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Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*

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ABSTRACT

Ethnopharmacological relevance: The ripe dried fruit of *Ficus carica* Linn. (Moraceae) commonly known as “Fig” has medicinal value in traditional system of medicine for its use in gastrointestinal and inflammatory disorders.

Aim of the study: To rationalize the medicinal use of Fig (*Ficus carica*) in gastrointestinal and inflammatory disorders.

Materials and methods: The aqueous-ethanolic extract of *Ficus carica* (Fc.Cr) was studied for antispasmodic effect on the isolated rabbit jejunum preparations and for antiplatelet effect using ex vivo model of human platelets.

Results: Fc.Cr tested positive for alkaloids, flavonoids, coumarins, saponins, sterols and terpenes. When tested in isolated rabbit jejunum, Fc.Cr (0.1–3.0 mg/mL) produced relaxation of spontaneous and low K⁺ (25 mM)-induced contractions with negligible effect on high K⁺ (80 mM) similar to that caused by cromakalim. Pretreatment of the tissue with glibenclamide caused rightward shift in the curves of low K⁺-induced contractions. Similarly, cromakalim inhibited the contractions induced by low K⁺, but not of high K⁺, while verapamil equally inhibited the contractions of K⁺ at both concentrations. Fc.Cr (0.6 and 0.12 mg/mL) inhibited the adenosine 5'-diphosphate and adrenaline-induced human platelet aggregation.

Conclusion: This study showed the presence of spasmolytic activity in the ripe dried fruit of *Ficus carica* possibly mediated through the activation of K⁺_{ATP} channels along with antiplatelet activity which provides sound pharmacological basis for its medicinal use in the gut motility and inflammatory disorders.

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1. Introduction

Ficus carica Linn. (Syn: *Ficus sycomorus*; family: Moraceae) is commonly referred as “Fig”. Its fruit, root and leaves are used in the native system of medicine in different disorders such as gastrointestinal (colic, indigestion, loss of appetite and diarrhea), respiratory (sore throats, coughs and bronchial problems), inflammatory and cardiovascular disorders (Burkill, 1935; Ponelope, 1997). Fig has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic and anti-inflammatory remedy (Duke et al., 2002; Werbach, 1993).

Phytochemical studies revealed the presence of numerous bioactive compounds: arabinose, β-amyrins, β-carotenes, glyco-

side, β-setosterols and xanthotoxol (Duke, 1992). *Ficus carica* has been reported to exhibit antioxidant (Solomon et al., 2006), anti-HSV (Wang et al., 2004), Haemostatic (Richter et al., 2002), hypoglycemic (Canal et al., 2000) and hypo-lipidemic activities (Perez et al., 1999). The 6-O-acyl-β-D-glucosyl-β-sitosterols along with its palmitoyl, linoleyl, stearyl and oleyl derivatives isolated from the fruit of *Ficus carica* exhibited strong cytotoxic effect (Rubnov et al., 2001). However, the plant has not been studied for antispasmodic and antiplatelet activities. This study was aimed at providing pharmacologic basis for its folkloric use in spasmodic and inflammatory disorders.

2. Materials and methods

2.1. Plant material, preparation of crude extract

Dried ripe fruits of *Ficus carica* were purchased from herbal store located in Multan and sample was identified with the kind

Abbreviations: Fc.Cr, *Ficus carica* crude extract; Syn, synonym; K_{ATP}, ATP-dependent K⁺ channels; ADP, adenosine 5'-diphosphate.

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cooperation of an expert taxonomist (Dr. Altaf A. Dasti) from the Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Punjab, Pakistan. A sample voucher (FC-F-09-05) was submitted to the herbarium of Department of Biological and Biomedical Sciences, the Aga Khan University, Karachi, Sind, Pakistan. The plant material was rendered free from soil and adulterated materials and coarsely ground by electrical device. The powdered material was soaked into aqueous ethanol (80%) for 72 h with occasional shaking. The soaked material was rendered free plant debris by passing 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 filtrates were 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 *Ficus carica*, yielding approximately 25% (w/w).

2.2. Phytochemical analysis

Phytochemical screening of the crude extract of the Fig was carried out qualitatively for the presence of alkaloids, anthraquinones, coumarins, flavonoids, saponins, sterols, tannins and terpenes according to the standard methods (Akinyemi et al., 2005; Evans, 1996).

2.3. Drugs and animals

Acetylcholine perchlorate, potassium chloride, Adenosine 5'-diphosphate (ADP), adrenaline and verapamil hydrochloride were purchased from Sigma Chemicals Co., St. Louis, MO, USA. Cromakalim and glibenclamide were purchased from Tocris, Ellisville, MO and RBI Chemicals Co., Natick, MA, USA, respectively. All chemicals used were of the analytical grade available and solubilized in distilled water/saline except cromakalim and glibenclamide which were dissolved in 10% DMSO. 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 the experiment.

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

2.4. Isolated tissue experiments

The spasmolytic activity of the plant materials was studied by using isolated rabbit jejunum preparations (Gilani et al., 2007a). Respective segments of 2 cm in length were suspended individually in 10 mL tissue baths containing Tyrode's solution, maintained at 37 °C and aerated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen). The composition of the Tyrode's solution in mM was: KCl 2.68, NaCl 136.9, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, CaCl₂ 1.8 and glucose 5.55 (pH 7.4). Intestinal responses were recorded isotonicity 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 acetylcholine (0.3 μM) 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 testing of the relaxant (spasmolytic) activity directly without the use of any agonist.

For elucidation of mechanism of spasmolytic effect, low K⁺ (25 mM) and high K⁺ (80 mM) concentrations were used to depolarize the isolated tissues which in turn produced sustained contractions. The plant material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses. The relaxation of isolated tissue preparations was expressed as percent of the control response mediated by added low and high K⁺ concentrations.

2.5. Preparation of human platelets

Blood was taken by vein puncture from normal human volunteers reported to be free of medications for 1 week. Blood samples were mixed with 3.8% (w/v) sodium citrate solution (9:1) and centrifuged at 260 × g, 20 °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 °C with PRP having platelet counts between 2.5 and 3.0 × 10¹¹ L⁻¹ of plasma. All experiments were performed within 2 h of PRP preparation.

2.5.1. Measurement of platelet aggregation

Aggregation was monitored using a Dualchannel Lumi Aggregometer (Model 400 Chronolog Corporation, Chicago, USA) using 0.45 mL aliquots of PRP (Shah et al., 1999). The final volume was made up to 0.5 mL with the test drug dissolved either in normal saline or appropriate vehicle known to be devoid of any effect on aggregation. Platelets aggregation was induced with the agonists (adrenaline and ADP). The concentration dependent anti-aggregatory effect was studied by pretreatment of PRP with plant extract for 1 min followed by addition of adrenaline or ADP. The resulting aggregation was recorded for 5 min by the change in light transmission as a function of time.

2.6. Statistical analysis

All the data expressed are mean ± standard error of mean (S.E.M., *n* = number of experiments) and the median effective concentrations (EC₅₀ values) with 95% confidence intervals (CI). The concentration–response curves were analyzed by non-linear regression (GraphPAD program, GraphPAD, San Diego, CA, USA).

3. Results and discussion

The results of our preliminary phytochemical analysis reveal that the aqueous extract of ripe dried fruit of *Ficus carica* contains alkaloids, flavonoids, coumarins, saponins and terpenes. Due to the folkloric reputation as a gut relaxant, Fc.Cr was tested for its possible spasmolytic effect in isolated rabbit jejunum preparations, where it inhibited the contractions at the concentration, thus showing the antispasmodic effect (Fig. 1). The relaxant effect was dose-dependent with an EC₅₀ value of 0.74 mg/mL (0.52–1.0, 95% CI, *n* = 5) like that of cromakalim at 0.01–10 μM with EC₅₀ value of 1.8 μM (1.5–2.3, 95%, *n* = 4). The observed antispasmodic effect of Fc.Cr was reversible returning to normal spontaneous contractions within 2–4 min of washing the tissue with fresh bathing physiological solution (Fig. 1).

In our earlier studies, we observed that the spasmolytic effect of medicinal plants is usually mediated through Ca⁺⁺ channel blockade or K⁺ channel opening mechanisms (Gilani et al., 2005a,b, 2008). To assess whether the spasmolytic effect of the Fig extract was also mediated via similar mechanisms, it was tested on low K⁺ (25 mM) and high K⁺ (80 mM)-induced contractions. Fc.Cr at lower doses completely inhibited the low K⁺ (25 mM)-induced contractions with EC₅₀ value of 0.37 mg/mL (0.22–0.62, *n* = 6) while it

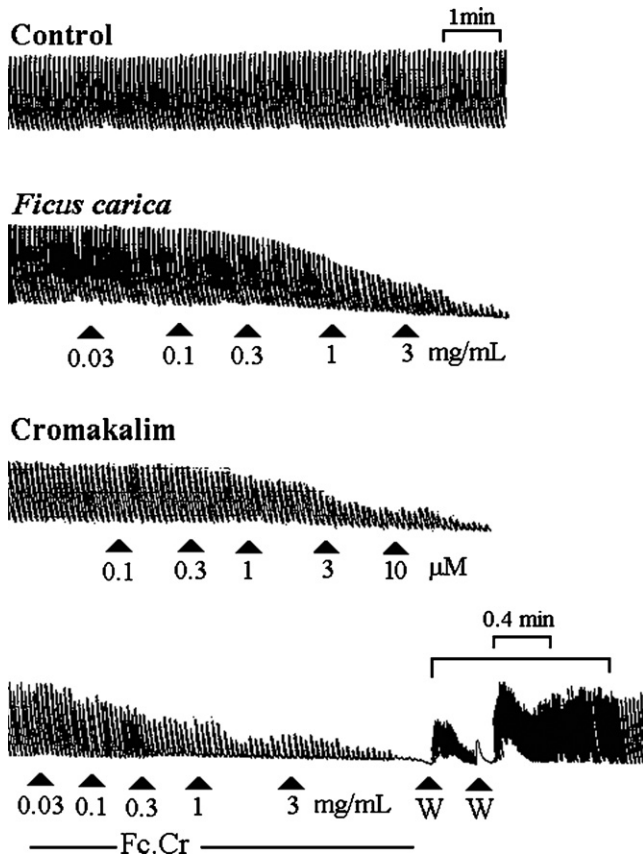


Fig. 1. Tracings showing reversible spasmolytic effect of the crude extract of *Ficus carica* dried fruit (Fc.Cr) and cromakalim on spontaneously contracting isolated rabbit jejunum preparation. (W) represents washing of the tissue.

produced mild effect against high K^+ (80 mM)-induced contractions but at high doses (1–10 mg/mL) (Fig. 3A). The substance that selectively relaxes the contractions induced by low K^+ is considered as K^+ ATP channel opener like, while Ca^{++} antagonists inhibit both low K^+ and high K^+ -induced contractions equally (Gilani et al., 2005a). The K^+ channel opening effect was confirmed, when the inhibition of low K^+ (25 mM)-induced contractions was prevented in the presence of glibenclamide, a specific blocker of the ATP-dependent K^+ channels (Frank et al., 1994). Fig. 2 presents the typical tracing showing the inhibitory effect of the Fc.Cr and cromakalim against low and high K^+ -induced contractions. Cromakalim, a prototypical K^+ ATP channel opener (Escande et al., 1988) produced similar results, causing selective and glibenclamide sensitive relaxation of the contractions induced by low K^+ (Fig. 3B), where as verapamil, a standard Ca^{++} channel blocker (Fleckenstein, 1977) inhibited low K^+ and high K^+ induced contractions at a similar concentration range as expected (Fig. 3C).

K^+ channel activators produce smooth muscle relaxation as a result of decrease in intracellular free Ca^{++} through respective mechanisms of membrane hyperpolarization via increase in K^+ efflux by opening of K^+ channels (Quest and Cook, 1998). *Ficus carica* fruits has been recommended in different hyperactive gastrointestinal disorders, such as colic and diarrhea, the observed spasmolytic effect of the crude extract of Fig, mediated possibly through K^+ ATP channel activation, may provide a plausible explanation for Fig as a part of the prescription under such diverse complaints.

Based on the medicinal use of *Ficus carica* as an anti-inflammatory remedy and knowing that some K^+ ATP channel

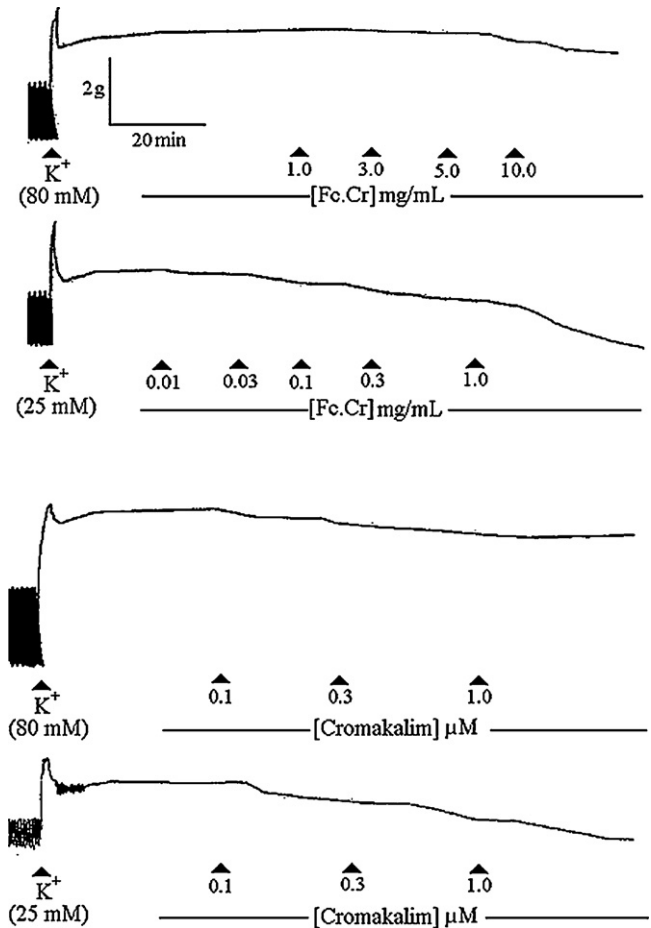


Fig. 2. Tracings showing the inhibitory effect of the crude extract of *Ficus carica* dried fruit (Fc.Cr) and cromakalim against high K^+ (80 mM) and low K^+ (25 mM)-induced sustained contractions in isolated rabbit jejunum preparations.

openers possess antiplatelet effect (Cho et al., 2005; Patelunas et al., 1994), Fc.Cr was tested for its possible antiplatelet effect in human platelet-rich plasma against adrenaline and ADP-induced platelet aggregation. Fig. 4 presents typical tracings, while the combined data from different experiments is plotted in Fig. 5. The observed inhibitory effect of Fc.Cr on adrenaline and ADP-induced platelet aggregation at relatively lower doses (0.6 and 1.2 mg/mL) presents an interesting picture. Adrenaline is known to cause platelet aggregation through activation of α_2 -adrenergic while ADP causes activation of P2 Y1 and P2 Y12 receptors resulting in inhibition of the adenylyl cyclase pathway, thus leading to decreased intracellular cAMP level which in turn raises the cytosolic free Ca^{2+} (Kimura and Okuda, 1994; Oury et al., 2006). Interestingly, an active principal (ficin) from this plant was shown to possess haemostatic effect through activation of Factor X (Richter et al., 2002). It is not surprising to observe that plant usually contain combination of constituents with opposing effects. For example, plants; *Fumaria indica*, *Hibiscus rosasinensis* and *Saussurea lappa* have been shown to possess the combinations of spasmogenic and spasmolytic activities thus explaining their use in constipation and diarrhea (Gilani et al., 2005b,c, 2007b). Similarly, turmeric and St. John's wort have been shown to contain combination of hypotensive and hypertensive constituents (Gilani et al., 2005d,e). Perhaps nature has placed such composition in plants to offset the side-effects in case of access doses (Gilani and Rahman, 2005). The Fig extract due to its K^+ ATP channel opening effects,

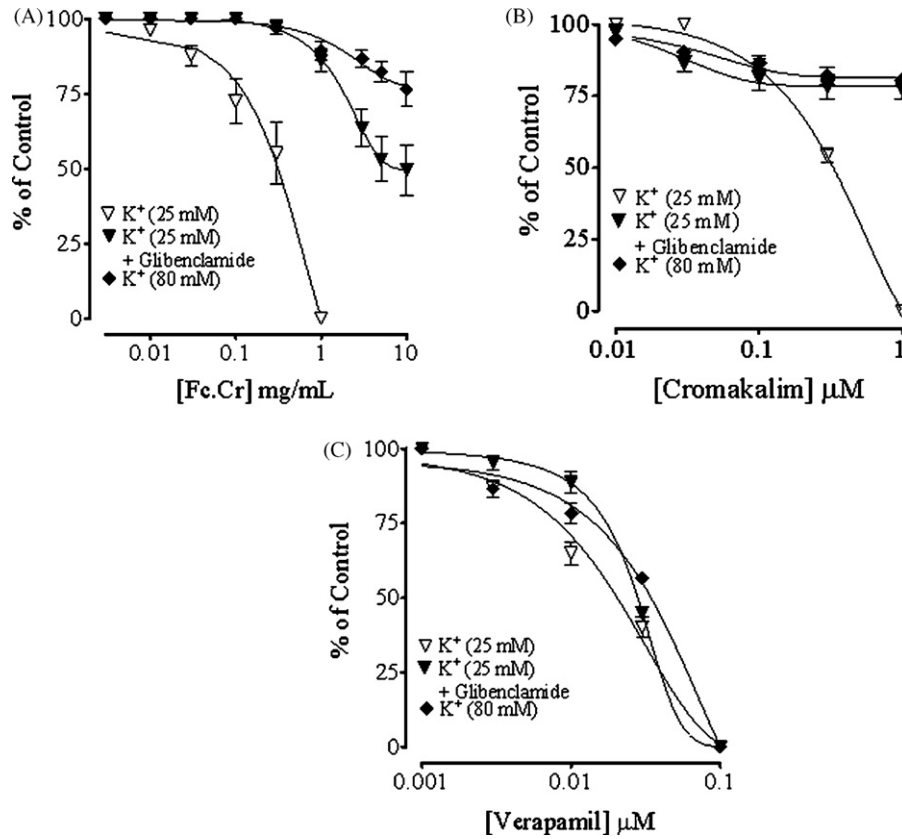


Fig. 3. Concentration–response curves showing comparison of (A) crude extract of *Ficus carica* fruit (Fc.Cr), (B) cromakalim and (C) verapamil for their inhibitory effects against low K⁺ (25 mM) with and without glibenclamide and high K⁺ (80 mM)-induced contractions in isolated rabbit jejunum preparations. The values shown are mean ± S.E.M. from 3 to 6 determinations.

is likely decrease the intracellular Ca⁺⁺, thus exerting non-specific inhibitory effect on adrenaline and ADP-induced platelet aggregation, which may explain in part its medicinal use in inflammatory disorders.

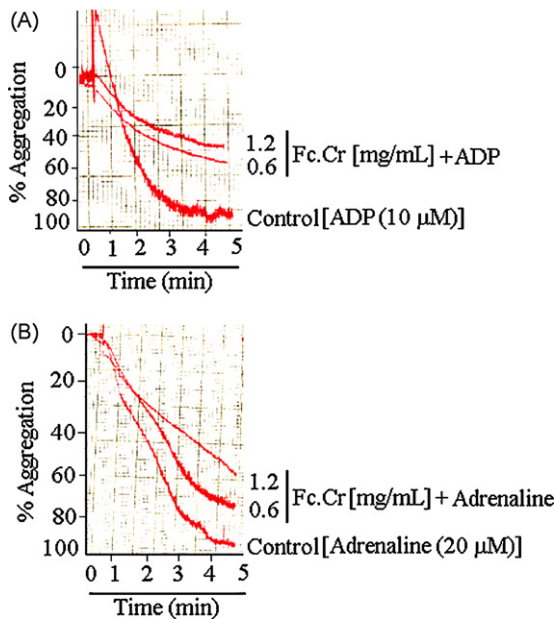


Fig. 4. Tracing showing the inhibitory response of different concentrations of the crude extract of *Ficus Carica* fruit (Fc.Cr) on (A) adenosine 5'-diphosphate (ADP) and (B) adrenaline-induced aggregation in human platelets.

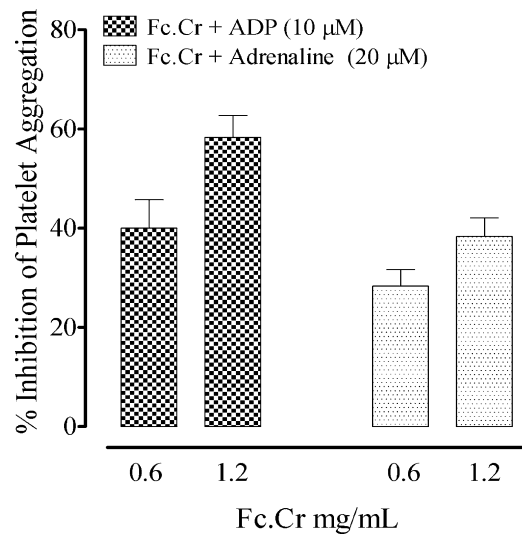


Fig. 5. Bar chart showing the concentration-dependent inhibitory effect of *Ficus carica* crude extract (Fc.Cr) on adenosine 5'-diphosphate (ADP) and adrenaline-induced human platelet aggregation. The values shown are mean ± S.E.M. from 3 determinations.

4. Conclusion

The data obtained in this study indicate that the Fig possesses spasmolytic effect mediated possibly through K⁺ATP channel activation, which explain some of its medicinal uses in hyperactive

gut disorders and its observed antiplatelet effect offers additional health benefits.

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