Pharmacodynamic study on acute hypotensive activities of *Carissa carandas* extract in normal rats

Sumbul Shamim[1*] **and Syed Iqbal Ahmad**[2]
[1]Faculty of Pharmacy, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[2]Dr. Hafiz Muhammad Ilyas Institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi, Pakistan

Abstract: This study aims to evaluate the effect of *Carissa carandas* extract on cardiovascular function of normal rats. Intravenous bolus injection of this extract in the doses of 5 mg kg^{-1}-45 mg kg^{-1}, produced dose dependent reduction in arterial blood pressure ($p<0.001$). The 45mg/kg dose caused a 50.75% ± 2.71 decrease in MABP which was highly significant with P value< 0.0005 when compared with its controls. Significant reduction in heart rate frequency was observed after CC injection at a dose of 45 mg kg-1 ($p<0.001$). The results were comparable with Acetylcholine 10^{-4} M. The receptor activity performed for which Atropine 10^{-4}M was administered I.V. and then the extract (45mg/kg) was administered. A highly Non Significant fall in Mean Arterial Blood pressure was observed 1.51% ± 0.22 ($P>0.05$).It was concluded that the *Carissa carandas* Ethanol extract possess potent acute hypotensive effect in normal rats. It stimulates the muscarinic receptors located on the endothelial cells of the vasculature. This stimulation results in the release of endothelial-derived relaxing factors (EDRFs) or nitric oxide that diffuses to vasculature smooth muscles and causes their relaxation.

Keywords: *Carissa carandas* extract, mean arterial pressure, hypotension, atropine, adrenaline, acetylcholine.

INTODUCTION

Cardiovascular disease is a major problem worldwide. The World Health Organization estimates that this disease is responsible for the deaths of approximately 30,000 people each day (Middlemiss and Watson 1994). The search for compounds that will prevent or retard progression of the disease and beneficially effect the impairment of patients with cardiac failure continues to attract much interest. There are a number of ways in which the heart can malfunction and, in many cases drugs, which alleviate these conditions are available (Reuben and Wittcoff 1989). The pharmacopoeias of many countries of the world include even today a large number of drugs of plant origin. While it is true that purely synthetic compounds are being employed in increasing measure, in clinical practice, interest in the examinations of plants as potential source of new drug has never waned (Behl and Arora, 1993).

Carissa carandas belongs to family apocynaceae which consists of 300 genera and 1000 species. It is a large shrub with simple thorn and commonly cultivated throughout Pakistan for hedges and is called "Kakronda". The different parts of this plant have been used for various systems of medicine. Cardiotonic activity was found in root of this plant. This plant has been mentioned in the old chemical literature as purgative, antihelmintics and antidote for snake-bite. The physical characteristics of oil from the fruits of Carissa carandas were determined by using standard methods (Morton 1987).

**Corresponding author:* e-mail: shamim.drsumbul@gmail.com

The literature search has shown that there was no scientific data regarding the cardiovascular effect of Leaves extract of *Carissa carandas* (Auct.). This study is a contribution to the cardiovascular activity of the plants use.

MATERIAL AND METHODS

Plant material

Carissa carandas (Auct.) was collected from the Herb Garden of Hamdard University and Identified from Department of Botany, University of Karachi, where a voucher specimen GH No. 97998 has been deposited.

Preparation of extract

Air dried leaves of *Carissa carandas* were crushed into coarse size in a mortor and pastle and soaked in a mixture of Ethanol and water (1:1) for six days then filtered. Solvents were evaporated using a Rotary Evaporator (R-114- Buchi) and freeze dried by the help of Lyophilizer (Tokyo, Rikakikai Co. Ltd.) (Fatima *et al.*,1990). This *Carissa carandas* leaves extract (CCLE) was used for the experiments related to cardiovascular studies.

Experimental Animals

Sprague-Dawley rats, of either sex weighing 220-250g, were purchased from animal house of *HEJ Research Institute of Chemistry, International Centre for Chemical and Biological Sciences (ICCBS), University of Karachi* and were given standard diet and water *ad-libitum* (Gilani 1991). They were housed in a controlled room with a 12 h light-dark cycles, at room temperature of 22±02°C at Dr. HMI Institute of Pharmacology and Herbal Sciences,

Hamdard University, Karachi, Pakistan. The Institutional Animals Ethics Committee approved all the protocols of animals maintenance and handling which were in accordance to internationally accepted standard guidelines for use of laboratory animals (Saleem *et al.,* 2005).

Surgery

Normotensive rats of either sex (180g-200g) were anaesthetized with intraperitoneal injection of Sodium pentothal (50mg/kg) (WHO Research guidelines 1993), (Lahlou, S *et al.,* 2002). An anaesthetized rat was fixed on a supine position on a dissecting table. Spontaneous respiration was maintained by inserting tracheal cannula after tracheotomy. The temperature of the animal was maintained at 37°C by the use of an overhead lamp (fig. 1).

A longitudinal midtracheal incision approximately 2 cm long was made in order to expose the trachea, the right jugular vein and left carotid artery.

Experimental procedure

The trachea was cannulated with polyethylene tube 92.75 mm diameter to maintain a free airway. The left jugular vein was cannulated with polyethylene tube for intravenous administration of drug and flushing of 0.9% NaCl (normal saline) (Lahlou *et al.,* 2002).

For the administration of drugs and isotonic saline solution, the right jugular vein was cannulated with a saline filled polyethylene tube (1 mm diameter). The exposed surface with the cannula was covered with cotton moist in warm saline.

The cannulation of the carotid artery was performed in the same manner as the cannulation of jugular vein and the polyethylene tube (1 mm diameter) filled with heparin sodium in saline solution was used (Aftab 1995) (fig. 2).

The arterial blood pressure was measured from the left carotid arterial cannula connected to a Research grade blood pressure transducer (Harvard, 60-3003) which was connected with Oscillograph (Harvard) for recording. The same apparatus was used for receptor activity (Ferreira *et al.,* 2007) (fig. 3). The animal was allowed to equilibrate for at least 30 min. before commencing the experiment. Blood pressure was monitored until steady baseline levels were obtained. Drugs were then administered by I.V. injections and flushed in with 0.2 ml saline (Zeggwagh *et al*., 2007).

Control response to intravenous injections of Acetylcholine, Atropine and Adrenaline were taken before the administration of any test material. 6 Rats were used for each dose testing.

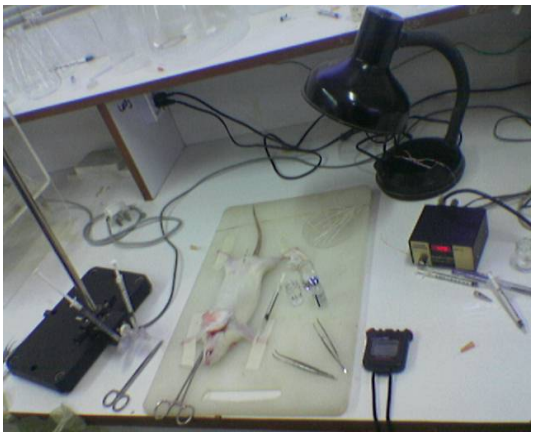

Fig. 1: A cannulated rat for Hypotensive activity.

Fig. 2: The blood pressure transducer connected with the left carotid arterial cannula and recorder

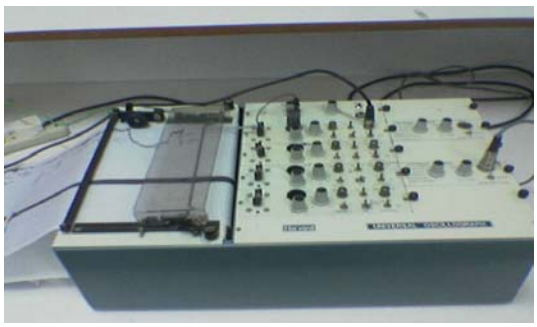

Fig. 3: A four channel recorder Oscillograph assembly, showing the tracing for Blood pressure monitoring.

Calibration

The Oscillograph was adjusted by keeping its channel amplifier's knob on DC, and +ve polarity. The pen position on chart paper was adjusted using zero knobs slightly above the lowest pen position. The pressure transducer was calibrated by using "Sphygmomanometer" to standerdize1 mm pen deflection on chart paper for its equivalency with pressure (mmHg) (Ahmed *et al.,* 2000)

Calculations

The mean arterial Blood pressure was calculated using the following formula (Ibarrola *et al.,* 1999):

MABP = DP + 1/3 (SP-DP)
Where, DP= diastolic pressure
SP= systolic pressure

The response of mean arterial blood pressure (MABP) was expressed as percent change from the control measurement taken immediately before injection (Adeboye *et al.,* 1999).

Preparation of Dilutions of Drugs

Pharmacodynamic screening was carried out by using Acetylcholine (10^{-6} M i.v.), Atropine (10^{-4} M i.v.), Adrenaline (10^{-5} M i.v.), All dilutions were freshly prepared in 0.9% saline (Gilani *et al.,* 1994).

Carissa carandas (CCLE) extract in doses 5mg/kg–45mg/kg (i.v.) were freshly prepared by dissolving in warm distilled water before each experiment.

STATISTICAL ANALYSIS

Data obtained during tests were statistically analyzed using Student's *t*-test, one-way analysis of variance (ANOVA) and two-way ANOVA. All differences are considered significant at 5% level, therefore *P*-values less than 0.05 (P<0.05) were considered statistically significant. Our results are expressed as mean ± S.E.M. (Walpole 1982; Hanif *et al*., 2004).

RESULTS

Effect on mean arterial blood pressure (MABP) of normal rats

1. The *Carissa carandas* leaves extract (CCLE) caused a decrease in Mean Arterial Blood Pressure (MABP) of rats (WHO Research guidelines 1993), in a dose dependent manner in anaesthetized rats, when compared with their controls (fig 4).
2. At the doses of 5mg/kg, 10mg/kg and 15mg/kg, this extract caused a statistically non-significant decrease in mean arterial Blood Pressure i.e., 5.77% ± 1.52, 13.89% ± 7.17, 15.15% ± 2.2 respectively with P value >0.05 when compared with their controls (figs. 4.1-4.3).
3. 20mg/kg dose showed reduction in blood pressure i.e., 41.05% ± 8.94 that was statistically significant (P<0.05) when compared with its controls (fig. 4.4).
4. 25mg/kg and 30mg/kg doses also showed reduction in blood pressure i.e., 42.36% ± 9.35, 43.76% ± 2.35 respectively that were statistically significant (P<0.025) when compared with their controls (figs. 4.5-4.6).

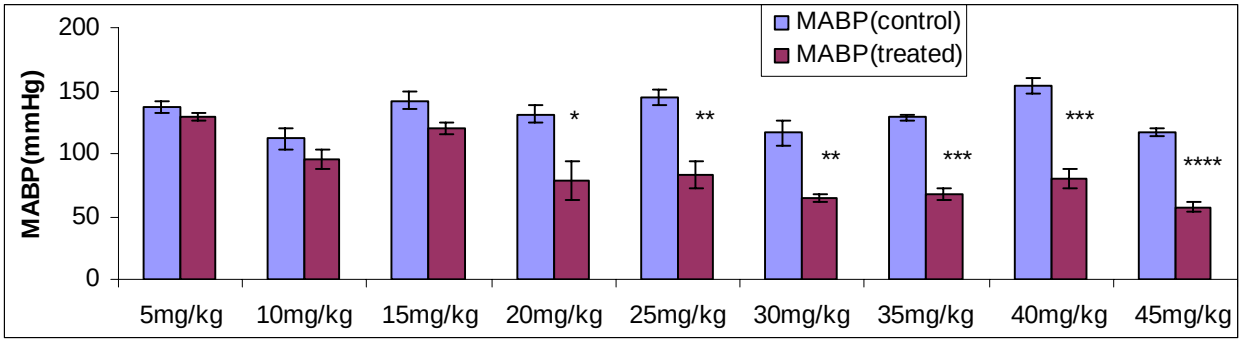

Fig. 4: Effects of C.C.L.E. on MABP of rats in different doses.
n= 6, mean± S.E.M., * = P<0.05, ** = P<0.025, *** = P<0.005, **** = P<0.0005

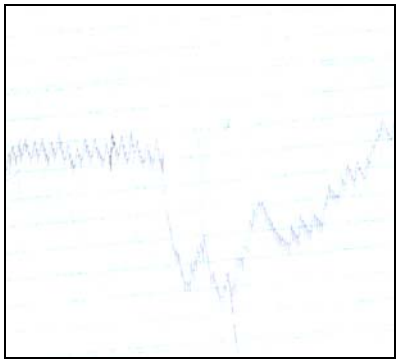

Fig. 4.1: Tracing showing the effect of CCLE (5mg/kg) on MABP of rats.

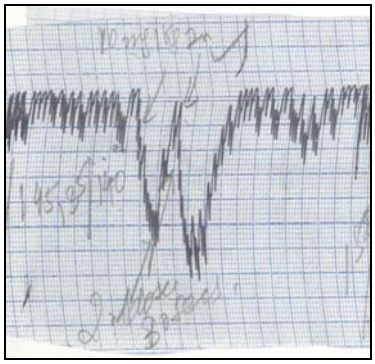

Fig. 4.2: Tracing showing the effect of CCLE (10mg/kg) on MABP of rats.

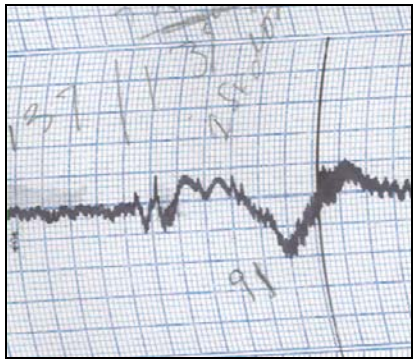

Fig. 4.3: Tracing showing the effect of CCLE 15mg/kg) on MABP of rats.

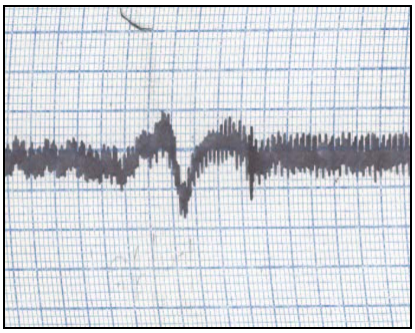

Fig. 4.4: Tracing showing the effect of CCLE (20mg/kg) on MABP of rats.

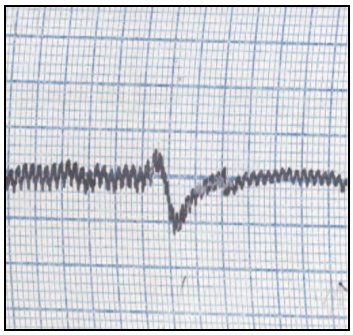

Fig. 4.5: Tracing showing the effect of CCLE (25mg/kg) on MABP of rats.

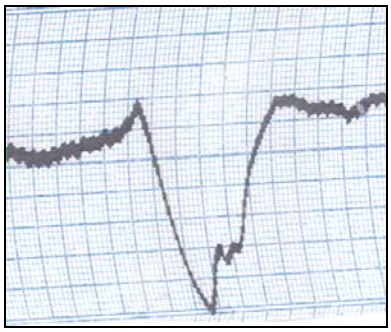

Fig. 4.6: Tracing showing the effect of CCLE (30mg/kg) on MABP of rats.

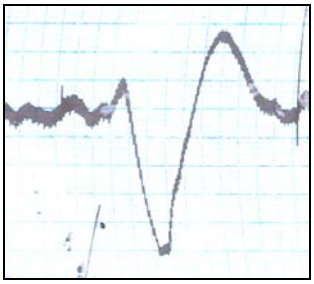

Fig. 4.7: Tracing showing the effect of CCLE (35mg/kg) on MABP of rats.

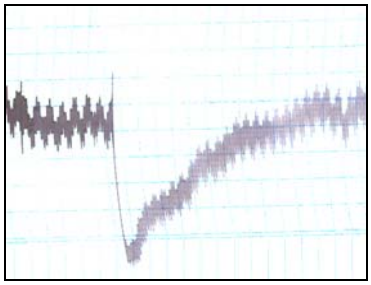

Fig. 4.8: Tracing showing the effect of CCLE (40mg/kg) on MABP of rats.

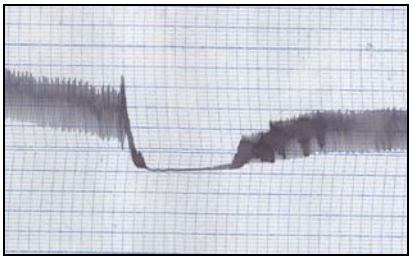

Fig. 4.9: Tracing showing the effect of CCLE (45mg/kg) on MABP of rats.

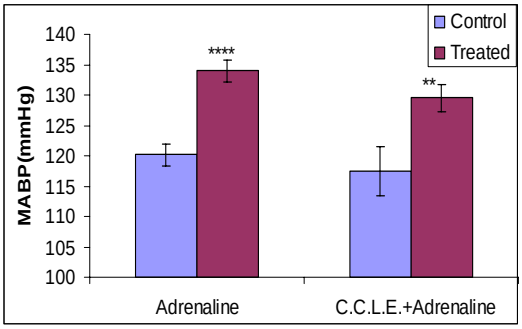

Fig. 4.10: Receptor Activity of CCLE (45mg/kg) for hypotensive effect.
n=6, Mean ± S.E.M. **=P<0.025 ****=P<0.005

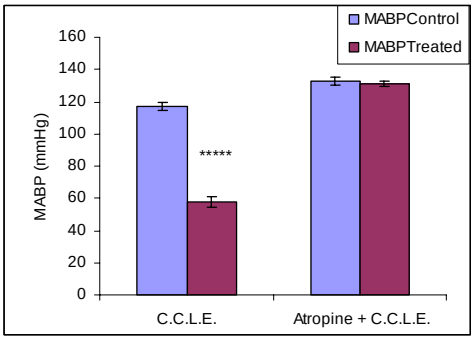

Fig. 4.11: Receptor Activity of CCLE (45mg/kg) with Atropine for hypotensive effect.
**** =P<0.0005

5. While at the dose of 35mg/kg and 40mg/kg reduction in MABP was 47.78%±2.6, 48.05%±3.27 respectively. These reductions were found to be statistically highly significant (p<0.005) when compared with their controls (figs. 4.7-4.8).
6. 45 mg/kg dose caused a 50.75% ± 2.71 decreases in MABP which was highly significant with P < 0.0005 when compared with its controls (fig. 4.9).

Receptor activity for hypotensive effect

To determine that by acting on which receptors the drug is lowering the Blood Pressure; the Receptor activity was performed with the help of Adrenaline 10^{-5} M i.v. A significant increase in Blood pressure was observed i.e., 11.69%±3.05 P<0.005) by Adrenaline. Again, adrenaline was administered I.V. after 30 seconds of administration of 45mg/kg of C.C Leaves Extract, but no Significant change in the action of Adrenaline was observed 10.32%±2.30 (P<0.025) (fig. 4.10). Adrenaline showed its effect in the C.C.L.E pretreated rats. This ruled out the action of drug as adrenoceptor antagonist for lowering the blood Pressure (Aftab, 1995)

For further Evaluation of Receptor Activity for the fall in blood pressure, the experiment was also performed with the help of Atropine Sulfate. Atropine 10^{-4}M was administered IV and then the extract (45 mg/kg) was administered. A highly Non Significant fall in Mean Arterial Blood pressure was observed 1.51%±0.22 (P>0.05) which was very less as was observed by the Extract alone i.e., 50.75% ± 2.71 (P < 0.0005) (fig. 4.11).

Comparative study with acetylcholine

The effect of *Carissa carandas* extract (45mg/kg) on MABP was compared with that of Acetylcholine (10^{-6}M). The reduction in MABP by CCLE leaves extract was 50.75% ± 2.71 (fig. 4.9) which was comparable with Ach i.e., 49.18%±4.02 (fig 4.12). Both drugs showed a highly significant decrease with P value <0.0005 when compared with their controls (fig. 4.13).

Comparative study with acetylcholine

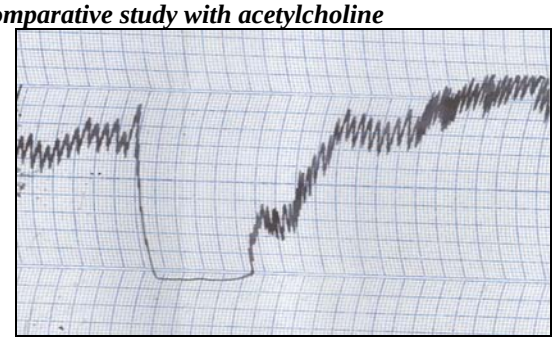

Fig. 4.12: Effect of Ach (10^{-6}M) on MABP of rats.

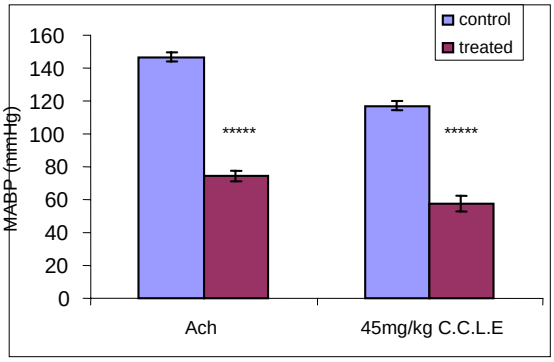

Fig. 4.13: Comparative study between the effect of C.C.L.E. (45mg/kg) and Ach 10^{-6}M
n= 12 mean± S.E.M. ****=P<0.0005

DISCUSSION

In the present study different doses of the Aqueous: Ethanol extract (50:50) of *Carissa carandas* leaves were tested and were found to have a dose dependent hypotensive effect on mean arterial blood pressure of Normotensive rats. Ach was used as positive controls or reference drug (Ahmed *et al.,* 2000) (Lahlou *et al.,* 2002). Different doses of the extract i.e., 5mg/kg, 10, 15, 20, 25, 30, 35, 40, and 45mg/kg were given I.V to obtain a dose response curve and the 45mg/kg dose caused a 50.75% ± 2.71 decrease in MABP which was highly significant with P value< 0.0005 when compared with its controls.

The literature survey of the plant has confirmed the hypotensive effect of *Carissa carandas* as the activity performed by (Zaki *et al*., 1983).

Physiologically, the reduction in blood pressure has been reported to be either due to their direct or indirect effect on the pumping ability of heart (Aftab 1995), (Kimura *et al.,* 1986) or associated with decrease in peripheral resistance (vascular muscle tone) leading to vasodilatation (Ebeigbe and Ezimokhai 1988). On these bases it is difficult to pin point the exact mechanism of extract. For this purpose the receptor activity is performed.

Receptor activity for hypotensive effect

It is reported that Ach causes a generalized vasodilatation that is an indirect effect mediated by the release of nitric oxide from vascular endothelial cells (Katzung 1998). That is why it is hypothesized that this extract might be acting like Ach and to confirm this opinion the receptor activity was performed.

But, firstly to rule out the action of drug as adrenoceptor antagonist for lowering the blood pressure. After 30 seconds of I.V. administration of 45mg/kg of CC leaves extract, adrenaline was administered IV, but no Significant change in the action of Adrenaline was observed 10.32% ± 2.30 (P<0.025). This ruled out the action of drug as adrenoceptor antagonist for lowering the blood pressure. Excluding the action as adrenoceptor blocker, the receptor activity was performed by using cholinergic agonist Ach and cholinergic competitive antagonist Atropine. The results demonstrate that Ach and different doses of CCLE when administered alone have significantly reduced the MABP. On the other hand use of Ach and different doses of CCLE on Atropine pre-treated rats did not show such decline in blood pressure as was shown earlier without Atropine Pre-treatment. These results clearly indicate that the effect of *Carissa carandas* leaves extract is mediated through same receptors and mechanism as established for the action of Ach. So, it is suggested that the Extract of *Carissa carandas* stimulates the muscarinic receptors located on the endothelial cells of the vasculature. This stimulation results in the release of endothelial-derived relaxing factors (EDRFs) or nitric oxide that diffuses to vasculature smooth muscles and causes their relaxation (Katzung 1998).

Comparative study with acetylcholine

The reduction in MABP by CCLE leaves extract was comparable with Ach i.e., both showed a highly significant decrease with P<0.0005 when compared with their controls. This experiment suggests that the drug is acting through the same mechanism and efficacy as that of acetylcholine, responsible for the hypotensive property of the extract when administered intravenously.

The Heart rate reduction caused by CCLE leaves was calculated as 25.99% ± 0.25 (P<0.0005).

It may therefore be concluded that M_2 receptors on cardiac muscles as well as vascular dilation by endothelium derived relaxing factor may contribute to the cholinergic hypotensive effect of theses extracts. This hypotensive efficacy of CCLE is comparable to that of Ach.

The documented literature for the activity and chemical constituents of *Carissa carandas,* also supports the above mentioned activity of *Carissa carandas.*

Rastogi isolated three glycosides from the plant by column chromatography over silica gel using 60:20 CHCl3- EtOAc as the eluant and by preparative thin layer chromatography and identified by comparison with authentic samples. Sugars were identified by paper chromatography. He reported that the cardiotonic activity of glycosidic fraction was due to the presence of glucoside of digitoxigenin (Rastogi 1967).

Further tested the action of aqueous extract of root of *Carissa carndas* on blood pressure, toad heart, isolated rabbit intestine, motility of Ascaris worms in dogs, and on isolated rat uterus (Zaki *et al*., 1983).

Dhawan investigated some new cardio-active glycosides as cardinolide from the plant and compared it with other commonly used cardiac glycosides for the safety margin (Dhawan 1985).

REFERENCES

Adeboye JO, Fajonyomi MO Makinde JM and Taiwo OB (1999). A preliminary study on the Hypotensive activity of *Persea americana* leaf extracts in anaesthetized normotensive rats. *Fitoterapia,* **70**:15-20.

Aftab K (1995). Pharmacological screening of natural products for their antihypertensive action. *Thesis,* Department of Pharmacology, Faculty of Pharmacy. University of Karachi, Karachi, Pakistan.

Ahmed M, Azeem MA, Navaidul-Zafar and Ahmad SI (2000). Hypotensive activity of Dilsukh (herbal drug). *Pak. J. Pharmacol*., **17**(1): 7-14.

Behl PN Arora RB and Srivastava G (1993). Herbs useful in dermatological therapy. CBS Publishers and Distributors, Dehli, India, pp.6-9, 20-24.

Dhawan BN and Patnaik GK (1985). Investigation on some new cardioactive glycosides. *Indian Drugs,* **2**(6): 285-290.

Ebeigbe A B and Ezimokhai M (1988). Vascular smooth muscle responses in pregnancy induced hypertension. *Trends Pharmacol. Sci.,* **9**: 455-457.

Fatima N and Navaidul Zafar (1993). Pharmacological and toxicological studies of Icterene: A herbal formulation of *Tamarix pakistanica*, prepared by Hamdard Laboratories (Waqf.) Pakistan in rodents. *Proc. Pakistan Congr. Zool.,* **13**: 185-194.

Ferreira HC, Serra CP and Lemos SV (2007). Nitric oxide-dependent vasodilatation by ethanolic extract of Hancornia speciosa via Phosphatidyl-inositol 3-kinase. *J. Ethnopharmacol* **109**: 161-164.

Gilani AH, Shaheen F and Saeed SA (1994). Cardiovascular actions of *Daucus carota. Arch. Pharm. Res.,* **17**: 150-153.

Gilani AH (1991). Antihypertensive activity of himbacine in anaesthetized cats. *Drug Dev. Res.,* **24**: 127-133.

Hanif M, Ahmad M and Ahmad AM (2004). Biostatistics for Health Students. Islamic Society of Statistical Sciences, Lahore, Pakistan, pp.145-177.

Ibarrola DA, -Ibarrola MH, Vera C, Montalbetti Y and Ferro EA (1996). Hypotensive effect of crude root extract of *Solanum sisymbriifolium* (Solanaceae) in normo- and hypertensive rats. *J. Ethnopharmacol.,* **54**: 7-12.

Katzung BG (1998). Antihypertensive drugs. *In:* Basic and Clinical Pharmacology. 7th Edn.. Appleton and Lange Stamford, Connecticut, pp.154-155.

Kimura Y, Fukui H, Tanaka M, Okamoto M, Morino A, Miura A, Kimura K and Enomoto H (1986). Anti-hypertensive effect of a new dohydropyridine calcium antagonist. *Arzneimittelforsch. Drug Res.,* **36**: 1329-1335.

Lahlou S, Galindo CAB, Leal-Cadoso JH, Fonteles MC, and Durate GP (2002). Cardiovascular effects of the essential oil of *Alpinia zerumbet* leaves and its main constituents, Terpinen-4-ol, in rats: Role of the autonomic nervous system. *Planta Medica*, **68**: 1097-1102.

Middlemoss D and Watson SP (1994). A medicinal chemistry case study: An account of an angiotensin II antagonist drug discovery programme. *Tetrahedron,* **50**: 13049-13080.

Morton J (1987). Karanda. *In*: Fruits of warm climates. Julia F. Morton, Miami, FL. pp.422-424.

Rastogi RC, Vohra MM, Rastogi RP and Dhar ML (1966). *Carissa carandas*. I. Isolation of the cardiac-active principles. *Indian J. Chem.,* **4**(3): 132-138.

Research guidelines for evaluating the safety and efficacy of herbal medicines (1993). World Health Organization, Regional Office for the Pacific Manila, pp.30-40.

Reuben BG and Wittcoff HA (1989). Cardiovascular Drugs, *In*: Pharmaceutical Chemicals in Perspective, Ch. 7, John Wiley and Sons, New York, pp.174-223.

Saleem R, Ahmed M, Azmat A, Ahmed SI, Faizi Z, Abidi L and Faizi S (2005). Hypotensive activity, toxicology and histopathology of Opuntioside-I and methanolic extract of *Opuntia dillenii. Biol. Pharm. Bull.,* **28**(10): 1844-1851.

Walpole RE (1982). Introduction to Statistics, 3rd ed. MacMillan Publishing Co., Inc. New York, pp.209-236, 386-424.

Zaki RC, Vohra MM, Rastogi RP and Dhar ML (1966). *Carissa carandas*. I. Isolation of the cardiac-active principles. *Indian J Chem.,* **4**(3): 132-138.

Zeggwagh NA, Michel JB and Eddouks M (2007). Acute hypotensive and diuretic activities of *Chamaemalum nobile a*queous extract on normal rats. *Am. J. Pharmacol. Toxicol.,* **2**(3): 140-145.