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Evaluation of acute toxicity and *in-vitro* anti-spasmodic activity of Caspa drops, an ayurvedic formulation

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Abstract

Infantile colic is a very common condition appears to pediatricians. It is also known as baby colic. Common factors involved in the etiopathogenesis of infantile colic are such as intolerance to food like milk protein and lactose; neuro-hormonal immaturity, maternal anxiety etc. Generally, conventional anti-spasmodics (like atropine, dicyclomine, hyoscyamine) are used to treat gastric spasm, but safety is always the major concern in that case. In the present study, an attempt was made to evaluate safety (by acute toxicity study) and efficacy (by *In-vitro* anti-spasmodic experiment) of Caspa drops – An Ayurvedic proprietary formulation. Acute toxicity study was carried out as per OECD guideline 425. *In-vitro* anti-spasmodic activity was evaluated by KCl, BaCl₂ and Acetylcholine (Ach) induced contraction in rat ileum. Test drug was evaluated for efficacy at two different concentration levels, 0.5% and 1.0%. As per the results, no animal manifested any signs of toxicity during cage side observation and mortality at 20 mL/kg. LD₅₀ of Caspa drops was higher than 20 mL/kg. Caspa drops showed smooth muscle relaxation in a dose dependent manner. 1.0% concentration showed highly significant effect against KCl, BaCl₂ and Ach - induced smooth muscle contraction. Test drug was also compared with established conventional standard drugs. From the data it can be assumed that Caspa drops have anti-spasmodic activity through inhibition of muscarinic effect or calcium channel and also it did not manifest any signs of toxicity.

Keywords: Caspa drops, acute toxicity, *In-vitro* anti-spasmodic activity, ileum smooth muscle contraction, infantile colic

1. Introduction

Infantile abdominal colic, also known as baby colic is defined as episodes of crying for more than three hours a day for more than three days in any week for 3 weeks. Colic begins at about 2 to 3 weeks of age and ends anywhere between 3 and 6 months of age. [1] The several factors involved in the etiopathogenesis of infantile colic such as food intolerance or allergy to milk protein and lactose; intestinal hyperperistalsis, neuro-hormonal immaturity, maternal anxiety, and familial stress. [2] Due to involvement of several factors, it is very difficult to manage infantile colic. Generally, conventional anti-spasmodics (like atropine, dicyclomine, hyoscyamine) are used to treat gastric spasm. But, they are reported for side effects in children like unusual excitement, nervousness, restlessness, rapid increase in body temperature and dryness [3]. Safety of conventional anti-spasmodics is a burning issue in the management of infantile abdominal colic.

Caspa drops is a proprietary Ayurvedic formulation which contains extracts of *Anethum sowa* (Soa) fruit, [4-6] *Acorus calamus* (Vacha) rhizome, [7, 8] *Plumbago zeylanica* (Chitrak) root, [9,10] *Apium graveolens* (Ajmoda) fruit, [11,12] *Mentha sylvestris* (Pudina) leaves, [13] *Aconitum heterophyllum* (Ativisha) root tuber, [14] *Caesalpinia crista* (Latakaranj) seed, [15] *Embelia ribes* (Vidang) fruit, [16] *Elettaria cardamomum* (Elaichi) fruit, [17] *Hedychium spicatum* (Shati) rhizome, [18] *Holarrhena antidysenterica* (Indrayav) seed [19, 20] and powders of Kala namak (Black salt), [21] Saindhav (Rock salt), [21, 22] *Ferula foetida* (Hingu) exudate, [23] *Citrus acida* (Nimbuk) fruit juice. [24] It is manufactured and marketed by Vasu Healthcare Pvt. Ltd., Vadodara. The Majority of the ingredients of Caspa Drops are well reported in Ayurvedic texts and scientific research publications for having carminative, digestive and anti-spasmodic properties. However, no such evidences were available which prove the safety and efficacy of their combination. Hence, in the present study, an attempt was made to evaluate acute toxicity and *In-vitro* anti-spasmodic activity of Caspa drops on experimental animals.

2. Materials and methods

2.1 Test drug and chemicals

For experimental purpose, test drug (Caspa drops) was used as it is. For acute toxicity study, single dose of test drug was administered at different dose level and LD₅₀ was evaluated. Two dose level of test drug were taken for experimental study i.e. lower dose - 0.5% and higher dose - 1.0%. Verapamil and atropine were used as standard drugs in suitable experimental model. An EC₅₀ value (concentration of drugs causing half-maximal responses) was established by regression analysis. Tyrode's solution was used as a physiological salt solution (PSS). The composition of Tyrode solution used was: 150 mM NaCl, 2.7 mM KCl, 2.00 mM MgCl₂, 12 mM NaHCO₃, 0.4 mM NaH₂PO₄, 1.8 mM CaCl₂ and 5.5 mM glucose.

2.2 Experimental animals

The experiment protocol described in present study was approved by the Institutional Animal Ethics Committee (IAEC) (Approval No.: SKPCPER/IAEC/2013-02/14) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.: 197/PO/c/2000/CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy adult wistar rats weighing 180-250 g were used for acute toxicity and *in-vitro* anti-spasmodic activity. Rats were housed in polypropylene cages, maintained under standardized condition (12-hour light/dark cycle, 24 °C, 35 to 60% humidity) and provided free access to 'Sabardan' pellet diet and purified drinking water *ad libitum*. The animals were deprived of food for 24 hour before experimentation but allowed free access to water throughout.

2.3 Acute toxicity study

For acute toxicity study, single dose of test drug was administered at different dose levels and LD₅₀ was evaluated. The general behavior and mortality of the rats were continuously monitored for 1 h after dosing periodically during first 24 h (with special attention given during the first 4 h.) and then daily for total 14 days. Changes in the normal activity of rats, sign and symptoms of toxicity and mortality were monitored and recorded. Acute toxicity study was carried out as per OECD Guidelines 425 [24].

2.4 Experimental protocol

The selected animals were sacrificed by cervical dislocation only. No anesthetic agent was used to avoid relaxation responses of the ileum tissue. Tissue of approximately 2 cm in length was cut and mounted in organ bath containing

physiological salt solution (Tyrode's solution). The tissue was maintained at 37 °C and constantly oxygenated. Test drug was directly added into organ bath. Responses were recorded on power lab. Test drug was evaluated against KCl, BaCl₂ and Acetylcholine (Ach) induced contraction in rat ileum. % E_{max} and pD₂ value were calculated [25].

2.5 Statistical analysis

All the data was converted to Mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's test. Significant difference was considered only when $p \leq 0.05$.

3. Results

3.1 Acute toxicity study

The animals did not manifest any signs of toxicity during cage side observation and mortality at 20 mL/kg. LD₅₀ of Caspa drops was higher than 20 mL/kg.

3.2 Effect of Caspa drops on KCl induced contraction in isolated rat ileum

Caspa drops concentration dependently inhibited contraction induced by 60 mM KCl with IC₅₀ = 0.5 ± 0.01%. E_{max} was found 80.98 ± 9.44% and 56.25 ± 2.09% at 0.5% and 1.0% concentration of Caspa drops respectively. 1.0% concentration of Caspa drops showed equivalent E_{max} and pD₂ value with respect to standard drug verapamil at 1 μM/L. Caspa drops at 1.0% concentration showed significant (p<0.001) relaxation in isolated rat ileum. Standard drug verapamil showed significant effect at both concentrations (1 μM/L and 2.5 μM/L) (Table 1). Effects of Caspa drops and verapamil on KCl induced contraction in isolated rat ileum were showed in Fig. 1 and Fig. 2 respectively.

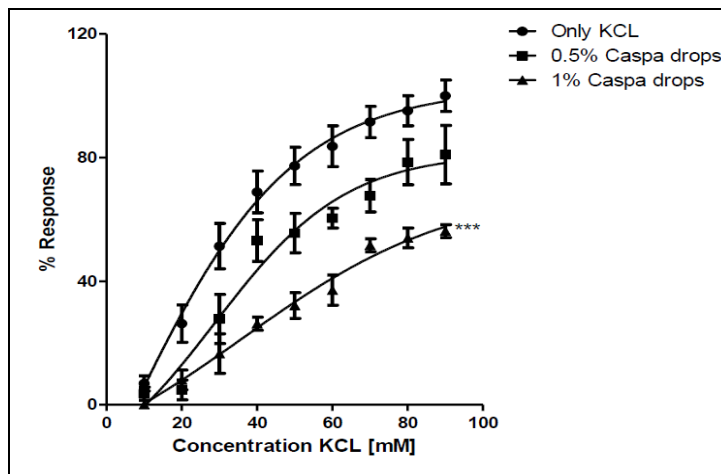
3.3 Effect of Caspa drops on BaCl₂ induced contraction in isolated rat ileum

Caspa drops relaxed the contraction induced by BaCl₂ (5 mM) in a concentration dependent manner, with an IC₅₀ = 0.73 ± 0.04%. Caspa drops showed significant (p<0.001) relaxation at 1.0% concentration in comparison to BaCl₂ induced contraction. At 0.5% concentration, Caspa drops attenuated contraction but not up to significant level. Standard drug verapamil showed significant effect at both concentrations (1 μM/L and 2.5 μM/L) (Table 2). Effects of Caspa drops and verapamil on BaCl₂ induced contraction in isolated rat ileum were showed in Fig. 3 and Fig. 4 respectively.

Table 1: %E_{max} and pD₂ value of Caspa drops and verapamil on KCl induced contraction

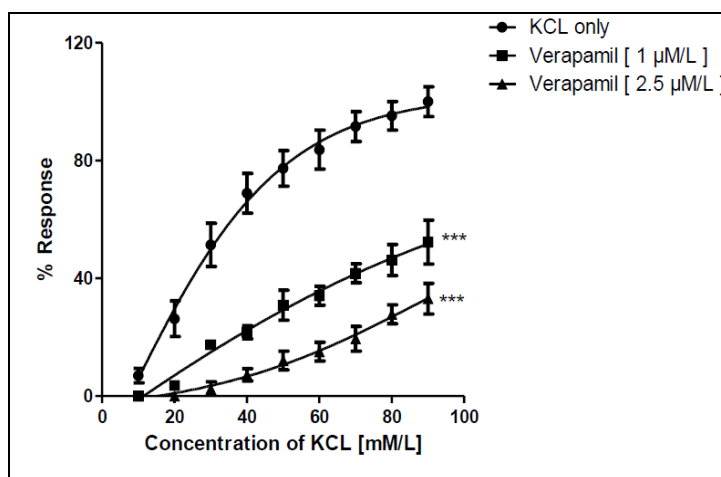
| Group | %E _{max} | pD ₂ value |
|----------------------|-------------------|-----------------------|
| KCl only | 100.01 ± 5.08 | 1.53 ± 0.03 |
| Caspa drops (0.5%) | 80.98 ± 9.44 | 1.44 ± 0.04 |
| Caspa Drops (1.0%) | 56.25 ± 2.09*** | 1.35 ± 0.02*** |
| Verapamil (1 μM/L) | 52.3 ± 7.44*** | 1.33 ± 0.03*** |
| Verapamil (2.5 μM/L) | 33.09 ± 5.21*** | 1.2 ± 0.03*** |

All the values are expressed as mean ± SEM (n=6). ***p<0.001 when compared to KCl only group.



All the values are expressed as mean \pm SEM (n=6). ***p<0.001 when compared to KCl only group.

Fig 1: Effect of Caspa drops on KCl induced contraction in isolated rat ileum



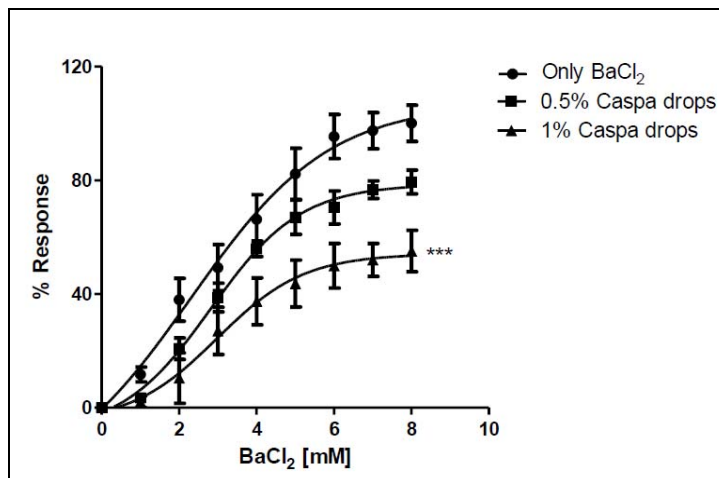
All the values are expressed as mean \pm SEM (n=6). ***p<0.001 when compared to KCl only group.

Fig 2: Effect of verapamil on KCl induced contraction in isolated rat ileum

Table 2: %E_{max} and pD₂ value of Caspa drops and verapamil on BaCl₂ induced contraction

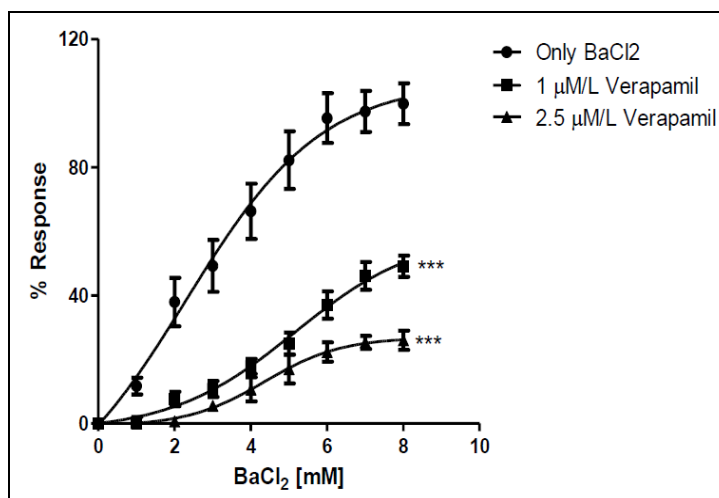
| Group | %E _{max} | pD ₂ value |
|------------------------|---------------------|-----------------------|
| BaCl ₂ only | 99.81 \pm 6.39 | 2.53 \pm 0.06 |
| Caspa drops (0.5%) | 79.36 \pm 4.16 | 2.42 \pm 0.085 |
| Caspa Drops (1.0%) | 55.07 \pm 7.27*** | 2.38 \pm 0.078*** |
| Verapamil (1μM/L) | 49.09 \pm 3.32*** | 2.30 \pm 0.069*** |
| Verapamil (2.5μM/L) | 25.98 \pm 3.04*** | 2.21 \pm 0.071*** |

All the values are expressed as mean \pm SEM (n=6). ***p<0.001 when compared to BaCl₂ only group.



All the values are expressed as mean ± SEM (n=6). ***p<0.001 when compared to BaCl₂ only group.

Fig 3: Effect of Caspa drops on BaCl₂ induced contraction in isolated rat ileum



All the values are expressed as mean ± SEM (n=6). ***p<0.001 when compared to BaCl₂ only group.

Fig 4: Effect of verapamil on BaCl₂ induced contraction in isolated rat ileum

3.4 Effect of Caspa drops on Ach induced contraction in isolated rat ileum

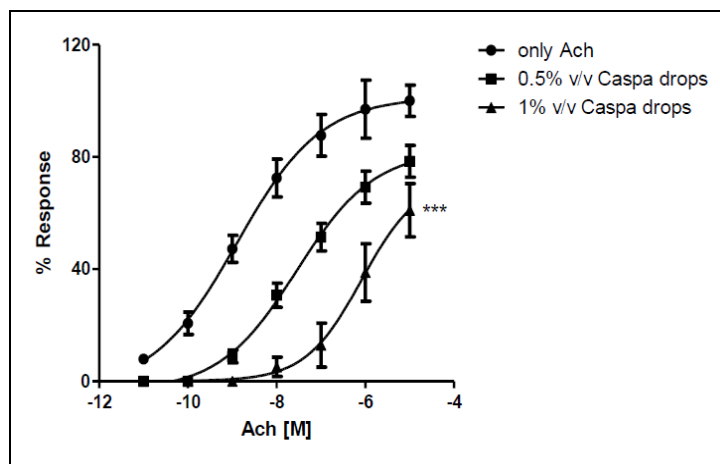
Caspa drops relaxed the contraction induced by Ach (2 µM) in a concentration dependent manner, with an IC₅₀ = 0.91 ± 0.07%. Caspa drops showed significant (p<0.001) effect at

1.0% concentration. Standard drug atropine showed significant effect at both concentrations (1 nM/L and 5 nM/L) (Table 3). Effects of Caspa drops and atropine on Ach induced contraction in isolated rat ileum are shown in Fig. 5 and Fig. 6 respectively.

Table 3: %E_{max} and pD₂ value of Caspa drops and atropine on Ach (acetyl choline) induced contraction

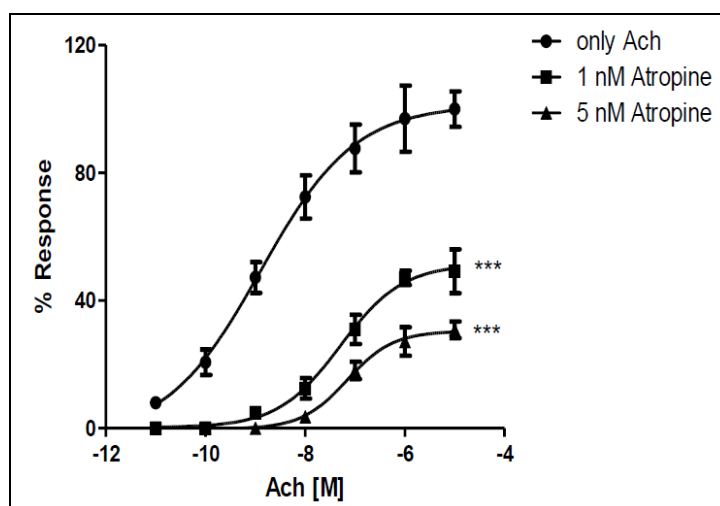
| Group | %E _{max} | pD ₂ value |
|--------------------|-------------------|-----------------------|
| Ach only | 100.00 ± 5.60 | 8.69 ± 0.24 |
| Caspa drops (0.5%) | 78.45 ± 5.68 | 7.45 ± 0.28 |
| Caspa Drops (1.0%) | 61.02 ± 9.48*** | 6.94 ± 0.195*** |
| Atropine (1nM/L) | 49.09 ± 6.85*** | 7.35 ± 0.29*** |
| Atropine (5nM/L) | 30.84 ± 2.59*** | 7.11 ± 0.23*** |

All the values are expressed as mean ± SEM (n=6). ***p<0.001 when compared to Ach only group.



All the values are expressed as mean \pm SEM (n=6). ***p<0.001 when compared to Ach only group.

Fig 5: Effect of Caspa drops on Ach induced contraction in isolated rat ileum



All the values are expressed as mean \pm SEM (n=6). ***p<0.001 when compared to Ach only group.

Fig 6: Effect of atropine on Ach induced contraction in isolated rat ileum

4. Discussion

Drugs that affect lower gastro-intestinal function may act on smooth muscles and/or work by modulating the activity of the enteric nervous system (ENS). Mainly acetylcholine, histamine, 5-hydroxytryptamine, bradykinins, prostaglandins and cholecystikinin are involved which achieve their contractile effect through an increase in cytosolic Ca^{2+} . [26, 27] To clarify the possible underlying mechanism, present study was initiated to check the influence of Caspa drops on KCl, $BaCl_2$ and Ach - induced smooth muscle contraction. Extracellular high K^+ is responsible for membrane depolarization and is the most common method for introduction of Ca^{+2} into cell without receptor stimulation. High concentration of K^+ cause smooth muscle contractions through opening voltage dependent calcium channels (VDCCs) [28]. A substance causing inhibition of high K^+ -induced contractions is considered as a blocker of calcium influx [29]. KCl is producing dose dependent contractile responses in isolated rat ileum. Verapamil is clinically used calcium channel inhibitor and diminished K^+ induced contraction of smooth muscle [30]. Caspa drops at 1.0% concentration causes significant inhibition of high K^+ induced

contraction possibly mediated through calcium channel blockage.

Barium ion (Ba^{+2}) has been known to induce contractions in smooth muscle tissues. Ba^{+2} depolarizes the smooth muscle membrane and opens the voltage-dependent Ca^{+2} channels resulting in a Ca^{+2} influx [31]. Caspa drops at 1.0% concentration exerted a statistically significant inhibition of the contractions induced by $BaCl_2$.

Acetylcholine, a neurotransmitter, is released by the parasympathetic nervous system, and plays an important physiological role in the regulation of gut movements [32]. The acetylcholine induced contractions of the rat ileum involve two different mechanisms coupled to muscarinic receptors. One mechanism activates non-selective cation channels in the plasma membrane, which results in membrane depolarization. The depolarization stimulates Ca^{+2} influx through voltage dependent Ca^{+2} channels. The other mechanism activates contraction by the release of intracellular calcium [33]. Standard drug, atropine abolished the contractile effect of acetylcholine. Caspa drops also showed concentration dependent decrease in percentage response of acetylcholine.

So, it may be assumed that Caspa drops have anti-spasmodic activity through inhibition of muscarinic effect or calcium channel. Another assumption could be that there are different constituents in the Caspa drops that are affecting the nonadrenergic-noncholinergic neurotransmitters. So far pharmacodynamic of any active chemical constituents of Caspa drops has not been established at cellular level however on basis of available scientific literature it can be inferred that constituents like tannins, flavonoids and alkaloids may be playing an important role.

5. Conclusion

The results obtained from the present study indicate that the Caspa drops has significant muscle relaxant effect on isolated rat ileum. The Caspa drops could inhibit ileum contractions induced by potassium, barium and acetylcholine ions. LD₅₀ of Caspa drops was observed more than 20 ml/kg at which it did not produced any signs of toxicity during acute toxicity study and confirmed its safety for human consumption. Further research for identification of compounds responsible for anti-spasmodic activity is recommended.

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