Folkloric uses of *Jatropha gossypifolia* in emesis and gut motility disorders: Pharmacological validation
Folkloric uses of *Jatropha gossypifolia* in emesis and gut motility disorders: Pharmacological validation

Musaddique Hussain¹, Shahid Masood Raza¹ and Abdul Majeed²

¹School of Pharmacy, The University of Faisalabad, Faisalabad, Pakistan; ²Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan.

**Abstract**

*Jatropha gossypifolia* is used in folkloric system to manage emesis and gastrointestinal motility disorders such as constipation and diarrhea. The present study was undertaken to provide pharmacological evidences for these folkloric uses by *in vivo* and *in vitro* experimental setting. Ethanolic extract of *J. gossypifolia* showed the significant antiemetic potential (p<0.05) against different emetogenic stimuli, when compared with chlorpromazine. The extract (0.01-1.0 mg/mL) on application to isolated rabbit jejunum preparation exerted spasmogenic effect, followed by the spasmolytic effect (3-10 mg/mL). In the presence of atropine, spasmogenic effect was blocked while spasmolytic effect was emerged, suggesting the involvement of muscarinic receptor activation. *J. gossypifolia* caused relaxation of high K⁺ (80 mM)-induced contraction and shifted the Ca²⁺ concentration-response curve to the right in a manner similar to verapamil, thus conforming its Ca²⁺-channel blocking activity. These findings provide pharmacological validation for the presence of antiemetic and gut modulator (spasmogenic and spasmytic) activates, validating its folkloric uses.

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**Introduction**

Constipation is a heterogeneous condition associated with infrequent bowel movements and/or difficulty in defecation or both (Talley et al., 2003). Untreated constipation may cause urinary and gastrointestinal disorders (Imanzadeh et al., 2012).

Emesis is protective reflex which expels out the toxic substances from the stomach and intestine, and is associated with indigestion of toxicants, cancer chemotheraphy, post-operative procedures and drug adverse effects (Gilman and Goodman, 2001). Commercially available antiemetic may cause excessive sedation, dysphoria, extra-pyramidal signs, and hallucination (Griffith et al., 1996).

*Jatropha gossypifolia* Linn. (Euphorbiaceae) is a bushy gregarious shrub known as tua-tua (English) and laal bagharenda (Local) and is native to Brazil but cultivated as an ornamental plant in all over world (Khare, 2007).

Phytochemical investigation revealed the presence of histamine, triterpenes, alkaloids, flavonoids, jatropholone A, jatrophastrione, tannins, macrocyclic diterpene, jatropline, jatrodien, gossypiline (Das et al., 1998; Matsuse et al., 1999), latex contains cyclogossine A and B (Morton, 1980) whereas seeds contain hydroxyl jatrophone and jatrophonolones A and B (Horsten et al., 1996).

*J. gossypifolia* has been reported to have hepatoprotective, anti-inflammatory, anticancer, anti-septic, anti-fertility and antipyretic activities (Panda et al., 2009; Falodun et al., 2012). Decoction of the plant has been used as purgative, anti-diarrheal, antiemetic, antidote for snakebite and blood purifier (Kirtikar and Basu, 1996; Khare, 2007). The present study was aimed to pharmacologically evaluate the possible mechanism
of action *J. gossypifolia* in emesis and gastrointestinal motility disorders such as constipation and diarrhea.

**Materials and Methods**

**Collection and extraction of *J. gossypifolia***

Fully grown aerial parts of *J. gossypifolia* were collected in the month of November (2013) from the botanical garden of The University of Faisalabad and were identified by the Curator, Botany Department of same University. Adulterants free and electrically grinded coarse powdered material (#40) was subjected to triple maceration (Hussain et al., 2013), with 80% ethanolic-aqueous mixtures to produce the ethanolic extract of *J. gossypifolia*.

**Animals used and their housing conditions**

Animals (♂/♀) including, Swiss albino mice (32-48 g, 4-5 weeks old), chicks (75-85 g, 10-15 days old) and rabbits (1.2-1.7 kg, 8-10 months old) were used and were provided with standard diet and *ad libitum* tap water under controlled environmental condition (25-28°C). Chicks were housed in plastic cages with sawdust as bedding with 12 hours/12 hours dark-light cycle.

**Chemicals and drugs used**

Research grade and highest purity chemicals, solvents, and drugs were used in the experimental study and were purchased from the Sigma Chemicals (USA), Merck (Germany), Sharlab Labs (Spain) and Highnoon Pharmaceutical (Pakistan).

**Acute toxicity test**

Acute toxicity testing was performed by orally administering the 2, 4 and 6 g/kg of ethanolic extract to the mice, as described previously (Hussain et al., 2015a).

**In vivo estimation of antiemetic potential**

Chick emetic model was adopted to estimate the antiemetic potential of the extract (Eda et al., 2005; Hussain et al., 2015b). Acclimatize and healthy chicks were separated in 8 groups of 4 chicks in each group. Chicks of Group 1 (control) and Group 2 (standard) were given an oral dose of normal saline (0.9%) and chlorpromazine (150 mg/kg body weight) respectively, while the chicks of Group 3 and 4 (experimental) were given an oral dose of 50 and 100 mg/kg body weight of ethanolic extract respectively, dissolved in 10 mL/kg of 0.9% saline containing 5% DMSO and 1% tween 80. After 15 min, 50 mg/kg body weight of copper sulfate was administered orally to chicks of all groups. Same process was repeated by using fresh juice of *Brasica campestris* (10 mg/kg; oral), instead of copper sulfate while other protocols remain same as before, then number of retches was counted for the next 15 min. The percentage retching inhibition was calculated as:

\[
\text{Retching inhibition (\%)} = \frac{(A-B)}{A} \times 100
\]

Where, A and B represents the frequency of retching in control and experimental groups respectively.

**In vitro assessment of spasmogenic and spasmolytic activities**

Possible presence of spasmogenic and spasmolytic activity was assessed by applying ethanolic extract on isolated rabbit jejunal segments (2-3 cm) hanged in tissue baths containing 10 mL of Tyrode’s solution with following composition (mM): KCl (2.68), NaCl (136.9), MgCl₂ (1.05), NaHCO₃ (11.90), Na₂HPO₄ (0.42), CaCl₂ (1.8) and glucose (5.55), aerated with carbogen (37°C) (Gilani et al., 1994; Janbaz et al., 2014). A preload of 1 g was applied and jejunum responses were recorded through isotonic transducers by Power Lab Data Acquisition System (AD Instrument, Sydney, Australia) attached to a computer installed with Lab Chart Reader Software (version 7). The Jejunum tissues were allowed to stabilized (spontaneous rhythmic contraction) for at least 30 min. After 30 min, ethanolic extract was added to stabilized jejunum segment in a cumulative fashion, without prior addition of any agonist for the testing of spasmogenic and/or spasmolytic effect (Hussain et al., 2014). Contractile effect of the extract was calculated as the percent of the maximum effect produced by 1.0 μM of acetylcholine.

**Assessment and conformation of Ca²⁺-channel blocking activity**

For the assessment of calcium channel blockade, jejunal preparations were depolarized by exposing to high K⁺ (80 mM), resulting in sustained contraction (Arshad et al., 2012; Hussain et al., 2015c). Ethanolic extract was applied in a cumulative manner to the sustained contractions to achieve concentration-dependent inhibitory response (Farre et al., 1991). The observed relaxant effect of the extract on K⁺ (80 mM)-induced contraction was expressed as percent of the control response mediated by K⁺.

Calcium channel blocking effect of the ethanolic extract was confirmed by the previously reported method (Gilani et al., 2005). The isolated rabbit jejunal preparations were allowed to stabilize in normal Tyrode’s solution, which were subsequently replaced with Ca²⁺-free Tyrode’s solution to which EDTA (0.1 mM) was added for 30 min, in order to remove calcium from the tissues. This bath solution was further replaced with K⁺-rich and Ca²⁺-free Tyrode’s solution, having the following composition (mM): MgCl₂ (1.05), KCl (50), NaHCO₃ (11.90), NaCl (91.04), glucose (5.55), Na₂HPO₄ (0.42), and EDTA (0.1). Subsequent to an incubation period of 30 min, cumulative Ca²⁺ concentrations were applied to the tissue bath to obtain control calcium dose-response curves. On achievement of the
superimposable control calcium dose-response curves (usually after two cycles), the tissues were then washed and incubated with the plant extract for 60 min. Then concentration response curves of Ca\(^{2+}\) were constructed and compared to the control curves. The concentration response curves for Ca\(^{2+}\) were developed in the presence of different concentrations of the plant to assess a possible Ca\(^{2+}\) channel blocking effect (Bolton, 1979).

**Statistics**

All data was expressed as mean ± SEM of triplicate. EC\(_{50}\) values were given with 95% confidence intervals (95% CI) and the logarithmic dose response curves of different treatments were then plotted using computer software “Graph pad Prism” version 6, (Graph Pad Software, San Diego, CA, USA). Concentration-response curves were analyzed by nonlinear regression sigmoidal response curve (variable slope). Unpaired Student t-test was used for assessment of the observations. p<0.05 and p<0.005 were believed to be statistically significant and most significant values respectively.

**Results**

**Acute toxicity**

Acute toxicity testing of *J. gossypifolia* (2, 4 and 6 g/kg) indicates that the extract is safe up to the maximal tested dose (6 g/kg) because there was no mortality and no toxic effects, such as anorexia, diarrhea and gastrointestinal spasms.

**Antiemetic potential**

The chicks of control groups, to whom normal saline (10 mL/kg) was administered, followed by copper sulfate and fresh juice of *B. compestris*, showed the numbers of retches 78 ± 1.10 and 77 ± 1.11 respectively. While in the chicks of the experimental groups (75 mg/kg), number of retches were reduced with %inhibition of emesis of 53.9 and 49.4, whereas at the dose of 150 mg/kg, number of retches were significantly reduced with %inhibition of emesis of 74.4 and 76.6 followed by copper sulfate and fresh juice of *B. compestris* respectively, which were comparable to standard drug chlorpromazine, indicating the antiemetic potential of *J. gossypifolia* (Table I).

**Spasmogenic and spasmolytic effect**

In spontaneously contracting isolated rabbit jejunum preparations, the ethanol extract showed the spasmodic effect in concentration range of 0.01-1.0 mg/mL, which did not sustained and was subsequently followed by spasmolytic effect at the next higher concentration (3-10 mg/mL) with EC\(_{50}\) value of 1.9 mg/mL (95% CI: 1.40-2.40, n=3) (Figure 1). The observed contractile responses to *J. gossypifolia* was expressed as percentage of the maximal response to acetylcholine (0.3 µM), i.e. 14.6 ± 1.7, 22.3 ± 0.8, 26.3 ± 1.7, 32.3 ± 1.2, and 10.2 ± 5.2 (mean ± SEM, n=3), at the concentration range of 0.01, 0.03, 0.1, 0.3 and 1.0 mg/mL, respectively (Figure 2). In

![Figure 1: Concentration-response curves, showing the effect of *J. gossypifolia* in the absence and presence of atropine (0.1 µM) on spontaneously contracting rabbit jejunum preparation. The spasmodic responses are expressed as percent of acetylcholine maximum (values are expressed as mean ± SEM, n=3)](image)

### Table I

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>Copper sulfate-induced</th>
<th>Brasica-induced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean number of retches</td>
<td>%Inhibition</td>
</tr>
<tr>
<td>Control (10 mL/kg)</td>
<td>78 ± 1.1</td>
<td>___</td>
</tr>
<tr>
<td>Chlorpromazine (150 mg/kg)</td>
<td>44 ± 0.3</td>
<td>43.6(^a)</td>
</tr>
<tr>
<td>Ethanolic extract of <em>J. gossypifolia</em> (75 mg/kg)</td>
<td>36 ± 1.3</td>
<td>53.8(^a)</td>
</tr>
<tr>
<td>Ethanolic extract of <em>J. gossypifolia</em> (150 mg/kg)</td>
<td>20 ± 0.8</td>
<td>74.4(^b)</td>
</tr>
</tbody>
</table>

\(^a\) p<0.05 and \(^b\) p<0.005 vs control showing significant and most significant values by using student’s t-test. 
In the presence of atropine (0.1 μM), spasmogenic effect was abolished while the spasmolytic effect was observed with EC₅₀ value of 1.7 mg/mL (95% CI: 0.32-2.95, n = 3).

J. gossypifolia was unable to relax the K⁺ (25 mM)-induced spastic contractions, but exhibited complete relaxation of K⁺ (80 mM)-induced contractions in isolated rabbit preparations, with EC₅₀ value of 1.4 mg/mL (95% CI: 0.72-1.95, n=3). Verapamil (standard drug) inhibited the K⁺ (80 mM)-induced contractions, with EC₅₀ value of 0.14 mg/mL (95% CI: 0.07-0.45, n=3) (Figure 3). Moreover, pretreatment of the isolated rabbit jejunum preparations with J. gossypifolia caused a rightward shift of the Ca²⁺-concentration response curves at the dose range of (0.1-1.0 mg/mL, n=3), in a manner similar to that of verapamil (Figure 4).

Discussion

Ethanolic extract of J. gossypifolia was tested for acute toxicity on healthy mice at oral dose ranges of 2, 4 and 6 g/kg and it was found that J. gossypifolia is safe because it did not produce lethality, behavioral changes and toxic effect, even at the dose range of 6 g/kg, which is more than normal therapeutic dose range. J. gossypifolia has folkloric repute for use in the management of emesis in traditional system of medicine; hence, in vivo pharmacological study was carried out by adopting chick emetic model to evaluate its possible antiemetic effect. Emesis is either caused by the activation of the vomiting centre located in medulla oblongata or by the activation of motor pathway. Additionally, signals from following four principal regions, i.e., chemoreceptor trigger zone, GIT, cortex,
thalamus, cerebral and vestibular) region may also cause emesis (Hussain et al., 2015a). The chemoreceptor trigger zone is in the proximity to medulla and it is not protected by the blood brain barrier (Becker, 2010). Results clearly indicate that J. gossypifolia (150 mg/kg) showed more significant antiemetic response against copper sulfate and B. compestris induced emesis which was comparable to chlorpromazine. So, it can be hypothesized that the antiemetic effect of J. gossypifolia can be likely mediated through inhibition of chemoreceptor trigger zone while other mechanism cannot be ruled out.

For the inspection of conventional uses of J. gossypifolia in gastrointestinal motility disorders, pharmacological study of J. gossypifolia was carried out on spontaneously contracting isolated rabbit jejunum preparations, to evaluate its possible effect and mechanism of action. Initially it produced the spasmodic effect (jejenum contractile effect), which is generally mediated through the action of acetylcholine on cholinergic system (Janbaz et al., 2012; Hussain et al., 2014). For the confirmation of this mechanism, spontaneously contracting rabbit jejunum preparations were pre-exposed to 0.1 mM of atropine (Muscarinic receptor blocker) (Ghayur and Gilani, 2005), which resulted in blocking the stimulatory effect of extract, indicating that the J. gossypifolia exerted the gut stimulation via cholinergic pathway (Hussain et al., 2015b). Acetylcholine (neurotransmitter) acts on the M3 muscarinic receptor and regulate the peristaltic movement of GIT while atropine antagonize the muscarinic receptors (Brown and Taylor, 1996). The observed spasmodic effect of J. gossypifolia validates its traditional use as anti-constipative agent in the hypomotility disorder of the gut. The spasmodic effect was followed by the spasmolytic effect at next higher doses of the extract, indicating the co-existence of spasmodic and spasmolytic constituent(s), which is probably meant by the nature not to allow the spasmodic effect going beyond the constipation, particularly at higher doses (Janbaz et al., 2012).

Addition of J. gossypifolia to the tissue bath completely relaxed the K+ (80 mM)-induced contractions, suggesting that relaxant activity might be due to blockade of Ca2+ channels (Gilani et al., 2005). The contractile elements in rabbit jejunum are activated through increase in cytoplasmic free Ca2+ concentration via opening of the voltage dependent Ca2+ L-type channels (Karaki et al., 1997) or release of Ca2+ from sarcoplasmic stores (Godfraind et al., 1986). The extract exhibited relaxant effect through Ca2+ channels blockade and further inhibition of sequences of events including decrease of cytosolic Ca2+ concentration, decrease in Ca2+ binding to calmodulin, decrease in Ca2+-calmodulin complex formation, decrease in activation of myosin light chain kinase, decrease in phosphorylation of myosin light chains, decrease in interaction between actin and myosin and inhibition of contractile process. These speculations were confirmed as pretreatment of rabbit jejunum preparation with extract caused rightward shift of Ca2+-concentration response curves, like that caused by verapamil (Fleckenstein, 1977).

J. gossypifolia exhibited the anticholinergic and Ca2+ channels blocking activities, which can be attributed due to presence of alkaloids and flavonoids, as these phyto-constituents have been reported to possess the anticholinergic (Broadley, 1996) and Ca2+ channel blocking (Revuelta et al., 1997) activities, respectively, while other mechanisms and phytoconstituents cannot be neglected.

**Conclusion**

This study provides the sound pharmacological background for the traditional use of J. gossypifolia as aperients (mild laxative) and anti-diarrheal drug, mediated possibly through the activation of M3-muscarinic receptors and blockade of voltage-dependent Ca2+ channels respectively. The observed results also validate its folkloric use in emesis.

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**Conflict of Interest**

Authors declare no conflict of interest

**References**


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Author Info

Musaddique Hussain (Principal contact)
e-mail: musaddique.ph@gmail.com