1.8 cineole decreases gastric compliance in anesthetized rats[1]

1.8 cineol diminui a complacência gástrica de ratos anestesiados

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ABSTRACT

Purpose: To study the effect of 1,8 cineoleee components of the essencial oil of *Croton nepetaefolius* - plant of North-East of Brasil, used in the popular medicine for riots of the gastrointestinal tract - on the motor behavior of the gut of Wistar rats. **Methods**: Used 16 male animals under jejun of 24h weighing 300-350g. The effect of 1.8 cineoleee (1 or 3mg/Kg) on gastric compliance had been lead in anaesthetized rats. The variations of the gastric volume (GV), had been measured by plethysmography, while AP, HR and CVP had been monitored continuously by a digital system of data acquisition. **Results**: Observe reduction of the GV, which was significant on 30, 40, 50 and 60min after treatment (2.0±0.1; 1.9±0.1; 1.8±0.1 and 1.7±0.1mL, versus 2.1±0.2mL). The AP presented significant fall after the administration of 1.8 cineoleee, remaining thus during 60min of monitorization (87.9±7.7; 87.6±7.1; 87.9±6.4; 87.8±5.7; 86.0±5.5 and 87.7±6.0mmHg, respectively versus 94.4±6.2 mmHg), as well as the HR (366.3±13.4; 361.7±11.5; 357.3±10.4; 353.0±10.4; 348.3±11.1 and 350.4±13.7bpm, respectively versus 395.2±11.1bpm). The CVP did not suffer significant variations after treatment. **Conclusion**: Observe the 1.8 cineoleee reduces the gastric compliance in anaesthetized rats besides presenting effect hipotensor and bradicardic; probably for direct action on the gastrointestinal and vascular smooth muscel and moduling the autonomic nervous system.

Key words: Croton. Gastrointestinal Motility. Stomach. Rats.

RESUMO

Objetivo: Estudar o efeito do 1.8 cineol, componente do *Cróton nepetaefolius* (planta do Nordeste) comumente usada na medicina popular para distúrbios do trato gastrintestinal (TGI), sobre o comportamento motor do TGI de ratos Wistar anestesiados. **Métodos**: Utilizamos 16 animais machos, pesando entre 300 a 350g. Os estudos de complacência gástrica foram conduzidos em animais sob jejum de 24h. As variações do volume gástrico (VG), foram medidas por pletismografia, enquanto a PA, FC e PVC foram monitoradas continuamente por um sistema digital de aquisição de dados. **Resultados**: Observamos diminuição do VG, o qual foi significativo aos 30, 40, 50 e 60min após o tratamento com 1.8 cineol quando comparado ao perído basal (2,0±0,1; 1,9±0,1; 1,8±0,1 e 1,7±0,1mL, *vs* 2,1±0,2mL). A PA apresentou queda significativa após a administração de 1.8 cineol, mantendo-se assim durante os 60min de monitoração (87,9±7,7; 87,6±7,1; 87,9±6,4; 87,8±5,7; 86,0±5,5 e 87,7±6,0mmHg, respectivamente *vs* 94,4±6,2; mmHg), bem como a FC (366,3±13,4; 361,7±11,5; 357,3±10,4; 353,0±10,4; 348,3±11,1 e 350,4±13,7bpm respectivamente *vs* 395,2±11,1bpm). Já a PVC não sofreu variações significativas durante após o tratamento. **Conclusão**: O 1.8 cineol diminui a complacência gástrica em ratos anestesiados além de apresentar efeitos hipotensor e bradicárdico; provavelmente por ação direta sobre a musculatura lisa gastrintestinal e vascular e modulação do sistema nervoso autônomo.

Descritores: Croton. Motilidade Gastrointestinal. Estômago. Ratos.

Introduction

Croton nepetaefolius Baill is the scientific denomination of a type and common shrub in the Brazilian Northeast popularly known as marmeleiro sabiá, used in popular medicine folk for riots gastrintestinais[1]. Intragastric administration of the essential oil of the Croton nepetaefolius (EOCN) increases the time of gastrintestinal transit of a liquid meal in mice[2]. Already in vitro preparations of the intestinal smooth muscle of cobaio the EOCN demonstrated miorrelaxantes properties, diminishing tônus basal and reducing the amplitude of the spontaneous contractions of segments of íleo as well as of gastro-esophagicus, pyloric and ileo-cecal sphincter of cobaio[3]. These data demonstrate that the EOCN present mio-relaxant and antispasmodic properties in vivo and in vitro, consistent with the use in popular medicine folk[1,4]. We decide, then, to study the actions of the administration i.v of the 1,8 cineoleee, main component of the EOCN[1], on the gastric compliance, arterial pressure, central venous pressure and cardiac frequency of anestesiados rats.

Methods

Male Wistar rats (300-350g, n=16) proceeding from the Central Breathing of the UFC was used. All procedures had been in accordance with the norms of the Brazilian College of Animal Experimentation (COBEA). The 1,8 cineoleee and EOCN was gentily granted by the Laboratory of the Prof. Jose Henrique Leal Cardoso - University State of the Ceará.

Surgical procedures

We kept the animals in jejum for 24h with free access to the water until 2h hours before the experiments. After anesthesia with uretana (1,2g/kg, I.P.), we carry through traqueostomie, followed of the cannula insertion, in order to facilitate the spontaneous ventilation. Next, we insert polyethylene cannulas (PE 50), full of saline solution 0.9% with heparina (500U/mL) in the left vein jugular and the right carotid artery. We destine the vein for administration of drugs or vehicle and monitoration of the central venous pressure while we use the artery for arterial pressure monitoration.

Gastric volume measurements

For the monitoration of the gastric volume (GV), we use the pletismometric model develop in ours laboratory[5]. Initially, we introduce per os, a polyethylene catheter (ED=2,0mm/ID=1,5mm) with a latex balloon (volume ~ 4mL), which was posioned carefully in the proximal stomach. Next, we connect the free extremity of the catheter to liquid reservoir (ID=2,5cm, maximum capacity of 30mL). The reservoir, the stomach and the balloon then, establish a comunicant system vassel, had been filled with conducting solution standard [45mg% of NaCl and 0.3mL% of polietilenoglicol (Imbebiente BBC Ornano®)] at 37°C. Variations in gastric tonus capable to modify the liquid volume in a balloon, had been detected by an electronic volume sensor was continuously registered for one plethysmometer (Ugo Basile®, Comerio, Italy). Given the constant pressure of the system, variations in gastric tonus, means variations on a gastric compliance[5]. In the beginning of each experiment, the stomach was submitted the distention for a pressure of 4cmH_2O, by rising the liquid level of the reservoir until 4cm above of the xifoide appendix of the animal. Variations on gastric compliance had been monitored continuously, written down to each 30s, express in mL.

Monitoration of the cardiovascular parameters

Mean arterial pressure (AP), central venous pressure (CVP) and cardiac frequency (CF) had been gotten by the connection of the arterial and venous cannules, respectively, the pressure transducers that had been connected to a acquisition system of data (PowerLab-ADInstrumesnts®).

Experimental protocols

After surgical procedures, animals had been kept in rest for 30min for the stabilization of the hemodinamics parameters. All the animals had been studied by a period at least 80min, twenty min initial was considered as Basal period, after that 1.8 cineoleee (1 or 3mg/kg - n=11) or vehicle (saline 0.9% - n=5) i.v. administration was perormed. We evaluated GV, AP, CVP and FC by more 60min, which had been divided in equal intervals of 10min, called 10, 20, 30, 40, 50 and 60.

Experimental avaliation

To the end of each experiment, all the animals had suffered, still in plain anaesthetic, medium laparotomie for inspection of the positioning of the balloon was performed, being to follow sacrificed for injection e.v. of solution of KCl. Excluded the data of animals with anomalous localization of the balloon.

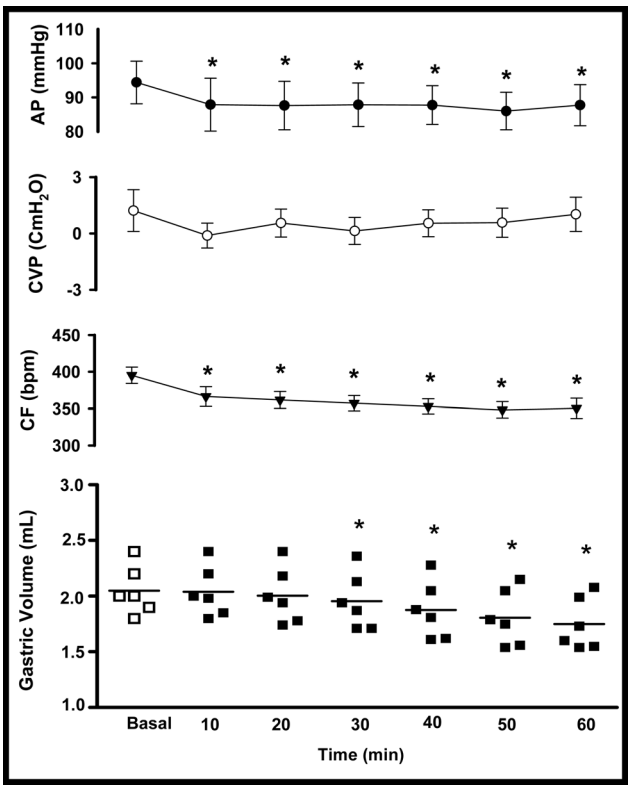
Statics analise

Individual values of GV, CF, CVP and AP of diverse experimental groups are express in the text in Mean±SD. Analysis of variance ("One way - ANOVA") followed of Dunnett's test was used to compare the differences between averages of diverse experimental periods, as well as between the studied groups. Differences with $p<0,05$ had been as significant.

Results

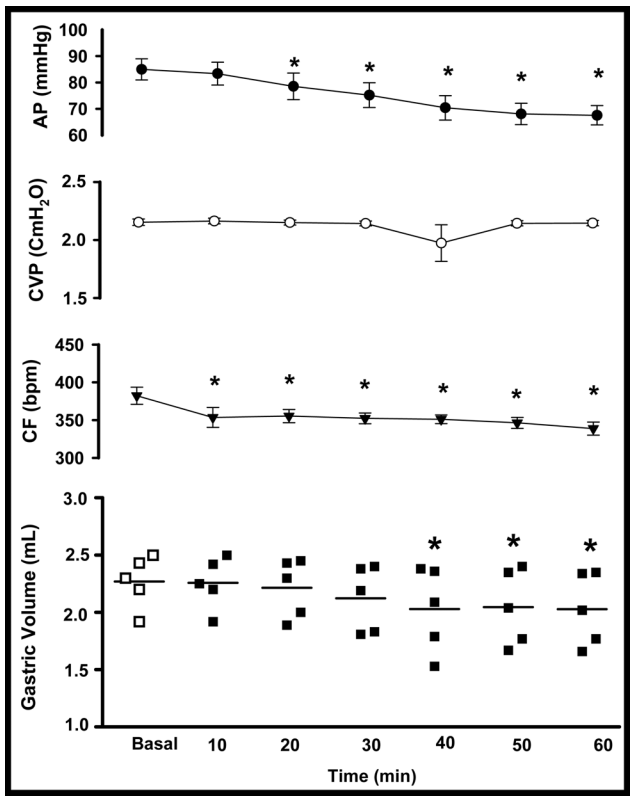
Figure 1 shows the variations of gastric volume (GV), arterial pressure (AP), central venous pressure (CVP) and cardiac frequency (CF) after and before 1.8 cineole treatmensts (1mg/Kg, i.v.). We observe reduction ($p<0.05$) on the GV, which was significant at 30, 40, 50 and 60min after 1.8 Cineoleee treatments when compared a basal

value (2.0±0.1; 1.9±0.1; 1.8±0.1 and 1.7±0.1mL respectivelly, vs 2.1±0.2mL). Mean arterial pressure (87.9±7.7; 87.6±7.1; 87.9±6,4; 87.8±5,7; 86.0±5,5 and 87.7±6,0mmHg, respectively vs 94.4±6.2mmHg) as well as the CF (366.3±13.4; 361.7±11.5; 357.3±10.4; 353.0±10.4; 348.3±11.1 and 350.4±13.7bpm respectively, vs 395.2±11.1bpm) had presented significant falls after 1.8 cineoleee treatments, remaining themselves thus during 60min of monitoration. Already, CVP did not suffer significant variations after 1.8 cineoleee treatments (0.1±0.7; 0.6±0.7; 0.1±0.7; 0.5±0.7; 0.6±0.8 and 1.0±0.9CmH_2O versus 1.2±1.1CmH_2O). Figure 2 shows the variations of GV, AP, FC and CVP after and before 1.8 cineole treatmensts (3mg/Kg, i.v.). We observe reduction ($p<0.05$) on the GV, which was significant at 30, 40, 50 and 60min after 1.8 Cineoleee treatments when compared a basal value (2.0±0.2; 2.0±0.1 and 2.0±0.1mL respectively, *vs* 2.3±0.1mL). Mean arterial pressure (83.4±4.4; 78.5±5.0; 75.2±4.7; 70.4±4.6; 68.0±4.0 and 67.5±3,7mmHg, respectively *vs* 85.0±4.0mmHg) as well as the CF (353.5±13.2; 355.3±8.7; 352.3±7.1; 351.0±5.7; 346.3±7.3 and 338.8±8.6bpm, respectively *vs* 382.3±11.4bpm) had presented significant falls after 1.8 cineoleee treatments, remaining themselves thus during 60min of monitoration. Already, CVP did not suffer significant variations after 1.8 cineoleee treatments (2.2±0.1; 2.2±0.1; 2.1±0.1; 2.0±0.1; 2.1±0.1 and 2.1±0.1CmH_2O, respectively *vs* 2.2±0.1CmH_2O). Figure 3 shows the variations of GV, AP, FC and CVP after and before placebo treatmensts (0.2mL of saline 0.9%, i.v.). We do not observe significant variations on GV, AP, CVP or FC along 80min of study (2.2±0.2; 2.2±0.1; 2.2±0.2; 2.1±0.2; 2.1±0.2 and 2.0±0.2mL *vs* 2.2±0.2mL), (92.2±3.9; 92.1±3.7; 91.0±3.5; 92.3±3.4; 92.6±4.0; 92.3±4.1 mmHg and 91.2±4.5mmHg), (0.1±1.1; -0.8±1.2; -0.6±2.7; 0.6±2.4; -1.9±1.5; -2.8±1.9 and 0.3±0.0CmH_2O *vs* 0.2±0.2CmH_2O) and (402.3±26.6; 383.5±34.9; 374.5±32.5; 359.0±20.6; 353.3±26.0; 347.0±27.1bpm and 366.7±27.2bpm), respectively.



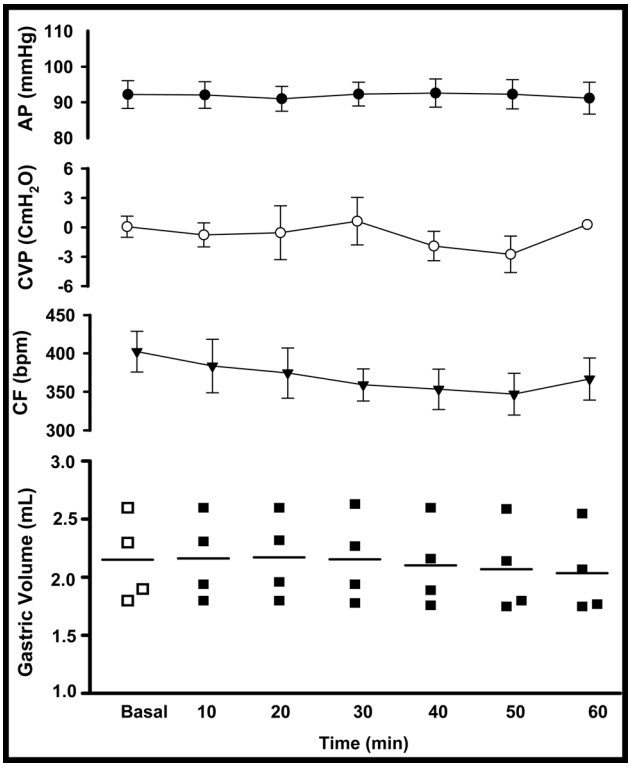
After a basal period of 20min, we treated the animals with 1.8 cineole (1µg/Kg, n=6). The values of VG, AP, CVP and CF monitored during 60min had been grouped in six consecutive intervals of 10min (10, 20, 30, 40, 50 and 60, respectively). The data of GV gotten by mean barostatic system are represented in a form of scatter with the horizontal line representing the median. The circles closed and opened and the closed triangles and the vertical lines represent mean±ED of the AP, CVP and CF, respectively. *, P<0,05 versus basal period (ANOVA and Dunnett's test).

FIGURE 1 - Effects of 1.8 cineole on Arterial Pressure (AP, in mmHg), Central Venous Pressure (CVP, in CmH2O), Cardiac Frequence (CF, in bpm) and gastric volume (GV, in mL) in an anesthetized rats.



After a basal period of 20min, we treated the animals with 1.8 cineole (3µg/Kg, n=5). The values of VG, AP, CVP and CF monitored during 60min had been grouped in six consecutive intervals of 10min (10, 20, 30, 40, 50 and 60, respectively). The data of GV gotten by mean barostatic system are represented in a form of scatter with the horizontal line representing the median. The circles closed and opened and the closed triangles and the vertical lines represent mean±ED of the AP, CVP and CF, respectively. *, P<0,05 versus basal period (ANOVA and Dunnett's test).

FIGURE 2 - Effects of 1.8 cineole on Arterial Pressure (AP, in mmHg), Central Venous Pressure (CVP, in CmH2O), Cardiac Frequence (CF, in bpm) and gastric volume (GV, in mL) in an anesthetized rats.



After a basal period of 20min, we treated the animals with saline 0,9% (0,2 mL, i.v., n=4). The values of VG, AP, CVP and CF monitored during 60min had been grouped in six consecutive intervals of 10min (10, 20, 30, 40, 50 and 60, respectively). The data of GV gotten by mean barostatic system are represented in a form of scatter with the horizontal line representing the median. The circles closed and opened and the closed triangles and the vertical lines represent mean±ED of the AP, CVP and CF, respectively. *, P<0,05 versus basal period (ANOVA and Dunnett's test).

FIGURE 3 - Effects of placebo (saline solution of 0,9%) on Arterial Pressure (AP, in mmHg), Central Venous Pressure (CVP, in CmH2O), Cardiac Frequence (CF, in bpm) and gastric volume (GV, in mL) in an anesthetized rats.

Discussion

This work shows that the 1.8 cineoleee i.v. administration reduces gastric compliance, arterial pressure, cardiac frequency of anestesiados rats, dose dependent, however does not modify central venosa pressure. Arterial pressure, CF and CVP had been monitored continuously. For this proposite, we use a data acquisition system (PowerLab-ADInstruments®), considered standard for literature. Basal results of AP are similar to gotten for others[6]. 1,8 cineoleee presente an hipotensor effect, AP returned to basal levels to soon after 10min of the treatment, similar to observed for others[7,8,9]. Data of literature show that i.v. administration of OECN or 1.8 cineole delays gastric emptying and gastrointestinal transit of liquid in awake rats as demonstrated by Magalhães *et al.,* indicating that OECN and 1.8 cineole retard gastrintestinal transit in mice and relaxes the intestinal smooth musculatura of cobaio[2,3]. According to a prevalent interpretation of specialists, delays of the gastric emptying of liquid, seamns a provoked by OECN and 1.8 cineole, folows a proximal stomach relaxation[10] and/or an incresement of the antroduodenal resistance to a liquid flow, call "duodenal brake "[11]. Proximal and distal stomach, can determine different standards of GE for varied types and compositions of meals[12]. The proximal segment functions as reservoir, capable to accomodate the meal while the fasic contractions of the antrum and the body promotes the gradient pressure for mix intraluminal contents, propulsioning a gastric content for duodenum[12,13]. In the present study, to evaluate the effect of .8 cineole on the gut motilidade, we use a plethismometric system. A system previously developed and validated for Graça and colegues[5,14], it showns adequate to continuously monitoring gastric tonus variations of the proximal stomach. It reflects variations of gastric volume, at last, the gastric compliance. Beyond hipotensor and bradicardic effect, that 1.8 cineole is capable to increase, a dose dependent, the tonus of stomach, diminishing, therefore, the gastric compliance of anesthetized rats. These results, can is related to findings of Magalhães and co-workers[2,3], which had described inhibitore effects of EOCN and 1.8 cineole on the gut muscels. The hipotensor and bradicardic effects of 1.8 cineole is hard. However these treatments do not modify the CVP of animals. In studies with waked up rats Lahlou and collaborators, that EOCN and 1.8 cineole, show bradicardic effect as well as hipotensor effect, probably for modulation of the nervous system autônomo[7], by vagal action. The Croton nepetaefolius is sufficiently known for popular medicine application of riots of the gastrointestinal system[16]. We present effects of 1.8 cineole on the gastric compliance can result of an autonomic reflex, or direct action of this essential oil on smooth muscel. In studies in vitro 1.8 cineole presents miorelaxant effect, what probably it justifies the job of this plant for the popular medicine, however the mechanisms of action of this oil is not well clarify[2]. In the isolated intestine of cobaio the inibitório effect of the EOCN is not reverted by an inhibitor of the NO-sintetase (L-NAME), inhibitor of cicloxigenase (indometacin), ganglionar bloker (hexametonium), sodium canals bloker (tetrodotoxin) pre-treatments. In this model, essential oil does not involve NANC mediation[2,3]. Already the effect of the OECN on AP and CF of awaked rats involve the integrity of the autonomic parassimpatic inervation (vagus nerve), while the effect on sanguineous vases if give for direct action of the oil on the smooth muscel vascular[7,8,9]. That effect of 1.8 cineole on the gastric compliance also is related to increase on autonomic activity. Bilateral vagotomy blocks the effect of OECN and the 1.8 cineole on AP and CF[8].

Conclusion

The 1.8 cineole, decreases the gastric compliance, arterial pressure, cardiac frequence in anestesiados rats. The inhibition of 1.8 cineole on the gut motilidade, may involves a systemic domain the modulation of the autonomic nervous system.

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Acknowledgments

We thank to Prof. Dr. José Henrique Leal Cardoso for cession of the essential oil in its pure form.

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Conflict of interest: none
Financial sources: Unimed-Sobral, UFC and CNPq

Received: August 08, 2006
Review: September 12, 2006
Accepted: October 24, 2006

How to cite this article:
Neves JRC, Lira GHS, Oliveira Neto RM, Graça JRV, Vasconcelos PRL, Souza MAN, Magalhães PJC, Rola FH, Santos AA. 1.8 cineole decreases gastric compliance in anesthetized rats. Acta Cir Bras. [serial on the Internet] 2007 Jan-Feb;22(1). Available from URL: http://www.scielo.br/acb.