Interaction of aqueous leaf extract of *Aegle marmelos* (L.) Corr. with cholinergic, serotonergic and adrenergic receptors: An *ex vivo* study

Sanjeev Kumar, Rakesh Kumar Mahaseth, Mukesh Tiwari, Ratika Sehgal, Preety Rajora, Rajani Mathur

**ABSTRACT**

**Objectives:** The aim was to study interaction of aqueous leaf extract of *Aegle marmelos* (AM) with cholinergic, serotonergic, and adrenergic receptor systems using appropriate rat tissues—ileum, fundus and tracheal chain, respectively.

**Materials and Methods:** Cumulative concentration-response curves (CRC) were constructed at various doses on each tissue for AM and respective standard agonist. The CRC was again plotted in presence and absence of respective standard antagonist to confirm the interaction of receptor system and AM.

**Results:** AM induced concentration-dependent contractions in isolated rat ileum (0.2–6.4 mg/ml) and fundus (0.2–3.2 mg/ml) that were inhibited significantly (*P* < 0.05) in the presence of atropine (10⁻⁷ M) and ketanserin (10⁻⁶ M), respectively. The relaxant effect, produced by AM (0.2 mg/ml) on carbachol (10⁻⁵ M) precontracted rat tracheal chain, was also inhibited significantly (*P* < 0.05) by propranolol (1 ng/ml).

**Conclusion:** It may be concluded that AM possesses agonistic activity on cholinergic, serotonergic and adrenergic receptors.

**KEY WORDS:** *Aegle marmelos*, fundus, ileum, single channel organ bath, tracheal chain

**Introduction**

The use of plants as a source of herbal medicine has been an innate and vital aspect of India’s healthcare system. Like allopathic drugs, herbal medicines also have different pharmacokinetic and pharmacodynamic properties, which ultimately lead to therapeutic responses, but sometimes cause adverse actions and/or drug-herbal interactions. Understanding the interaction of herbal medicine with various receptor systems can provide the basis for their rational therapeutic use. Many adverse effects of drugs and drug toxicities can be anticipated by understanding the mechanism(s) of action of the drug, its pharmacokinetics, and its interactions with various receptor systems.[1]

*Aegle marmelos* (AM) (L.) Corr. (Rutaceae) is commonly known as bael/bilva, which is considered as a sacred tree by the hindus. The bael tree has great mythological significance, and the leaves of the tree are traditionally used as sacred offerings to Lord Shiva. Various pharmacological properties of AM such as hypoglycemic, hypolipidemic, antioxidant, analgesic, antiinflammatory, hepato-protective have been known for a long time, and these have been corroborated in pharmacological studies.[2-7]

Despite wide variety of therapeutic use in the traditional system of medicine and their validation in pharmacological studies, no information about its interaction with receptor system is available. Due to the presence of multiple active constituents in medicinal plants, receptor binding studies are not suitable for evaluating their interactions with receptor systems. The objective of this study was to carry out preliminary investigation using *ex vivo* preparations to study the interaction of aqueous leaf extract of AM (L.) Corr. with cholinergic, serotonergic and adrenergic receptor systems.

**Materials and Methods**

**Drugs and Reagents**

The drugs used were 5-hydroxytryptamine and ketanserin (+) tetratrate (Sigma Eldrich, India), acetycholine hydrochloride and atropine sulphate (Himedia, India), isoprenaline and propranolol
HCl (Samarth Pharma, India) and carbachol (CCh) (TCI Chemicals, India). Other chemicals, including the reagents used in the preparation of physiological solutions were of analytical grade.

**Plant Material and Preparation of Extract**

Fresh leaves of the plant were collected, identified (NHCP/NBPGR/2009–21), weighed, washed, dried in the shade and powdered. The powder was mixed with distilled water (1:10 w/v) and stirred at room temperature for 2 h. The extract was then filtered, frozen at -20°C and lyophilized at 0.8, 1.6 and 3.2 mg/ml) alone and AM in presence of atropine (10⁻⁶ M) on CCh (10⁻⁵ M, as identified) precontracted tracheal chain alone and also in the presence of propranolol (1 ng/ml) was recorded. Further response of AM (0.2 mg/ml) on CCh (10⁻⁵ M) contracted tracheal chain alone and also in the presence of propranolol (1 ng/ml) was recorded.

Doses were selected by titrating various concentrations of AM starting from 1 μg/ml and increasing by ×10 in each step.

**Statistical Analysis**

All the data were expressed as mean ± SEM (n = 3). Student *t*-test was used to compare the two groups. Significance was accepted at *P* < 0.05.

**Results**

AM tested positive for the presence of alkaloids, cardiac glycosides, terpenoids, reducing sugars, saponins, tannins, steroids, flavonoids, and proteins.

In isolated rat ileum, ACh as well as AM induced concentration-dependent contractions. In the presence of atropine (10⁻⁷ M), a rightward shift in the CRC of ACh [Figure 1a] as well as AM [Figure 1b] was recorded. The EC₅₀ of ACh in presence of atropine (4.56 ± 0.73 × 10⁻⁸ M) was significantly higher (*P* < 0.05) than EC₅₀ of ACh alone (8.63 ± 4.38 × 10⁻⁹ M). Similarly, the EC₅₀ of AM in the presence of atropine (3.30 ± 0.39 mg/ml) was significantly higher (*P* < 0.05) than EC₅₀ of AM alone (0.78 ± 0.12 mg/ml).

The CRC of AM was on the right side of ACh [Figure 1c] and the EC₅₀ of AM (0.78 ± 0.12 mg/ml) was significantly higher (*P* < 0.05) than that of ACh (1.57 ± 0.8 × 10⁻⁶ mg/ml). Moreover, the maximal response of AM (1053.82 ± 18.40 mg) was found to be significantly less (*P* < 0.05) than that of ACh (1491.01 ± 79.60 mg) [Figure 1c].

Concentration-dependent contractions were induced by 5-HT as well as AM in isolated rat fundus and both showed a rightward shift in CRC in the presence of ketanserin (10⁻⁶ M) [Figure 2a and b]. The EC₅₀ of 5-HT after ketanserin (3.6 ± 0.15 × 10⁻⁸ M) was significantly higher (*P* < 0.05) than EC₅₀ of 5-HT alone (1.0 ± 0.37 × 10⁻⁹ M). The EC₅₀ of AM after ketanserin (10⁻⁶ M) (0.62 ± 0.03 mg/ml) was significantly higher (*P* < 0.05) than EC₅₀ of AM alone (0.28 ± 0.03 mg/ml). The CRC of AM was shifted to the right side of 5-HT [Figure 2c] and the EC₅₀ of AM (0.28 ± 0.03 mg/ml) was significantly higher (*P* < 0.05) than that of 5-HT (4.1 ± 1.5 × 10⁻⁶ mg/ml). The maximal response of AM (827.44 ± 69.24 mg) was found significantly lesser (*P* < 0.05) than that of 5-HT (1110.93 ± 35.83 mg) [Figure 2c].

Isoprenaline (10⁻⁶ M) exerted relaxant effect of 81.45% on rat tracheal chain precontracted with CCh (10⁻⁵ M). In the presence of propranolol (1 ng/ml), the relaxant effect of these rings were connected in series by means of short loops of silk thread to form a tracheal chain according to the method described in Kulkarni. The tracheal chain was mounted in organ bath and was allowed to equilibrate in Kreb’s solution maintained at 37°C and constantly bubbled with carbogen for 45 min and a CRC was obtained with CCh (10⁻⁸–10⁻¹ M) to identify the sub-maximal concentration. The response of isoprenaline (10⁻⁶ M) on CCh (10⁻⁵ M, as identified) precontracted tracheal chain alone and also in the presence of propranolol (1 ng/ml) was recorded.

**Animals**

Male Wistar albino rats weighing 150–250 g were selected for the study and housed in standard environmental conditions (temperature 25°C ± 2°C and 12 h dark and light cycle) and fed with rodent pellet diet and drinking water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC/2013/18).

**Tissue Preparation**

Overnight fasted rats with free access to water were euthanized under CO₂ and dissected. After isolation, ileum was placed in Tyrode’s solution, whereas trachea and fundus were placed in Kreb’s solution. Tissue preparation was performed as per the method described below and responses of the tissues were recorded using single channel organ bath (50 mL) (Radnoti Single Channel Organ Bath, USA) and data was acquired using polyVIEW 16 (version 1.1) Data Acquisition System (Grass Technologies, USA).

**Ileum**

Ileum was cleaned off from extraneous tissue and lumen was cleaned with gentle care by flushing the Tyrode’s solution into it. Ileum (1 cm) was mounted in the organ bath maintained at 37°C and constantly bubbled with carbogen and allowed to equilibrate for 30 min. Subsequently, concentration response curves (CRC) of acetylcholine (ACh) (10⁻⁵–10⁻¹ M) alone, ACh in the presence of atropine (10⁻⁷ M), AM (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) alone and AM in presence of atropine (10⁻⁷ M) were recorded.

**Fundus**

The fundus portion of the stomach was cut and opened along the lesser curvature into a sheet, and a 1 cm long strip was prepared by cutting along the longitudinal fibers. The strip was mounted and allowed to equilibrate in Kreb’s solution maintained at 37°C and constantly bubbled with carbogen for 45 min in the organ bath. Subsequently, CRC of 5-HT (10⁻⁸–10⁻³ M) alone, 5-HT in the presence of ketanserin (10⁻⁶ M), AM (0.2, 0.4, 0.8, 1.6 and 3.2 mg/ml) alone and AM in presence of ketanserin (10⁻⁸ M) were recorded.

**Tracheal Chain**

The tracheal tissue was cleaned of extraneous tissue and cut into 5–6 rings, each containing 2–3 cartilaginous rings.
Kumar, et al.: Interaction of Aegle marmelos with receptor systems

isoprenaline ($10^{-6}$ M) was reduced significantly ($P < 0.05$) to 32.97% [Figure 3a]. AM (0.2 mg/ml) produced relaxant effect of 66.81% on CCh ($10^{-5}$ M) precontracted tracheal chain. The relaxant effect was reduced significantly ($P < 0.05$) to 36.23% in the presence of propranolol (1 ng/ml) [Figure 3b].

**Discussion**

In the present study, the activity of AM was significantly inhibited in the presence of atropine confirming the presence of cholinergic (ACh-like) components in AM. Moreover, the CRC of AM being on the right side of ACh and higher $EC_{50}$ of AM than that of ACh indicate lesser potency of AM when compared with ACh and also, the lower maximal response of ileum to AM indicates its lesser efficacy than ACh. The ileum is supplied with cholinergic nerves that produce contractions via muscarinic receptors, and these cholinergic nerves play an important role in the regulation of gastrointestinal motility.[9] The agonistic action of AM on cholinergic receptor may explain the medicinal use of leaves of AM as mild laxative.

The contractile effect of 5-HT on rat fundus is mediated through serotonergic receptors[10] and is competitively blocked by ketanserin.[11] The dose-dependent contractile effect of AM on isolated rat fundus was inhibited significantly by ketanserin confirming its action on serotonergic system as one of the possible mechanisms. It has been reported that methanolic extract of AM leaves possesses potential anxiolytic and antidepressant activities.[12] Further, it has been proposed, that the stimulation of 5-HT$_2$ receptor produces anxiolysis.[13] The agonistic action of AM on serotonin receptor as demonstrated by this study may explain the basis for reported use of AM in anxiety and depression.

Isoprenaline as well as AM exerted relaxant effects on precontracted (with CCh) isolated rat trachea. Furthermore, the effects of isoprenaline as well as AM on tracheal chain were reduced significantly in the presence of propranolol. Inhibition of relaxant effect in the presence of a β-blocker, shows that AM possesses an adrenergic component, which relaxed the precontracted tracheal muscle. In a previous study, alcoholic extract of leaves of AM showed relaxant effect on guinea pig tracheal chain and the effect was attributed to the presence of one or more antihistaminic constituents.[14] In our study, aqueous extract was investigated and the relaxant effect on rat tracheal chain may be due the presence of some adrenergic components in the plant. It may be hypothesized that AM shows anti-asthmatic activity due to presence of both components, that is, antihistaminic as well as adrenergic.
Conclusion

In this preliminary study, it was demonstrated that aqueous extract of AM possesses agonistic activity on cholinergic, serotonergic and adrenergic receptors using isolated rat ileum, fundus and tracheal tissue preparations.

References

1. Blumenthal DK, Garrison JC. Pharmacodynamics: Mechanisms of drug action and the relationship between drug concentration and effect. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman’s the Pharmacological Basis of Therapeutics. 12th ed. New York: McGraw-Hill; 2011. p. 31.
2. Ponnachan PT, Paulose CS, Panikkar KR. Effect of leaf extract of Aegle marmelose in diabetic rats. Indian J Exp Biol 1993;31:345-7.
3. Narender T, Shweta S, Tiwari P, Papi Reddy K, Khalig T, Prathipati P, et al. Antihyperglycemic and antidyslipidemic agent from Aegle marmelos. Bioorg Med Chem Lett 2007;17:1808-11.
4. Sabu MC, Kuttan R. Antidiabetic activity of Aegle marmelos and its relationship with its antioxidant properties. Indian J Physiol Pharmacol 2004;48:81-8.
5. Shankarananth V, Balakrishnan N, Suresh D, Sureshpandian G, Edwin E, Sheeja E. Analgesic activity of methanol extract of Aegle marmelos leaves. Fitoterapia 2007;78:258-9.
6. Arul V, Miyazaki S, Dhananjayan R. Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr. J Ethnopharmacol 2005;96:159-63.
7. Singanan V, Singanan M, Begum H. The hepatoprotective effect of bael leaves (Aegle marmelos) in alcohol induced liver injury in albino rats. Int J Sci Tech 2007;2:83-92.
8. Kulkarni SK. Handbook of Experimental Pharmacology. 5th ed. New Delhi (India): Vallabh Prakashan; 2005. p. 95-6.
9. Makhlouf GM, Murthy KS. Cellular physiology of gastrointestinal smooth muscle. In: Johnson LR, editor. Physiology of the Gastrointestinal Tract. London: Elsevier Acad Press; 2006. p. 499-522.
10. Cox DA, Cohen ML. 5-HT2B receptor signaling in the rat stomach fundus: Dependence on calcium influx, calcium release and protein kinase C. Behav Brain Res 1996;73:289-92.
11. Béjar E, Malone MH. Inhibitory effect of 5-hydroxytryptamine on rat stomach fundus: Mediated indirectly by activation of noradrenaline release. J Pharm Pharmacol 1995;47:637-42.
12. Kothari S, Minda M, Tonpay SD. Anxiolytic and antidepressant activities of methanol extract of Aegle marmelos leaves in mice. Indian J Physiol Pharmacol 2010;54:318-28.

13. Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, et al. SB 242084, a selective and brain penetrant 5-HT2C receptor antagonist. Neuropharmacology 1997;36:609-20.

14. Arul V, Miyazaki S, Dhananjayan R. Mechanisms of the contractile effect of the alcoholic extract of Aegle marmelos Corr. on isolated guinea pig ileum and tracheal chain. Phytomedicine 2004;11:679-83.

Cite this article as: Kumar S, Mahaseth RK, Tiwari M, Sehgal R, Rajora P, Mathur R. Interaction of aqueous leaf extract of Aegle marmelos (L.) Corr. with cholinergic, serotonergic and adrenergic receptors: An ex vivo study. Indian J Pharmacol 2015;47:109-13.

Source of Support: Government of N.C.T. of Delhi. Conflict of Interest: No.