmacology and therapeutics 2006; 5: 35-41.
7. Ahmed M, et al., Analgesic principles from the bark, Pharmazie 2002; 57: 698-701.
8. Rahman MT, et al, Antidiarrhoeal activity of the bark extract of Careya arborea Roxb. Fitoterapia 2003;74: 116-118.
9. Kumar RS et al., Hepatoprotective and in vivo Antioxidant effects of Careya arborea againt Carbon tetrachloride Induced Liver Damage in Rats, International Journal of Molecular Medicine and Advance Sciences **2005** ;1: 418-424.
10. OECD/OCDE, October 2000, OECD Guidelines for the Testing of Chemicals. Revised draft guidelines 423; Acute oral toxicity – Acute toxic class method, Revised document.
11. Kulkarni SK, Handbook of Experimental Pharmacology. 2nd Edn. Vallabh prakashan; 1993: 78-81.
12. Remington, The Science and Practice of Pharmacy. Pennsylvania, Mack publishing company Eastern; vol.1. 19th Edn. 1995: 112.
13. Lee L et al. Isolation of a detergent solubilized maltase / glucoamylase from rat intestine and its comparison with a maltase /glucoamylase solublized by papain. Biochem. J. 1980; 187: 437-446.
14. Ahmad G, The Inhibitory effect of some Iranian plant extracts on the Alpha- glucosidase. Iranian Journal of Basic Medical Sciences 2008; 11(1): 1-9.