ISSN: 2186-8476, ISSN: 2186-8468 Print
Vol. 1. No. 1. March 2012

ETHNOPHARMACOLOGICAL STUDIES ON ANTISPASMODIC, BRONCHODILATOR AND ANTIPLATELET AGGREGATION ACTIVITIES OF *BLEPHARIS EDULIS* , PERS

Fatima Saqib[1], Khalid Hussain Janbaz[1], Muhammad Farhaj Latif[1], Anwarul Hassan Gilani[2], Samra Bashir[2]

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, &
[2]Department of Biological and Biomedical Sciences,
Aga Khan University Medical College, Karachi,
PAKISTAN
fatima_pharmacology@hotmail.com

ABSTRACT

Blepharis edulis, Pers. is traditionally used for gastrointestinal, respiratory and inflammatory disorders. The Aim of the studywas to rationalize the medicinal use of Blepharis edulis in gastrointestinal, respiratory and inflammatory disorders. The aqueous-ethanolic extract of Blepharis edulis seed (Be.Cr) was studied for its antispasmodic and bronchodilator effect on the isolated rabbit jejunum and tracheal preprations respectively and for antiplatelet effect using ex vivo model of human platelets. Results were: Be.Cr tested positive for alkaloids, flavonoids, tannins, sterols, terpenes ,phenolic compounds and saponins. Be.Cr (0.01-3.0mg/ml) produced relaxation of spontaneous and K+ (80mM)-induced contractions .The calcium channel blocking (CCB) effect was confirmed when Be.Cr shifted the Ca++ dose-response curves (DRCs) to right similar to verapamil. In isolated rabbit tracheal preparation, it caused inhibition of high K+-induced contractions at low dose(0.03-1.0 mg/ml) and carbachol (1μM)-induced contraction at high dose(0.01-3.0 mg/ml). Verapamil produced similar effect on high K+ and carbachol(1μM)-induced contraction suggestive of bronchodilatory effect mediated possibly through CCB. Be.Cr inhibited ADP-induced platelet aggregation (0.5-1.5 mg/ml) at relatively high concentration than epinephrine induced aggregation (0.125-1.0 mg/ml). The study showed the presence of spasmolytic and bronchodilator activity in dried seed of Blepharis edulis mediated possibly through blockade of Ca++ channels along with antiplatelet activity which provides sound pharmacological basis for its medicinal use in gut motility, respiratory and inflammatory disorders.

Keywords: **Keywords**: e Blepharis edulis ; calcium channel blocker ; antispasmodic; bronchodilator and antiplatelet aggregation

Abbreviations: Be.Cr Blepharis edulis crude extract; CCB, calcium channel blocker; ADP, adenosine 5'-diphosphate

INTRODUCTION

Blepharis edulis, Pers. (family: Acanthaceae) is commonly known as "Utangan", a spinescent, woody herb found in Pakistan, Iran, India, Afghanistan, Arabia and Egypt (Kapoor, 1990). It is used in folk medicine to treat asthma, cough, fever, inflammation of throat (Kirtikar et al., 1987). It is applied locally to heal fastering wounds and ulcers .It is appetizer, astringent to bowels(Usmanghani et al., 1997). Seeds contain Allantoin, a bitter glycoside and Blepharin, a

Leena and Luna International, **Oyama, Japan.**

glucoside (Said, 1972). Catechol and tannin are also known to be present in this plant (Baqur, 1989) .Dihydrofurano-dihydrocoumarin was first detected as occurring in nature in this plant (Dymock et al., 1972).

Phytochemical studies revealed the presence of benzoxazine glucoside, banzoxazolone (Chatterjee et al., 1990) and blepharin (β-D-glucoside of 2-hydroxy-(2H, 4H)-1, 4-benzoxazine-3-one (Sahu et al., 1990). *Blepharis edulis* has been reported to exhibit antibacterial and antifungal activity (Keymanesh et al., 2009). Antioxidant activity of *Blepharis edulis* is due to the presence of phenolic compounds; benzoxazine glucoside, benzoxazolone, phenolic acids and flavone glycosides (Surveswaran et al., 2007).It is also screened to possess anticancer activity (Hussein et al., 1982).However, the plant has not been studied for antispasmodic, bronchodilator and antiplatelet aggregation activities. This study was aimed at providing pharmacological basis for its folkloric use in spasmodic, asthmatic and inflammatory disorders.

MATERIALS AND METHODS

Plant material and preparation of crude extract

Whole plant of *Blepharis edulis*, Pers. was collected from botanical garden of Bahauddin Zakariya University Multan was identified with the kind cooperation of an expert taxonomist (Professor Dr. Mumtaz Hussain Bukhari) from the Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan, Punjab, Pakistan. A sample voucher (BE-SE-675-1) was submitted to the herbarium of Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan, Punjab, Pakistan. The plant material was shade-dried and rendered free from soil and adulterated material and coarsely ground by electrical device. The powdered material was soaked into aqueous-ethanol (80%) for 72h with occasional shaking. The soaked material was rendered free from plant debris by passaing through a muslin cloth and fluid portion was filtered through a fine filter paper (Williamson et al., 1998). The above mentioned extraction procedure was repeated twice on the plant debris and filtrate was subsequently combined before subjecting to evaporation under reduced pressure on a rotary evaporator to thick paste like mass of dark brown colour, i.e., crude extract of *Blepharis edulis* , yielding approximately 17% (w/w).

Phytochemical Analysis

Phytochemical screening of crude extract of *Blepharis edulis* was carried out qualitatively for the presence of alkaloids, flavonoids tannins, phenols, saponins, coumarins, anthraquinones, sterols and terpenes (Tona et al., 1998)

Drugs and animals

Acetylcholine (Ach), carbachol (CCh), Verapamil hydrochloride, Adenosine 5'-diphosphate, Epinephrine bitartrate salt and Sodium citrate were purchased from Sigma Chemical Company, St. Louis, MO, USA..Chemicals used for making physiological salt solutions were potassium chloride(Sigma Chemicals Co.), calcium chloride, glucose, sodium bicarbonate, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium dihydrogen phosphate(Merck, Darmstadt, Germany) and sodium chloride from BDH Laboratory supplies, Poole England. All chemicals used were of the analytical grade available and solubilized in distilled water/saline. The vehicle used for solubilization of drugs had no effect on tissue

contractility in the control experiments. Stock solutions of all chemicals were made fresh in normal saline on the day of experiment,

Animals used in this study, adult rabbits (1.0-1.5kg) of either sex and local breed were housed at the Animal House of The Aga Khan University, Karachi, maintained at temperature of 23-25 ^{0}C and were given standard diet and tap water. Experiments performed complied with the rulings of the Institute of Laboratory Animals Resources, Commission on Life Sciences, National Research Council (NRC, 1996) and approved by the Ethical Committee of the Aga Khan University Karachi.

ISOLATED TISSUE EXPERIMENTS

Rabbit Jejunum

The spasmolytic activity of plant material was studied by using isolated rabbit jejunum preparations (Gilani et al.,2007) .Respective segments of 2cm in length were suspended individually in 10ml tissue baths containing Tyrode's solution, maintained at 37 ^{0}C and aerated with a mixture of 95% Oxygen 5% and Carbon dioxide (Carbogen).The composition of the Tyrode's solution in mM was NaCl 136.9, $NaHCO_3$ 11.90, KCl 2.68, $MgCl_2$ 1.05 , NaH_2PO_4 0.42 , $CaCl_2$ 1.8 and glucose 5.55 (pH 7.4). Intestinal responses were recorded isotonically using Bioscience transducers and Oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with a sub-maximal concentration of Ach (0.3μM) and the bath fluid was subsequently replaced with normal Tyrode solution before starting the experiment. Under these experimental conditions, rabbit jejunum exhibits spontaneous rhythmic contractions, allowing the testing of relaxant (spasmolytic) activity directly without the use of any agonist.

For elucidation of mechanism of spasmolytic effect, high K^+ (80mM) was used to depolarize the isolated tissue which in turn produced sustained contractions (Farre et al., 1991). The plant material was then added in a cumulative fashion to obtain concentration-dependant inhibitory responses (Van-Rossum, 1963). The relaxation of isolated tissue preparations was expressesd as percent of control response mediated by added high K^+ concentrations.

To confirm the Ca^{++} antagonist property of the test substances, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca^{++}-free Tyrode solution containing EDTA (0.1mM) for 30 minutes in order to remove Ca^{++} from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Tyrode's solution having the following composition (mM): KCl 50, NaCl 91.04, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, glucose 5.55 and EDTA 0.1. Following incubation period of 30min, control concentration-response curves (CRCs) of Ca^{++} ($CaCl_2$) were obtained. When the control CRCs of Ca^{++} were found superimposable (usually after two cycles) the tissue was pretreated with the crude extract for 60 min to test the possible CCB effect. The CRCs of Ca^{++} were reconstructed in the presence of various concentrations of test material. Verapamil was used as a positive control.

Rabbit trachea

The trachea was dissected out and kept in Kreb's solution. The tracheal tube was cut into rings 2-3mm wide. Each ring was opened by longitudinal cut on ventral side opposite to the smooth muscle layer, forming a tracheal strip with a central part of smooth muscle in between cartilaginous portions on the edges. The preparation was suspended in a 20 ml tissue bath

containing Kreb's physiological salt solution at 37 ^{0}C aerated with carbogen. The composition of Kreb's solution was (mM): NaCl, 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). A tension of 1g was applied to each of tracheal strip and was kept constant throughout the experiment. The tissue was equilibrated for one hour before the addition of any drug. High K^+ (80 mM) and carbachol(1μM) were used to stabilize the respective preparations until constant responses of each agonist were achieved. Then their sustained contractions were obtained and relaxant effect of the crude extract was assessed by adding in a cumulative fashion .Isometric responses were recorded on a Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA). Verapamil was used as a positive control.

Preparation of human platelets

Blood was taken by vein puncture from normal human volunteers reported to be free of medication for one week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) in order to prevent coagulation .Blood samples were centrifuged at 260×g, 20 ^{0}C for 15 min to obtain platelet rich plasma (PRP) Platelet count was determined by phase contrast microscopy and all aggregation studies were carried out at 37 ^{0}C with PRP having platelet count between 2.5 and 3.0×10^{11} L^{-1} of plasma. All experiments were performed within 2 hour of PRP preparation (Saeed et al., 2004).

Measurement of platelet aggrrgation

Aggregation was monitored using a Dualchannel Lumi aggregometer (Model 400, Chronolog Corporation, Chicago, USA) using 0.45mL with the test drug dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Platelet aggregation was induced with the agonist (ADP and epinephrine).The concentration dependent anti-aggregatory effect was studied by pretreatment of PRP with plant extract for 1 min followed by addition of ADP and epinephrine. The resulting aggregation was recorded for 5 min by change in light transmission as a function of time. (Shah et al., 1999).

STATISTICAL ANALYSIS

All the data expressed are mean ± standard error of mean (S.E.M., *n*=number of experiments) and the median effective concentration (EC_{50} values) with 95% confidence interval(CI) while inhibitory effects on platelet aggregation are expressed as Median Inhibitory concentration(IC_{50}). The concentration-response curves were analysed by non-linear regression (GraphPAD program, GraphPAD, San Diego, CA, USA).

RESULTS AND DISCUSSION

The results of our preliminary phytochemical analysis revealed that crude extract of *Blepharis edulis* contain alkaloids, sterols, terpenes, tannins, saponins, flavonoids and phenolic compounds. Due to the folkloric reputation as gut relaxant, Be.Cr was tested for its possible spasmolytic effect on spontaneously contracting isolated rabbit jejunum preparation, where it inhibited spontaneous contractions, thus showing an antispasmodic effect .The relaxant effect was dose-dependant at the dose range of 0.01-3.0mg/ml with an EC_{50} of 0.61mg/ml (0.46-0.99, 95% CI, n=5) like that of verapamil, a standard calcium channel blocker (Flekenstein, 1977) at a dose range of 0.03-1.0 μM, with an EC_{50} of 0.35 μM (0.13-0.61, n=5) (Fig.1).The observed

antispasmodic effect of Be.Cr was reversible returning to normal spontaneous contractions within 2-4min of washing the tissue with fresh bathing physiological solution. .

In our earlier studies, we observed that the spasmolytic effect of medicinal plants is usually mediated through Ca^{++} channel blockade (Gilani et al., 2005). To assess whether the spasmolytic effect of *Blepharis edulis* was also mediated via similar mechanism, it was tested on the high K^+ (80mM)-induced contractions. K^+ at high doses(>30mM) is known to cause smooth muscle contractions through opening of voltage-dependent L-type Ca^{++} channels, thus allowing the influx of extracellular Ca^{++} causing a contractile effect (Bolton, 1979) and a substance causing inhibition of high K^+-induced contractions is considered as a blocker of Ca^{++} influx(Godfrained et al.,1986). Following the peak effect of K^+, Be.Cr was added into the tissue bath in a cumulative fashion which caused dose-dependent relaxation at dose range 0.01-3.0 mg/ml with EC_{50} value of 0.58 mg/ml (0.35-0.99, n=5) like that of verapamil at dose range of 0.03-0.3 µM with an EC_{50} of 0.13 µM/ml (0.04-0.23, n=5) (Fig.2). This can be visualized as a result of restricted Ca^{++} entry through voltage-dependent calcium channels. Fig. 2 shows the dose-response curves of Ca^{++}, constructed in Ca^{++}-free and K^+-rich medium. Pre-treatment of tissue with Be.Cr produced rightward shift in the Ca^{++} DRC_S at concentration range 0.1-0.3mg/ml similar to verapamil which produced a shift in Ca^{++} curves at dose range of 0.03-0.1µM. The observed effect of Be.Cr to inhibit K^+ contractions, followed by displacing effect of high concentrations of Ca^{++} suggest the presence of calcium antagonistic activity of this plant, therefore the speculation of possible involvement of Ca^{++} influx antagonistic mechanism is confirmed which might explain the traditional use of plant in hyperactive disease state of gut because the calcium channel blockers are well known to be effective in hyperactive gut diseases (Brunton, 1996).

Diverse plants are used in traditional medicine for respiratory tract diseases, bronchitis, cough, bronchopneumonia, whooping cough, inflammation of oropharynx and hiccocough (Oliver-Bever, 1983). Based on the use of *Blepharis edulis* in hyperactive respiratory ailments, the plant extract was evaluated for its possible bronchodilator activity. Sustained contractions were induced by adding Carbachol (1µM) and high K^+ (80mM) to the tissue bath containing tracheal preparations. Be.Cr exerted inhibitory effect on high K^+(80mM) -induced contractions at 0.03-1.0mg/ml with EC_{50} value of 0.33mg/ml (0.17-0.94) while inhibited carbachol (1µM)- induced contraction at higher dose range 0.01-3mg/mL with EC_{50} value of 0.47mg/ml (0.24-0.94), suggestive of bronchodilator effect mediated possibly through CCB. These results were comparable to verapamil which inhibited of K^+(80mM) and carbachol(1µM)-induced contraction with EC_{50} value of 0.027µM(0.019-0.037,n=3) and 0.104µM(0.068-0.159,n=3) respectively.

Interestingly, calcium channel blockers known to be useful as tracheal relaxants in disorders characterized by hyper responsiveness of respiratory tract (Kamei et al., 1992). This was also supported by our experimental settings.

Based on the medicinal use of *Blepharis edulis* as an anti-inflammatory remedy and knowing that platelets participate in inflammation responses (Levy-Toledano, 1999). Be.Cr was tested for its possible antiplatelet effect on human platelet-rich plasma against epinephrine and ADP-induced aggregation. Fig. 4 represents typical tracings, while the combined data from different experiments is plotted in Fig. 5. The observed inhibitory effect of Be.Cr on epinephrine-induced aggregation at relatively lower doses (0.125-1.0 mg/ml) with IC_{50} value 0.49 mg/ml than ADP-induced aggregation at high dose range0.5-1.5 mg/ml with IC_{50} 0.74mg/ml presents an

interesting picture. Epinephrine is known to cause platelet aggregation through activation of α_2-adrenergic while ADP causes activation of P2Y1, P2Y12 receptors on platelets, resulting in inhibition of adenyl cyclase pathways, thus leading to decreased intracellular cAMP level which in turn raises cytosolic free Ca^{++} (Oury et al., 2006). Be.Cr possesses mixture of components likely to block both ADP and α_2-adrenergic receptors by blocking calcium influx. Several studies have reported that calcium plays a crucial role in platelet aggregation .Platelet agonists are reported to cause a rise in intracellular Ca^{++} (Vinge et al., 1988) leading to platelet aggregation and this effect has been shown to be blocked by verapamil and diltiazem, standard calcium channel blocker. Studies have shown Calcium Channel Blockers have antiplatelet activity (Shah et al., 1997) .This fact was supported by crude extract whose antiplatelet activity may be attributed to calcium channel blocking effect.

The results of our preliminary phytochemical analysis revealed that presence of alkaloids, flavonoids amongst many other constituents, which have been shown to possess antispasmodic and calcium antagonist activities(Khalid et al., 2004). Flavonoids also possess bronchodilator activity (Ghyur et al., 2007) and antiplatelet aggregation activities (Pignatelli et al., 2002). Tannins have beneficial role in diarrhea due to their astringent action (Heinrich et al, 1992). Phenolic compounds have also effect on platelet aggregation (Ruf, 1999) .Presence of such constituents in *Blepharis edulis* seeds may be responsible for some of the pharmacological activities observed in this study.

CONCLUSION

The data obtained in this study indicate that *Blepharis edulis* possesses spasmolytic and bronchodilator effect mediated possibly through Ca^{++} antagonist property, which provides pharmacological basis for its usefulness in hyperactive gastrointestinal and respiratory disorders. Moreover, the observed antiplatelet aggregation activity may validate its folkloric use in inflammatory conditions by traditional users.

ACKNOWLEDGEMENT

This study was supported by funds made available by Higher Education Commission of Pakistan under the program “Indiginous Scholarship” awarded to Ms.Fatima Saqib.

REFERENCES

Baquar, S. R. (1989). Medicinal and poisonous plants of Pakistan(1st ed.) Karachi, Pakistan, pp.58-59.

Bolton, T.B. (1979). Mechanism of action of transmitters and other substances on smooth muscles. *Journal of Physiology Review* ,59, 606-718.

Brunton, L. L., 1996. Agents effecting gastrointestinal water flux and motility; emesis and antiemesis; bile acids and pancreatic enzymes, in: Hardman, J.G, Limbird L. E., Molinoff, P. B., Ruddon, R.W., Gilmann, A.G.(Eds.), *Goodman and Gillman’s the Pharmacological basis of Therpeutics* (pp.917-936). New York: Mc Graw Hill.

Chatterjee, A., Sharma, N. J., Banerji, J.,& Bas, S. C., (1990). Studies on Acanthaceae-Benzoxazine glucoside and benzoxazolone from *Blepharis edulis* Pers. *Indian Journal of Chemistry,* 29(2), 132-134.

Dymock, W., Warden C. J. H., & Hooper D. (1972). in, Pharmacographia Indica: A history of principal drugs of vegetable origin met within British India. Vol II., (Hakim Said M., Eds.) Institute of Health and Tibbi Research., Karachi, Pakistan, pp. 40-42.

Farre, A. J., Colombo, M., and Gutierrez, B., 1991. Differential effects of various Ca^{2+} antagonists. *General Pharmacology,* 22, 177-181.

Flecknstein, A., (1977). Specific pharmacology of Ca^{++} in myocardium cardiac pacemakers and vascular smooth muscle. Review of Pharmacology and Toxicology 17, 149-166.

Ghyur, M. N., Khan, H., & Gilani, A. H., (2007). Antispasmodic, bronchodilator and vasodilator activities of (+)-catechin, a naturally occurring flavonoid. *Archives of Pharmacal Research*, 970-975.

Gilani, A. H.., Bashir, S., Janbaz, K.H ., & Khan, A.,(2005). Pharmacological basis for the use of *Fumaria indica* in constipation and diarrhea. *Journal of Ethnopharmacology,* 96,585-589.

Gilani, A. H., Bashir, S., Khan, A., (2007). Pharmacological basis for the use *Borago officinalis* in gastrointestinal, respiratory and cardiovascular disorders. *Journal of Ethnopharmacology* 114, 393-399.

Godfrained , T., Miller, R.,& Wibo. M., (1986). Calcium antagonism and calcium entry blockade. *Pharmacology Review* 38, 312-416.

Heinrich, M., Rimpler, H., & Barrera, N. A., (1992). Indigenous phytotherapy of gastrointestinal disorders in a low and mix community (Oaxaca, Mexico): ethnopharmacologic evaluation. *Journal of Ethnopharmacology,* 36, 63-80.

Hussein, A.S.M., Kingston, D. G. I., (1982). Screening of plants used in Sudan folk medicine for anticancer activity (II). *Fitoterapia* , 53(4), 119-123.

Kamei, J. Kasuya, Y., (1992)**.** Antitussive effects of Ca^{++} channel antagonists. *European Journal of Pharmacology* ,212, 61-66.

Kapoor, L. D., (1990)**.** Handbook of Ayurvedic medicinal plants. CRC Press, Florida, U. S, pp.77.

Keymanesh, K., Hamedi, J.,& Moradi, S., (2009). Mohammadipanah, F., Sardari, S.,. Antibacterial, antifungal and toxicity of rare Iranian plants. *International Journal of Pharmacology,* 5, 81-85.

Khalid, A., Haq, Z., Ghayur, M.N., Feroz, F., Rahman, A., Gilani, A. H., &Choudhary, M. I.,(2004).Cholinestrase inhibitory and spasmolytic potential of steroidal alkaloids. *Journal of Steroid Biochemistry and Molecular Biology,* 92, 477-484.

Kirtikar, K. R., Basu, B. D., (1987). in , Indian Medicinal plants,(Blatter, E., Caius, J. F., Mahaskr, K. S., eds), Dehradun, India,.Vol III ,2nd ed, pp.1872-1873.

Levy-Toledano, S., (1999). Platelets signal transduction pathways: could we organize them into a hierarchy? *Haemostasis* , 29, 4.

National Research Council, NRC,(1996)**.** Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington D. C., U.S.A, pp.1-7.

Oliver-Bever., (1983), Medicinal plants in tropical West Africa III. Anti-infective therapy with higher plants. *Journal Ethnopharmacology* ,9, 1-80.

Oury, C., Toth-Zsamboki, E., Vermylen, J., & Hoylaerts, M.F.,(2006).The platelet ATP and ADP receptors. *Current Pharmaceutical Design* ,12,859-875.

Pignatelli, P., Pulcinelli, F. M., Celestini, A., Lenti, L., Ghiselli, A., Gazzanga, P.P., & Violi, F., (2002). The flavonoids quercitin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *American Journal of Clinical Nutrition*. 72, 1150-1155.

Ruf, J.C., 1999. Wine and polyphenols related to platelet aggregation and atherothrombosis. *Drugs Experimental Clinical Research*. 25, 125-131.

Saeed, S. A., Rasheed, H., Fecto, F.A.W., Achakzai, M.I., Ali, R., Connor, J.D., and Gilani, A.H., (2004). Signalling mechanisms mediated by G-protein coupled receptors in human platelets. *Acta Pharmacologica Sinica* , 25 (7), 887-892.

Sahu, A., Chatterjee, A., (1990). New synthesis of blepharin, the naturally occurring glycoside of 2-hydroxy-(2H, 4H)-1, 4-benzoxazine-3-one. Indian *Journal of Chemistry* ,29 (7), pp. 119-123.

Said, H. M., (1972). Hamdard Pharmacopoeia of Eastern medicine. Institute of Health and Tibbi Research., Karachi, Pakistan, pp.364

Shah, B. H. Safdar, B., Virani, S.S. Nawaz, Z., Saeed, S.A.,& Gilani, A. H, (1997). The antiplatelet aggregatory activity of *Acacia nilotica* is due to blockade of calcium influx through membrane calcium channels. *General Pharmacology*, 29, 251-255.

Shah, B. H., Siddiqui, A ., Qureshi, K. A., Khan, M., Rafi, S., Ujan, V. A., Yaqub, Y., Rasheed, H. & Saeed , S. A., (1999). Co-activation of Gi and Gq proteins exert synergistic effect on human platelet aggregation through activation of phospholipase C and Ca^{2+} signaling pathway. *Experimental &Molecular Medicine,* 31 (1), 42-46.

Siess, W., (1989). Molecular mechanisms of platelet activation. *Physiological Review* 69, 58-78.

Surveswaran, S., Cai, Y.Z., Corke, H. & Sun, M., (2007). Systemic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Journal of Food Chemistry* 102, 938-953.

Tona, L., Kambu, K., Ngimbi, N., Cimaga, K., & Vlietnick, A. J., (1998). Antoamoebic and phytochemical screening of some Congolese medicinal plants. *Journal of Ethnopharmacology* 61, 57-65.

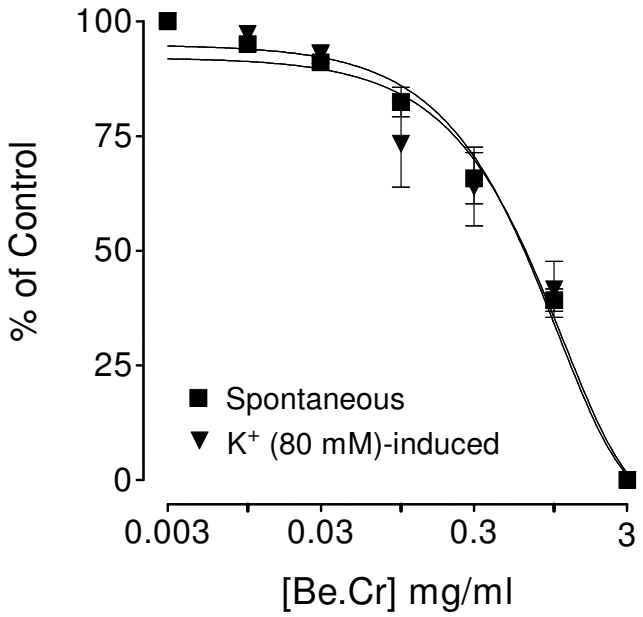
Usmanghani, K., Seed, A.,& Alam, M. T., (1997). Indusyunic Medicine; Traditional Medicine of Herbal, Animal, and Mineral Origin in Pakistan, University of Karachi Press, Pakistan, pp.123-124.

Van-Rossum, J. M., (1963). Cumulative concentration-response curves II. Techniques for the making of concentration-response curves in isolated organs and the evaluation of drug parameters. *Archives Internationales de Pharmacodynamie* , 143, 299-330.

Vinge, E., Anderson, T .L. G., and Larsson, B., (1988). Effects of some calcium antagonists on aggregation by adrenaline and serotonin on alpha adrenergic radioligand binding in human platelets. *Acta Physiol Scand* 133, 407-416.

Williamson, E.M., Okpako, D.T., Evans, F.J., (1998). Selection, Preparation and Pharmacological Evaluation of Plant Material. (pp.15-23) John Wiley&Sons, Chichester,.

(a)



(b)

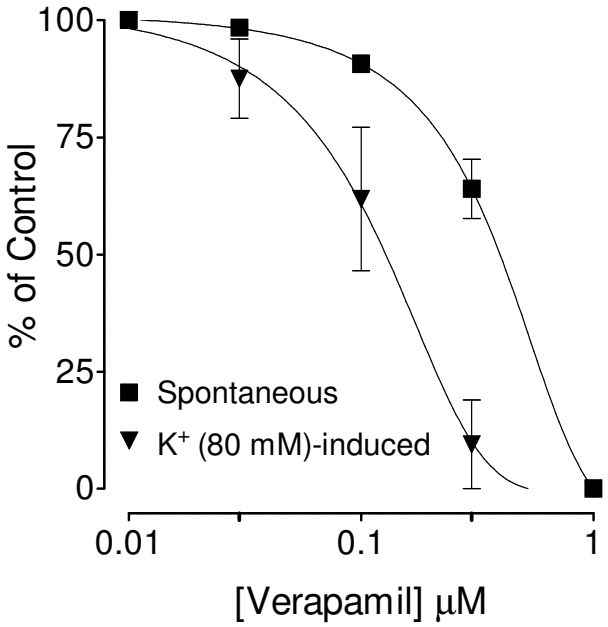
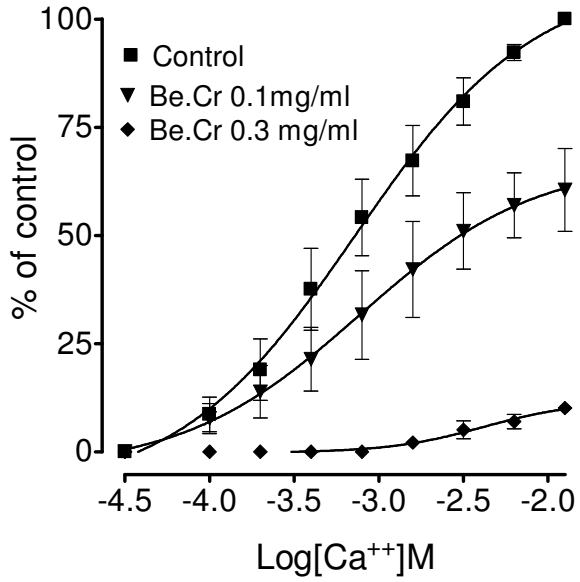


Fig.1. Concentration response curves showing the inhibitory effect of **(a)** *Blepharis edulis,* Pers. crude extract (Be.Cr) and **(b)** verapamil on spontaneous and K^+ (80mM)-induced contractions in isolated rabbit jejunum preparations. Values shown are mean ± S.E.M of 3-5 observations.

Leena and Luna International, **Oyama, Japan.**

(a)



(b)

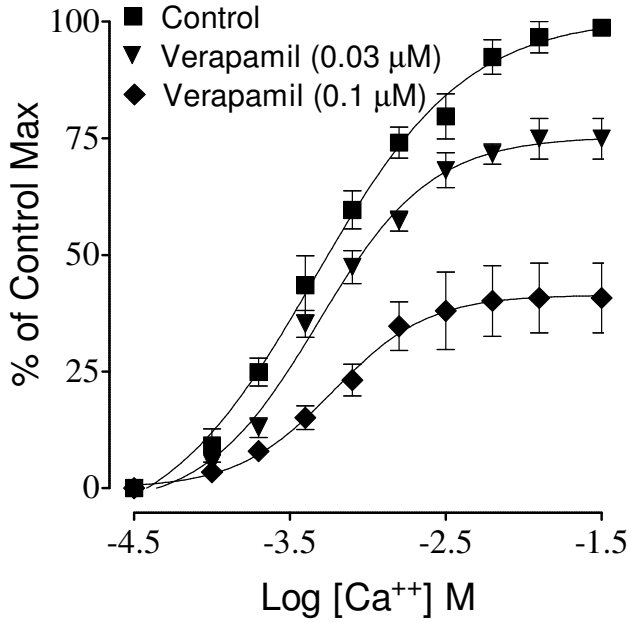
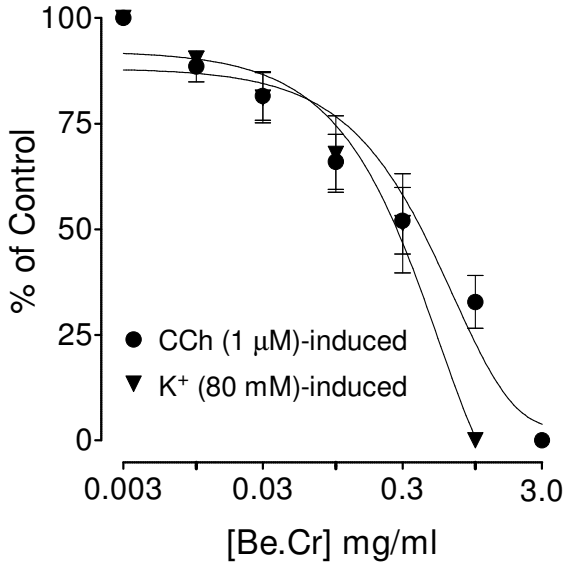


Fig. 2. Concentration response curves of Ca^{++} in the absence (control) and presence of different concentrations of crude extract of **(a)** *Blepharis edulis,* Pers. (Be.Cr) and **(b)** verapamil in isolated rabbit jejunum preparations. Values shown are mean ± S.E.M of 5-6 observations.

(a)



(b)

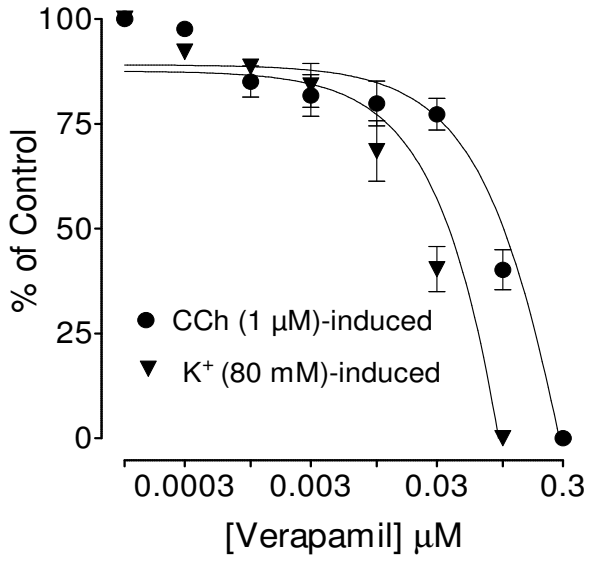


Fig. 3: Concentration response curves showing the inhibitory effect of **(a)** crude extract of *Blepharis edulis* (Be.Cr) and **(b)** verapamil on K^+ (80mM)-induced and Carbachol(1µM)-induced contractions in isolated rabbit trachea preparations. Values shown are mean ± S.E.M of 3-5 observations.

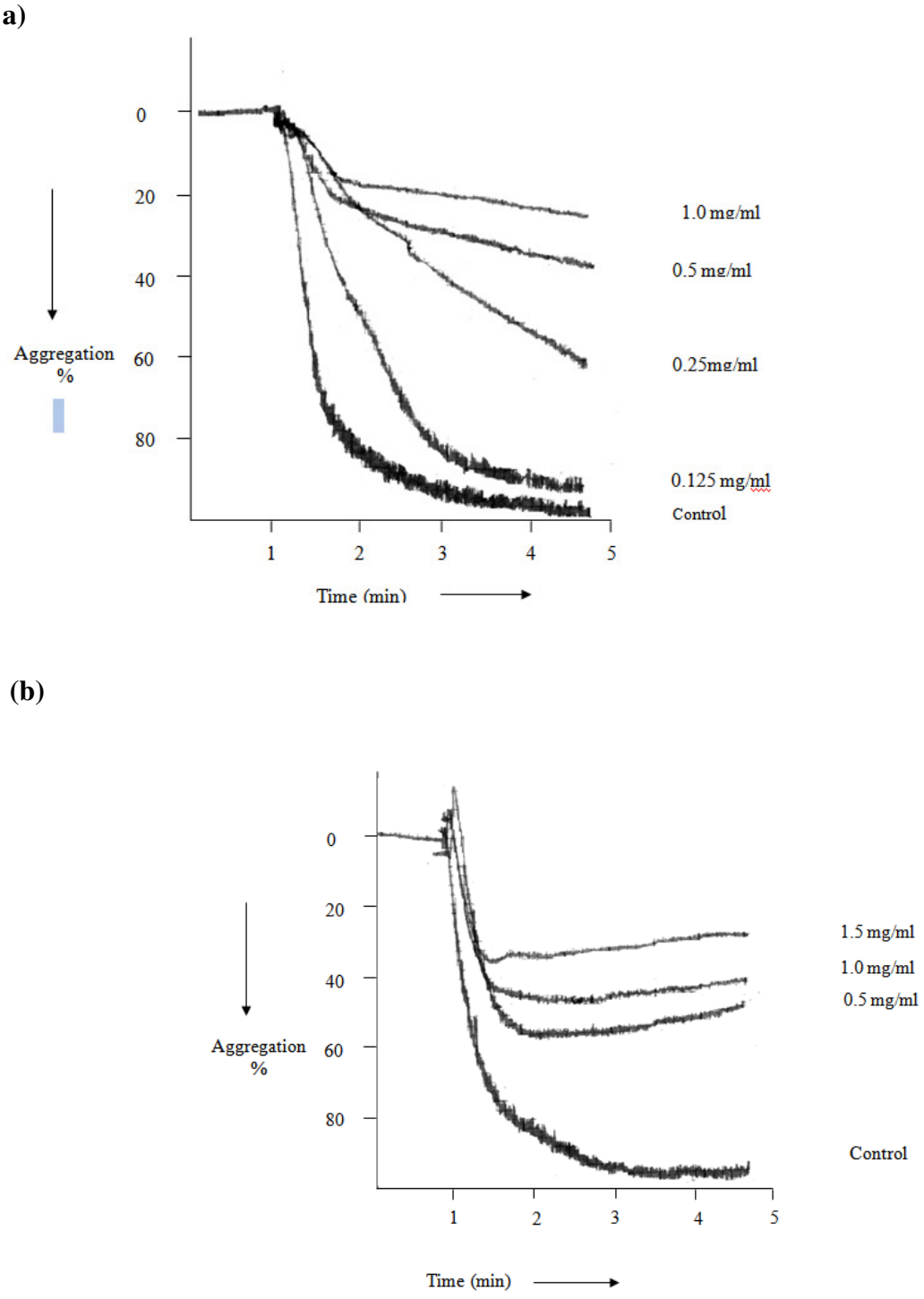


Fig. 4. Tracing showing the inhibitory response of different concentrations of crude ethanolic extract of *Blepharis edulis*, Pers (Be.Cr) on human platelet aggregation induced by (a) Ephinephrine (b) ADP.

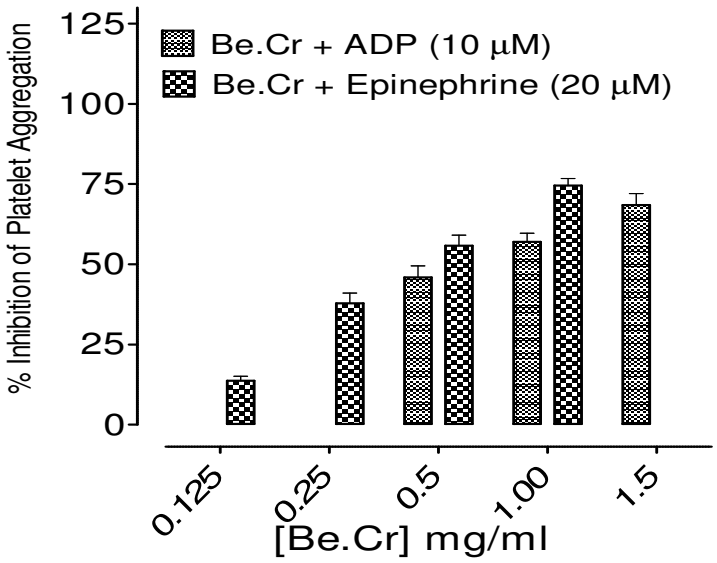


Fig.5 . Bar Chart showing the concentration-dependent inhibitory effect of *Blepharis edulis* crude extract (Be.Cr) on adenosine 5'-diphosphate (ADP) and epinephrine-induced human platelet aggreagation. The values shown are mean ± S.E.M., n=3-4