IN VITRO AND IN VIVO BIOLOGICAL APPROACH TO VALIDATE FOLKLORIC CLAIMS OF TRIANTHEMA TRIQUETRA ROTTLER & WILLD

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Abstract

Trianthema triquetra Rottler & Wild (Tt.Cr) is used in traditional practices as a remedy for various ailments. Hence current research was commenced to authenticate the folkloric uses. To discover spasmylytic potential, Tt.Cr was applied to isolate jejunum, while isolated tracheal and aorta tissues were used to determine the tissue relaxing properties of the extract. Anti-lipoxygenase activity was determined in vitro using Buicalein as standard. In vivo testing was carried to examine the potentiality of the herb to treat pyrexia and pain. Tt.Cr showed dose-dependent (0.01 - 3.0 mg/ml) spasmylytic effects in jejunum tissues and relaxed K+ (80 mM)-induced spasm and triggered rightwards shift of Ca2+ concentration-response curves. Carbachol (1μM)- together with K+ (80 mM) - induced tracheal spasm was also relaxed by Tt.Cr (0.01 to 1.0 mg/ml). Additionally, Tt.Cr (0.01 - 1.0 mg/ml) relaxed phenylephrine (1 μM) and K+ (80 mM) - treated constricted rabbit aorta. Tt.Cr (0.5 mM) inhibited lipoxygenase enzyme. Tt.Cr (80 mg/kg) settled pyrexia in rabbits comparable to aspirin and prolonged tail deflection time in mice (100 mg/kg) hence proving analgesic activity. The Tt.Cr demonstrated antispasmodic, bronchodilation and vasodilation properties probably by blocking calcium channels. These outcomes generate logic behind ancient application of herb for numerous ailments such as asthma, cough, heart problems and spasm.

Introduction

Trianthema triquetra (Aizoaceae) is commonly known as Alettie, Chulani (Ahmad et al. 2015), Choti Ulwaiti (Ahmad et al. 2014), Loonaki in Pakistan (Zarin et al. 2016), Naayi Soppo, Lunki, Lutanki in India, red spinach, small hogweed in Australia (Quattrocchi 2012). Plant paste has been applied on swellings by population for rheumatism (Katewa and Galav 2005) and possessed anti-inflammatory potential both in vitro and in vivo bioactivity test (Sathyaraj and Indumathy 2018). It exhibited hepatoprotective effects when investigated on rats (Chitra and Nithyanandhi 2007) and its roots showed significant gastric anti-secretory and acid neutralizing effects (Ghori 2016). The plant can cure cold, flu, cough and asthma (Akbar and Khatoon 2012, Ahmad et al. 2015) thus rendering its tendency towards its potential as an antitussive, bronchodilator and antipyretic but as it has not been searched for these activities. Chemically plant comprises thiobarbituric acid, reduced glutathione, glutathione peroxidase, and catalase (Chitra and Nithyanandhi 2007). Preliminary phytochemical testing (Tona et al. 1998) revealed that plant extract possesses saponins, tannins, and flavonoids.

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Irregular contractions of smooth muscles in GIT, respiratory tract or cardiovascular system results in diarrhoea, asthma and hypertension. Cytosolic Ca\(^{2+}\) plays a vital part in the functioning of smooth muscle and calcium exposure of contractive components alters depending on the milieu neighbouring the cell (Karaki et al. 1997). For inspection of probable smooth muscle relaxant effects of a plant extract, isolated organs from a rabbit might be deployed, as rabbits illustrate numerous structural and functional resemblances of human organs. Saqib et al. (2019) did investigations using isolated tissues of different animals.

**Materials and Methods**

The whole herb of *Trianthema triquetra* was collected fresh from BZU Multan, Pakistan, during August 2011 and Dr. Altaf A. Dasti (taxonomist) identified the plant and Voucher No. (P.Fl.235-4) was retained there. Fresh herb (1 kg) was subjected to shade drying followed by coarse grinding. Coarse powder was macerated in 80% methanol-aqueous for one week followed by filtration and whole technique was repeated twice. Combined extracts were subjected to evaporate on rotary evaporator resulting brownish extract which was stored at \(-20^\circ\text{C}\). On day of experimentation, fresh dilutions of extract were prepared (Saqib et al. 2018).

All the chemicals, solvents, and drugs used were of reagent analytical grade. Acetylcholine, carbachol, verapamil chloride and potassium chloride were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Methanol, EDTA, glucose, magnesium chloride, magnesium sulfate, phenylephrine, potassium dihydrogen phosphate, sodium bicarbonate, sodium dihydrogen phosphate and calcium chloride were purchased from Merck, Darmstadt, Germany. Sodium chloride, ammonium hydroxide and sodium hydroxide were obtained from BDH laboratory supplies, Poole, England.

For studies on isolated organs, adult rabbits weighing between 1.0 and 1.5 kg of either sex were used. In *in vivo* studies, albino rabbits and mice were used as animal model to find out antipyretic and analgesic potential of plant. All animals accommodated properly in animal house were normally fed with standard diet and water and were starved a night before experiment. Entire experimentations were accomplished by fulfilling instructions of the Institute of Laboratory Animals Resources, Commission on Life Sciences (Council 2010) and by getting authorization from Ethical Committee of the BZU Multan (EC/03/2011 dated 16.02.2011).

Tissues were prepared in laboratory and were subjected to Tt.Cr in accordance to the already reported method (Saqib and Janbaz 2016). Involvement of CCB phenomenon for antispasmodic characteristics was investigated by method and verified by developing CRCs using tyrode rich (Farre et al. 1991).

Tissues were prepared in laboratory and were evaluated for bronchodilator action according to the method by Saqib et al. (2019). Tissues were also prepared in laboratory and were evaluated for vasodilator action according to described method (Saqib et al. 2015). Lipoxgenase inhibiting potential of Tt.Cr was explored according to the technique (Tappel 1953) with little adjustments. Pyrexia resolving characteristics of Tt.Cr was evaluated by using albino rabbits according to cited technique (Khan et al. 2008). Characteristics of Tt.Cr in depressing nociception were evaluated by using albino mice according to cited technique (Asongalem et al. 2004, Adedapo et al. 2008).

Facts and figures were articulated as the mean ± SEM, EC\(_{50}\) values along with 95% CI were calculated by using the computer software “Graph pad Prism Program” (version 5.0), San Diego CA, USA. Concentration response curves were analysed by the non-linear regression of sigmoid response curve (variable slope). The statistic applied was the students t-test and p < 0.05 was considered quite noteworthy.
**Results and Discussion**

Addition of Tt.Cr to jejunum tissues yielded relaxation in a cumulative dose dependant manner (Fig. 1a) with EC$_{50}$ of 0.12 mg/ml. (95% CI: 0.01 - 0.28; n = 5). It induced prominent relaxation of high K$^+$ contracted tissues (Figs 1b & 2a) with EC$_{50}$ of 0.20 mg/ml. (95% CI: 0.01 - 0.61; n = 5). These outcomes were analogous to the effects of verapamil used as standard drug and relaxed both spontaneously contracting and high-K treated jejunum (Fig. 2b) with EC$_{50}$ value of 0.65 μM (95% CI:0.5551 - 0.8601, n = 5) and 0.70 μM (95% CI:0.5777 - 0.8881; n = 5), respectively.

The capability of Tt.Cr to block calcium channels was additionally verified on Calcium response curves (CRCs). The Ca$^{2+}$ free media fully exterminated the spontaneous constrictions of tissues but on adding Ca$^{2+}$ in a cumulative manner (0.1 - 6.4 mM), restoration of tissue contractions was obtained progressively, and best contraction was made at 6.4 mM concentration of Ca$^{2+}$. Afterwards, the jejunum tissue incubation with Tt.Cr (0.1 - 0.3 mg/ml) promoted the rightwards shifting of CRCs, and these effects were found equivalent to those yielded by verapamil (Fig. 3).

![Fig. 1](image1.png)

**Fig. 1.** Tracings showing (a) the relaxant effect of a crude methanolic extract of *Trianthema triquetra* (Tt.Cr) on spontaneous and (b) high K$^+$ (80 mM)-induced tissue contraction. Tt.Cr was added at increasing concentrations and values listed were the final tissue bath concentrations (n = 5).

![Fig. 2](image2.png)

**Fig. 2.** Spasmylytic effects induced in concentration-dependent manner by (a) a methanolic extract of *Trianthema triquetra* (Tt.Cr) and (b) verapamil on spontaneous- and high K$^+$ (80 mM)-induced contractions in isolated rabbit jejunum preparations. Values are the mean ± SEM, n = 5.
Normal tracheal tissue remained unaffected by adding Tt.Cr. But cumulative dosing of extract on 80mMol KCl treated tracheal tissue, relaxation resulted (Fig: 4a) with EC$_{50}$ of 0.14 (95% CI: 0.02 - 0.28; n = 5). Further testing on carbachol treated tracheal preparation, again tissue dilation was obtained (Fig. 4b) with EC$_{50}$ 0.31 (95% CI: 0.01 - 2.20; n = 5) showing bronchodilator potential of plant. Likewise, verapamil also relaxed carbachol (1 µM) - and K$^+$ (80 mM) - exposed tracheal preparations with EC$_{50}$ of 0.114 (95% CI: 0.057 - 0.227 mg/ml; n = 5) and 0.048 µM (95% CI: 0.028 - 0.083 mg/ml; n = 5), respectively (Fig. 4c, d).

Fig. 3. Calcium antagonizing effect of (a) a methanolic extract of *Trianthema triquetra* (Tt.Cr) and (b) verapamil on concentration response curves of Ca$^{2+}$. in isolated rabbit jejunum preparations. Values are the mean ± SEM, n = 5.

Fig. 4. Concentration dependent bronchodilator effect of a methanolic extract of *Trianthema triquetra* (Tt.Cr) (a, b and c) and (d) verapamil on carbachol (CCh: 1 µM)- and high K$^+$ (80 mM)- induced contractions in isolated rabbit tracheal preparations. Values are the mean ± SEM, n = 5.
Plant extract produced vasodilator effects when tested on high K\(^+\) (Fig. 5a) and phenylephrine (Fig. 5b) treated constricting aortic tissues with EC\(_{50}\) of 1.74 mg/ml (95% CI: 0.10 - 3.0; n = 5) and 0.24 mg/ml (95% CI: 0.01 - 0.82; n = 5) correspondingly. Similarly, verapamil too demonstrated vasodilation on phenylephrine (1 μM) and K\(^+\) (80 mM) treated aortic tissues, with EC\(_{50}\) of 0.90 μM (95% CI: 0.03 - 5.02 μM; n = 5) and 0.43 μM (95% CI: 0.03 - 1.98 μM; n = 5), respectively (Fig. 5c, d).

T. triqueta caused *in vitro* lipoxygenase inhibition thus showing activity up to 50 ± 1.23% analogous to baicalein 93.79 ± 1.27% (Table 1).

Tt.Cr (80 mg/kg; i.p) significantly resolved pyrexia comparable with Aspirin hence signifying a probable antipyretic potential of Tt.Cr (Table 2).

Tt.Cr (75 mg/kg showed significant (p < 0.01) expansion of tail deflection time which was comparable with the effects of aspirin (Table 3).

In current study, DRCs were constructed to assess how *Trianthema triqueta* relaxed smooth muscles. Seeing that free Ca\(^{2+}\) in cytoplasm promotes smooth muscles contractions as evidenced by acetylcholine, histamine, and serotonin (Karaki *et al.* 1997). There might be an opening of L-type calcium or discharged from intracellular reservoirs (Van-Rossum 1963). Uninterrupted depolarization and repolarization induce rhythmic contractions of jejunum and inwards Ca\(^{2+}\) movements by L-type Ca\(^{2+}\) channels lead to depolarization (Bolton 1979). So, it may be claimed that by stopping either calcium influx or hindering calcium release from reservoirs within cells may stop the impulsive contractions in jejunum.

To understand the underlying mechanism, 80 mM KCL was applied to spontaneously contracting jejunum which led to persistent contraction due to calcium influx thus depolarizing the cell membrane and resulting in generation of smooth muscle spam (Hill-Eubanks *et al.* 2011).
Tt.Cr relaxed the contracted tissues at even lower dose. So it may be proposed that plant possesses calcium channel blocking potential (Karaki et al. 1997). It was further confirmed when extract shifted CRCs towards right on jejunum preparation as verapamil does (Bolton 1979).

Folkloric use of Tt.Cr in cough was reported by Akbar and Khatoon (2012) and CCBs are well known to resolve cough (Saqib and Janbaz 2016). Mostly, spasmolytic components are absorbed due to their non-polar and lipophilic nature and produce systemic effects thus acting on trachea (Saqib et al. 2018). Tt.Cr relaxed carbachol (1 μM) - and K+ (80 mM) treated constricted tracheal tissues in dose dependent manner and it is supposed by mediating Ca²⁺ channel blocking action (Bolton 1979).

### Table 1. Inhibitory activity of Tt.Cr on lipoxygenase enzyme in comparison to standard.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Compound under study</th>
<th>Conc./well</th>
<th>% inhibition</th>
<th>IC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoxygenase</td>
<td>Tt.Cr</td>
<td>0.5 mM</td>
<td>50 ±1.23</td>
<td>0.34 ± 0.06 mg/ml</td>
</tr>
<tr>
<td></td>
<td>Baicalin</td>
<td>0.25 mM</td>
<td>93.79 ± 1.38</td>
<td>0.13 ± 0.015 mM</td>
</tr>
</tbody>
</table>

### Table 2. Effect of Tt. Cr on pyrogen-induced pyrexia in rabbit.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Tail deflection before treatment (sec)</th>
<th>Tail deflection after treatment (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>2 ± 0.2</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Aspirin</td>
<td>75 mg/kg</td>
<td>2.5 ± 0.34</td>
<td>3.86 ± 0.14</td>
</tr>
<tr>
<td>Tt.Cr</td>
<td>100 mg/kg</td>
<td>2.3 ± 0.24</td>
<td>5.1 ± 0.25</td>
</tr>
</tbody>
</table>

### Table 3. Effect of Tt. Cr and comparison with aspirin on tail deflection time (s) in mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose</th>
<th>Rectal temperature (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>Pyrogen + saline</td>
<td>38.62 ± 0.08</td>
</tr>
<tr>
<td>Aspirin</td>
<td>Pyrogen + aspirin 10 mg/kg</td>
<td>38.75 ± 0.08</td>
</tr>
<tr>
<td>Tt.Cr</td>
<td>Pyrogen + Tt.Cr 80 mg/kg</td>
<td>38.64±0.45</td>
</tr>
</tbody>
</table>

CCBs are famous for settling down cardiovascular issues, Tt.Cr was applied to rabbit aortic tissues contracted with phenylephrine- and K+ (80 mM) to elucidate the mechanism of calcium channel blocking activity (Saqib and Janbaz 2016). Tt.Cr displayed vasodilation when added on K⁺ (80 mM) - treated aortic tissues by Ca²⁺ channels blockade plus inhibition of Ca²⁺ discharge by endoplasmic reticulum. Phenylephrine-exposed aorta was relaxed too due to inactivation of α-adrenergic receptors and consequent no Ca²⁺ influx (Saqib et al. 2018).

Furthermore, Tt.Cr when examined in vitro led to inhibition of Lipoxygenase enzyme thus interfering the conversion of arachidonic acid into leukotrienes (Schaible et al. 2013) which in turn are well-known as constrictor of respiratory tract and also play role in inflammation. Hence it may be hypothesised that *T. triquetra* may prove useful in management of bronchoconstriction in asthma and inflammation as well as increased mucus secretion during allergic rhinitis.

The Tt.Cr demonstrated antipyretic and analgesic effects when tested in animal models. Prostaglandin plays vital role in inducing pyrexia (Loux et al. 1972) and perception of pain (Geusens et al. 2013). The prostaglandins are products of enzymes COX-1 and COX-2 on ω-3 and
ω-6 C20 fatty acids (Lone and Taskén 2013). Therefore, it may be suggested that Tt.Cr can inhibit cyclooxygenase enzymes thus limiting the amount of prostaglandin available in hypothalamus and at nociceptors.

The witnessed spasmolytic outcomes of *T. triquetra* on gastrointestinal, respiratory and cardiovascular systems might be facilitated by blocking Ca\(^{2+}\) channels and possibly provides logical foundation to authenticate the traditional remedial usage of herb. The detected lipoxygenase inhibition could endorse its ancient use in pain and inflammation. In the current study, crude extract is used, which comprises numerous components. Thus, advanced research must be conducted to isolate principal bioactive constituents accountable for the activities mentioned earlier and establish the test compound’s safety and toxicity.

References


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