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Pharmacological basis for the antihypertensive activity of *Grewia asiatica* fruit extract

Zuneera Akram[1], Rehana Perveen[1]*, Hasan Salman Siddiqi[2], Muzammil Hussain[1], Shahlla Imam[3], Hina Abrar[4] and Syed Mohammad Mahmood[5]

[1]Department of Pharmacology, Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan
[2]Natural Product Research Division, Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi, Pakistan
[3]Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Karachi, Pakistan
[4]Department of Pharmacology, College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[5]Department of Medicine (oncology), The Aga Khan University Medical College, Karachi, Pakistan

Abstract: In the management of cardiovascular disorders, medicines from herbal sources have played a vital role through centuries. The following study was commenced in order to lay possible pharmacological foundation associated with medicinal uses of edible fruit of *Grewia asiatica* in hypertension through in-vitro method. In this study isolated atrial preparation of Guinea pig was used where crude ethanolic extract of *Grewia asiatica* fruit (Ga.Cr) decreased the force and rate of spontaneous atrial contractions (0.03-10mg/kg). In isolated rat aortic ring preparations previously vasoconstricted by phenylephrine and High K^+, it also resulted in dose dependent vasodilation (0.01-10 mg/kg).In the presence of L-NAME, the relaxation curve of Ga.Cr was partially inhibited showing involvement of Nitric oxide (NO) mediated pathway. The speculative analysis contemplated that Ga.Cr has blood pressure reducing potentials through inhibition of Ca^{++} influx via Ca^{++} channels, its release from intracellular stores and through other means like NO mediated pathways.

Keywords: *Grewia asiatica* fruit, hypertension, vasodilation, NO- mediated, Ca^{++} influx.

INTRODUCTION

The major associated risk factors of cardiovascular disease are hypertension, stroke and ischemic heart disease (World Health Organization, 2002; American Diabetes Association, 2021; Lawes *et al.*, 2006). Blood pressure regulation is key factor to reduce risks of cardiovascular events. WHO analytically reported in 2012 that 1 out of every 3rd person is affected by BP fluctuation and approximate 50% deaths occur due to consequences of cardiovascular collapse (World Health Statistic, 2012) and until 2025 global population suffering from hypertension will increase up to 60%. In developing countries hypertension is major population burden and a leading problem for younger population as compared to developed countries (Kearney *et al.,* 2012). Rate of prevalence of hypertension is 18% above the age of 15 years and every 3rd individual is hypertensive in Pakistan by 45 years of age or above (Pakistan National Research Council, 1994).

Around 69 drugs in different classes are suggested for managing hypertension in United States, also accessible in single pill combinations (Food and Drug Administration, 2011). The side effects connected with these pharmacologic treatments have been often found in many patient surveys (Kiriyama *et al.,* 2016), having an adverse impact on patient compliance (Neutel and Smith, 2003). Many hypertensive patients often need more than one antihypertensive medication to regulate their blood pressure (Black *et al.,* 2001; Cushman *et al.,* 2002).If one drug is unsuccessful in controlling blood pressure, consequently use of multiple drugs becomes necessary (Chobanian *et al.,* 2003) resulting in high rates of unwanted side effects as well as high treatment expense. Many current medicines are costly and inaccessible to local population. In addition, hypertension pharmacotherapy needs lifelong treatment in most cases. All these determinants add to lack of compliance at patients' end which leads to doubtful effective management of blood pressure in developing countries and even in developed countries like the United States of America (Wang *et al.,* 2002). Noncompliance is identified as the main cause for hypertension therapy failure because victims generally regard hypertension as a symptomatic situation, affecting compliance in absence of symptoms though blood pressure remains high (Sharkness and Snow, 1992).Various reports have revealed that almost 50% hypertensive patients do not entirely follow the recommended treatment (Cushman *et al.,* 2002). In Pakistan, only 3% hypertensive patients are well managed due to defiance of plan for management as compared to a proper control seen in 27% patients in USA (Pakistan Hypertension League, 1998). Many people opt for General Practitioners (GPs) and are unsuccessful in managing hypertension properly or don't have enough

*Corresponding author: e-mail: rperveen@hotmail.com

information and thus are unable to tackle appropriately(Jafar *et al.,* 2005) The people have to undergo expensive treatment which they usually can't manage to pay for as there is no health insurance system. Hardships, lack of education and knowledge and fright of the drugs side effects are major factors resulting in poor treatment and consequently there is no proper hypertension management in Pakistani population at national level (Jafar *et al.,* 2011).

From preceding discussion it is evident that proper management of hypertension is required in order to reduce CVD risks as there are issues in management like side effects and costly drugs leading to non-compliance. Despite the availability of a vast range of medicines, hypertension continues to remain a public health challenge. It is therefore the need of the hour to search for some alternative therapeutic option like use of medicinal plants that can be logical choice keeping in view the eminent role of herbal medications, in history of mankind for thousands of years.

In cardiovascular field herbal medicines play a vital role which has increased through centuries (Siddiqui and Siddiqui, 1931).Approximately 70% of all medicines are of herbal source (Verma and Singh, 2008). Various plants extracts and their components have been used in hypertension management playing the roles of cardio-suppressants and vasodilators (Khan and Gilani, 2015; Gilani *et al.,* 2010; Eddouks *et al.,* 2017).

The fruit of *Grewia asiatica* (locally known as Phalsa) belongs to family Tiliaceae. Ten varieties of genus *Grewia* namely *G. asiatica* L.,*G. glabra* Blume, *G. damine* Gaertn, *G.tenax* (Forssk.) Fiori., *G. elastic* Royle., *G. sapida* Roxb., *G. helicterifolia* Wall., *G. optiva* J. R. Drumm. ex Burret, *G.microcos* L. and *G. villosa* are indigenous to Southern Asia, found from Pakistan to Cambodia but are also cultivated in other tropical areas. Botanical features of *G. asiatica* have been described in the Journal of medicinal plants research (Sinha *et al.,* 2015) According to research scientist *G. asiatica* medicinally have a significant value along with nutrition (Zia-Ul-Haq *et al.,* 2013) Its fruits have low calories and fat but is rich in minerals, vitamins and fibers (Yadav, 1999). Several approved researches demonstrated various pharmacological effects like antimicrobial, anti-cancerous and antiplatelet via different parts of *G. asiatica*. Leaves possess antiemetic, antimicrobial, anti-platelet, and anti-cancerous properties (Sinha *et al.,* 2015) while fruit has ethno-botanical significance (Jabeen *et al.,* 2009) as an astringent, stomachic, anti-diabetic, and is also used in treatment of cancer and inflammations (Shukla *et al.,* 2016).The unripe fruit is anti-inflammatory, anti-pyretic and used in cardiac, respiratory, and blood disorders (Morton, 1987; Gupta *et al.,* 2006; Akram *et al.,* 2019).

In this study we focused on the blood pressure lowering potential of *G. asiatica* which has not been evaluated previously although the plant has revealed multiple medicinal properties in various scientific studies.

MATERIALS AND METHODS

In line with the Laboratory Animal Resources Institute, Committee on Life Sciences, NRC statements, all experiments were conducted (National Research Council, 1996). Ethical approval of this study was provided by BASR Baqai Medical University, Karachi, as part of the M. Phil research project of Zuneera Akram.

Chemicals and standards

All the chemicals used had highest purity and all stock solutions were prepared using distilled water. All dilutions and extracts were prepared fresh before starting the experimentation. Phenylephrine (PE), Isoproterenol, Acetylcholine chloride (Ach), Potassium chloride (KCl), and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) collected from "Sigma Aldrich Chemical Company (St Louis, MO, USA)". The Krebs Solution used as a physiological solution and its composition expressed in (mM): Glucose (11.7), NaCl (118.2), KCl (4.7), NaHCO3 (25.0), CaCl2 (2.5), KH2PO4 (1.3), and MgSO4 (1.2) [pH 7.4]. These were purchased from "E.MerckKGaA (Darmstadt, Germany)".

Plant extract

The fruit of *Grewia asiatica* was obtained from the local market, authenticated and prepared extract in ethanol

Animals

Sprague-Dawley (SD) rats (weight 180-250 gms) and guinea-pigs (weight 450-500 gms) were housed for *in-vitro* studies under uniform and controlled conditions 12/12 hours light and dark cycle at a temperature of 25°C with free access to food and water in Animal House of Baqai Medical University.

Experiment on isolated atrial preparation

High dose Isoflurane anesthetized Guinea-pigs were used and dissected. After right atrium identification, isolation was done and fatty tissues were gently cleaned. It was immediately placed in tissue bath filled with 15mL of Kreb's solution bubbled with carbogen gas (95% O_2 with 5% CO_2) at a temperature of 32°C (Gilani *et al.,* 1997). Lower end of atrial preparation was stabilized through small loop of thread attached to metallic hook. Upper end was tied by another piece of thread that joined it to an isometric sensor to convey impulses to an amplifier linked to Power Lab that was in connection with computer to monitor the alterations in tension (1.0 gm) of the isolated tissue. The experiment was recorded at the same time. Spontaneously beating atrial preparation was allowed to beat for about 30minutes as a stability period. To monitor

positive control responses, isoproterenol (1μM) and acetylcholine (1μM) were used as standards. The ethanolic extract of *G. asiatica* was given in ascending dosage manner (0.01, 0.03, 0.1, 0.3, 3, 5 and 10 mg/dl) where effects were observed and recorded.

Experiments on isolated rat aorta

Previously established Furchgott and Zawadski experimental design was followed with some modification (Furchgott and Zawadski, 1980) where SD-Rats were sacrificed by high dose Isoflurane. Dissection was initiated by opening thoraco-abdominal cavity, separating aorta carefully and immediately placing in Kreb's solution. Each aortic ring was propped in an isolated tissue bath filled with 5mL of Kreb's solution with sustained temperature of 37 °C with carbogen gas. 1-2gms of preload was applied and the tissue was allowed a period of 60 minutes for stabilization.

For isometric tension, each tissue was joined to a separate force transducer coupled to a "Trans-bridge TBM4M, World Precision Instruments, Hertfordshire, UK" connected to the "Power Lab data acquisition system (model ML845, AD Instruments)" and a computer. The charts were displayed on monitor by using "the Chart software (version 5.3)".

Phenylephrine (1 μM) was used for stabilization of rat aortic preparations in tissue bath resulting in vasoconstriction. Then fresh solution replaced Kreb's solution in the bath (containing PE) producing vasodilation. To observe the endothelium integrity, Ach (0.3 μM) was added when the PE-induced contraction was sustained. Relaxation produced in the aortic tissue produced by adding Ach indicated intact endothelium. Such tissues with intact endothelium were used for some experiments. In some aortic rings, the endothelium lining was disrupted by gentle rubbing which was shown by lack of relaxation on adding Ach.

To observe vasodilator effects the ethanolic extract of fruit *G.asiatica* was added in increasing dose (0.01, 0.03, 0.1, 0.3, 3, 5 and 10 mg/dl) on a PE-induced sustained contraction. If PE induced sustained contractions in testing material are inhibited, it indicates obstruction of Ca^{++} inflow through receptor-operated Ca^{++}channels. For further evaluation of the vasodilator effect of the extract and to study possible involvement of endothelium-mediated activity, pre-incubation with L-NAME (0.1 mM) was done in PE-induced contractions for 20 minutes (Vanhoutte *et al.,* 1986).

Another spasmogen high potassium (K^+80 mM) was also used for obtaining sustained contraction. Inhibition of high K^+-induced contractions indicates blockage of voltage-dependent membranous Ca^{++} channels (Karaki *et al.,* 1997).

Data analysis

Data is ± standard error of mean (SEM, n = number of experiments)". Graphing, calculations and data analysis was done using "Graph Pad Prism version4.00 for Windows, (Graph Pad Software, San Diego, CA, USA, http://www.graphpad.com)".

RESULTS

Effects of ga.cr on isolated guinea-pig atrial preparations

Blood pressure can be defined as: "the product of cardiac output and peripheral resistance" (Johansen, 1992; Garofalidou and Munroe, 2020); hence, a decrease in any one and/or the other can lead to a fall in blood pressure. Keeping in view the cardiac component, effects of plant extracts on spontaneous atrial beating with respect to rate and force of contraction were screened.

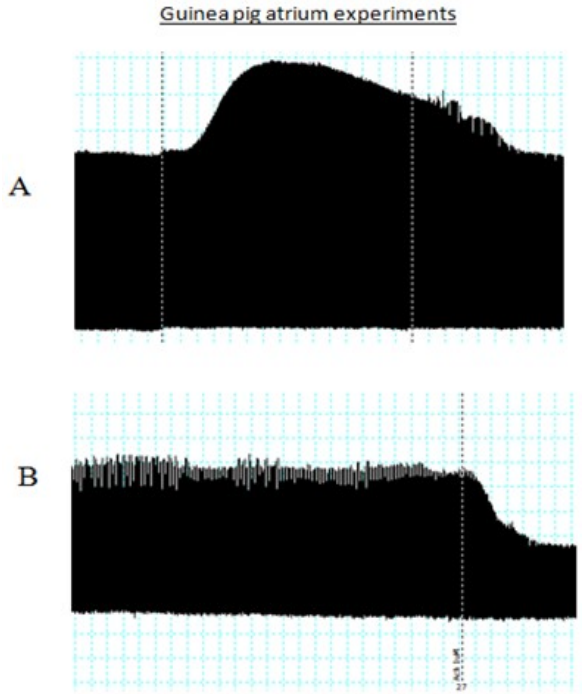


Fig. 1:Portion of tracing taken from a single experiment displaying the effect of (A) isoproterenol (1 μM) and (B) acetylcholine (1 μM) on the isolated guinea pig atrial preparation.

β-agonist, Isoproterenol (1μM), raised force and rate of atrial contractions as shown in fig. 1 (A) while muscarinic agonist, acetylcholine (1μM), reduced the force and rate of atrial contractions as shown in fig. 1(B).

As the dose of *G.asiatica* fruit increased (0.01, 0.03, 0.1, 0.3, 1, 2, 5, 10), depressant effect resulted on experimental preparation on force and rate of beating as shown in fig. 2 (A) and Graph 1(A). Verapamil, a known Ca^{++} channel blocker standard drug, also showed inhibition of force and rate of same preparation as shown in fig. 2(B) and Graph 1(B).

Effect of ga.cr on sustained contractions evoked by phenylephrine (pe) and high K^+ in experimental preparations of isolated rat aortae

Thoracic portions of Male Sprague–Dawley rats (weight 200–280 grams) aortae were obtained. PE (1 µM) and K^+ (80 mM) were used to obtain sustained aortic contractions as shown in figure 3(A) and 4(A).

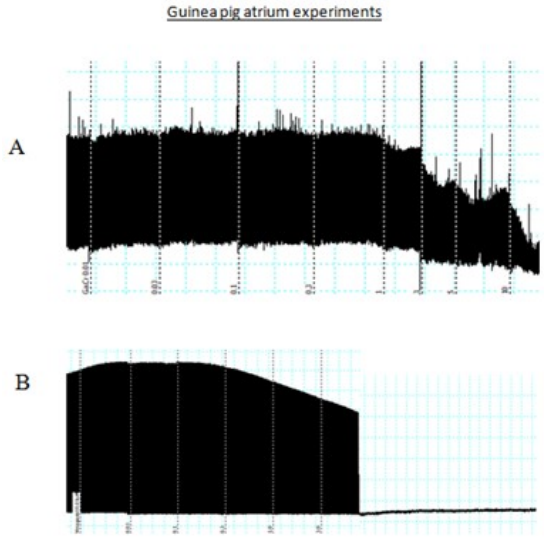
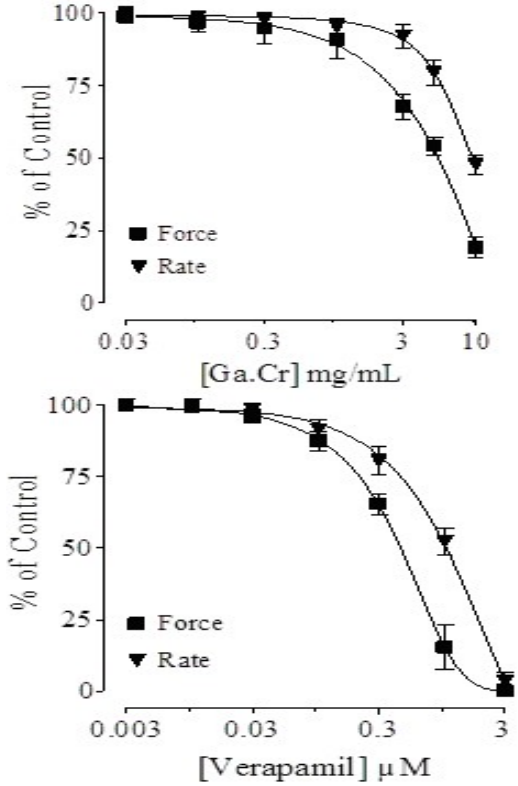


Fig. 2: Portion of tracing taken from a single experiment displaying the effect of increasing doses of (A) the *Grewia asiatica* crude extract (Ga.Cr) on isolated guinea pig atrial preparation and, (B) verapamil.

Fresh dilutions of the crude extract of *G.asiatica* fruit (Ga.Cr) in the form of cumulative dosing were prepared and added to isolated tissue bath on contracted aortic preparations for observing the effects of *G. asiatica*. The crude extract produced significant dose-dependent relaxant effect on the contractions evoked by both PE (1 µM) and K^+ (80 mM) as shown in figure 3(C), 4(C) and Graph 2(A).While Verapamil, a known hypotensive agent, produced complete dose-dependent relaxation of aortic rings contracted by both PE (1 µM) and K^+ (80 mM) exhibiting vasodilation as shown in figure 3(B), 4(B) and Graph 2 (B).

Effect of Ga.Cr on sustained contractions evoked by phenylephrine (pe) in intact endothelium and denuded endothelium experimental preparations of isolated rat aortae

Procured thoracic portions of aortae from Male Sprague–Dawley rats (200-280gms) through previously recognized procedure suitable for screening the effects of the plant extracts on PE-evoked contractions in intact endothelium and denuded endothelium aortic preparations and also on endothelium-intact preparations incubated with L-NAME. PE (1µM) which is a sympathetic agonist produced a sustained contraction in the isolated aortic ring preparation. After attaining plateau, acetylcholine (0.3µM) was added to the tissue to observe integrity of the endothelial lining. Immediate relaxation was an indication of intact endothelium. Some tissues were gently rubbed in order to remove endothelial lining. When acetylcholine (0.3µM) was added to PE-evoked contraction on such aortic tissues, failure of relaxation was observed indicating damaged endothelium. For observing mechanism of action of vasodilator effect of Ga.Cr, the PE-induced contraction obtained in an endothelium intact tissue was pre-incubated with L-NAME (0.1 mM) for 20 minutes. This experiment helps in exploration of endothelium-dependent vasodilator effect as the tissue now behaved as an endothelium denuded tissue.



Graph 1: Concentration-response curves illustrating depressant effect of (A) Ga.Cr and, (B) verapamil on the force and rate of spontaneously contracting isolated guinea pig atria. Values shown are mean ± SEM, n = 3.

Ga.Cr vasodilatory effect was observed as a dose-dependent complete relaxation of PE (1 µM)-evoking contraction in endothelium intact tissue while the relaxation was incomplete in the case of endothelium denuded preparation and in endothelium intact tissue pre-incubated with L-NAME (0.1mM) as in graph 3 (A).

DISCUSSION

In 1975, Solecki reported that the use of plants for medicinal purposes dates back to 60,000 years (Solecki *et al.,* 1975). In 2005 Gilani *et al* reported that herbs play a vital role in treating various diseases (Gilani and Atta-Ur-Rahman, 2005). To evaluate the effect of herbal drugs,

trials of several plant extracts and their constituents are being done for management of hypertension in modern world (Vakil, 1949).

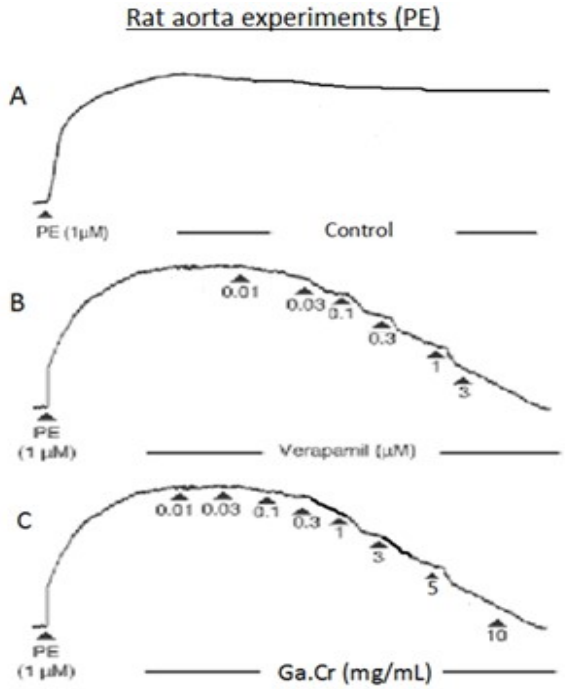


Fig. 3: Portion of tracing taken from a single experiment displaying (A)Control PE-induced contraction (B) the response to cumulative dosing of verapamil and (C)Ga.Cr on PE- induced contractions in isolated rat aorta.

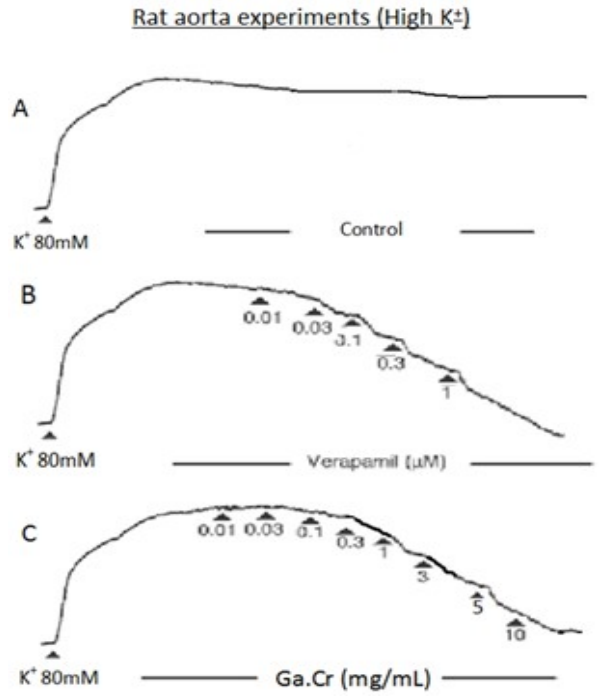
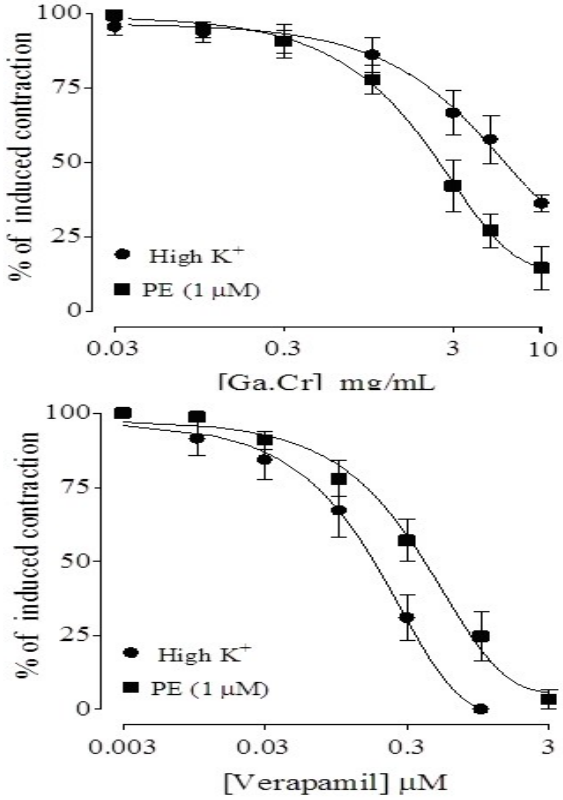
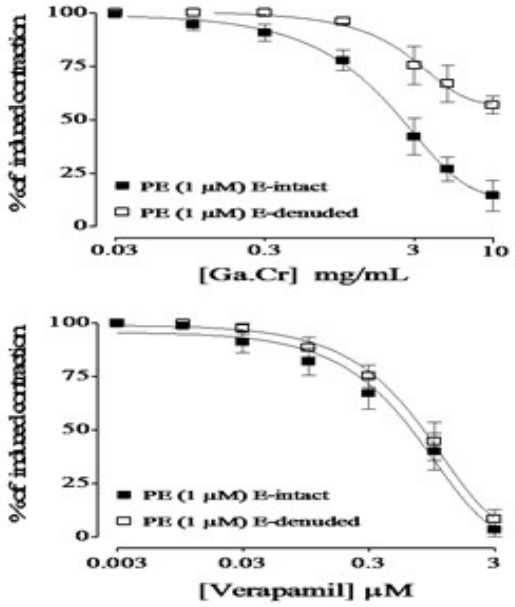


Fig. 4:Portion of tracing taken from a single experiment displaying (A) Control K^+80mM-induced contraction (B) the response to cumulative dosing of verapamil and (C) Ga.Cr on K^+80mM- induced contractions in isolated rat aorta.



Graph 2:The curves of concentration-response illustrating inhibition action of (A) the *Grewia asiatica* crude extract Ga. Cr and (B) verapamil on Phenylephrine (PE) and high K+- induced contractions in isolated rat aorta. The shown values are mean ± SEM; n = 3



Graph 3: Concentration-response curves illustrating inhibitory effect of **(A)** Ga.Cr and **(B)** verapamil on PE-evoked contractions in endothelium (E)-intact and E-

denuded isolated rat aorta. Values shown are means ± SEM; n = 3.

Analgesic, anticancer, antifungal, anti-hyperglycemic, antiplatelet, antipyretic, antioxidant, antimalarial, radio-protective, antiviral and immune-modulatory effects (Sastri, 1956)along with therapeutic efficacy for heart, blood and liver disorders are the known medicinal activities of the *G.asiatica* extract (Fleckenstein, 1977).

The research was conducted to study the effects on heart and blood vessels of the crude extract of *G.asiatica* (Ga.Cr) since blood pressure is the product of cardiac output and peripheral resistance (Garofalidou and Munroe, 2020).The effects of Ga.Cr were observed on heart and blood vessel by performing experiments on isolated tissue preparations of guinea-pig right atrium and SD rat aortic rings, consequently a drop in one and/or the other can lead to a decrease in blood pressure (Bp).

In spontaneous beating guinea-pig right atrial preparation, the extract restrained rate as well as force; similar effect was produced by a renowned Ca^{++} antagonist verapamil (Godfraind *et al.,* 1986) used in clinical practice (Sampson and Kass, 2011) producing cardio-depressant effect(Palmer *et al.,* 1988) demonstrating thatthe test material might be responsible for cardiac output diminution, eventually dropping blood pressure. For effect on blood vessel, Ga.Cr was tested on rat aortic tissues that were in pre-contracted state causing inhibition of PE and high K^+-induced vasoconstrictions.

Receptor-operated channels (ROCs) are activated by PE while high K^+ triggers voltage-dependent channels (VDCs), both causing Ca^{++}influx from extracellular to intracellular space, which causes a rise in the Ca^{++}concentration inside the cell. Increase in Ca^{++}concentration produces contraction[35] and can be inferred from mentioned observation of extract's effect that Ca^{++}influx is being blocked both through VDCs and ROCs, resulting vasodilation.

For more insight in vasodilation by Ga.Cr, some experiments performed on both endothelium intact and denuded vascular preparations revealed that in absence of endothelial lining, there was vasodilator effect diminution thus suggesting mediation of endothelium vasodilator effect.

As reported previously, endothelium-derived factor nitric oxide (NO) is responsible for vasodilation (Furchgott and Zawadski, 1980),synthesized by nitric oxide synthase (NOS) from L-arginine endothelium ((Palmer *et al.,* 1988). To ascertain the role of NO, incubated rat vascular tissue preparations with intact endothelial lining were used with L-NAME, a known inhibitor of NOS (Fantel *et al.,* 1997).

Partial inhibition was observed on the vasodilator effect lined with previous results and graph was almost super imposable to the one of endothelium-denuded preparations proposing that vasodilator effect in endothelium-intact preparations was being mediated by NO-dependent pathways.

CONCLUSION

The ethanolic extract of Ga.Cr fruit reduced the rate and force of the guinea pig atrial contractions hence decreasing cardiac output. It also demonstrated vasodilation in rat aortic rings. Consequently these can be the possible mechanisms for lowering blood pressure providing bases for using the plant in hypertensive cardiovascular disorders.

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