



ORIGINAL ARTICLE

Comparison of the anesthesia with thiopental sodium alone and their combination with *Citrus aurantium* L. (Rutaseae) essential oil in male rat

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ABSTRACT

The aim of this study was to comparison of anesthesia with thiopental sodium alone and their combination with citrus aurantium L. essential oil in male rat. In this study 24 male Wistar rats (250 – 300 g) randomly assigned in 3 groups, Citrus aurantium L. essential oil alone (CA)(500mg/kg,i.p.), combination of Citrus aurantium L. essential oil – thiopental sodium (CAT)(500mg/kg and 50mg/kg, i.p., respectively)- and thiopental sodium alone (T)(50mg/kg,i.p.) (n=8). Heart and respiratory rate, induction and duration of surgical anesthesia (SA) walking times, withdrawal reflexes (pedal withdrawal, lip and tail pinches) were measured. Our results showed that all of groups induced SA. Thiopental had rapid induction time, short duration of SA and fast recovery. Instead, CAT group has late induction time and long duration of SA and late walking time. Heart rate and temperature were not changed significantly between groups ($p>0.05$). Respiratory rates was decreased in CA group compared to other groups (Between 0-40 min) ($p<0.05$). Lip, tail and pedal pinches not significant changed between groups (except in T group between 35-55min), but T group weaker than other groups inhibited pain ($p<0.05$). In conclusion, anesthesia with CAT combination prefer to long time of surgeries and it seems GABAergic(GABAA) pathways involved CA anesthetic activity. But, further studies needed for determine this exact mechanism of action.

Keywords: *Citrus aurantium* L.; essential oil, thiopental; anesthesia, rat.

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INTRODUCTION

Safe and reliable injectable anesthesia is necessary for surgical procedures in veterinary and scientific practices. Although, inhalation anesthetics are safer than injectable anesthetics, but complexity, lack and cost of the equipment needed to administer the anesthesia, and potential hazards to personnel limited their use (1 & 2). A wide variety of different injectable anesthetic techniques have been used for induction of anesthesia in the rat, including pentobarbital, thiopental, ketamine and tiletamine. Thiopental sodium is a rapid-onset short-acting barbiturate that is an analogue of thiobarbital(3& 4).It has been used commonly in the induction phase of general anesthesia in all species (e.g. laboratory rat) (4). In other hand, general anesthesia most often is initiated by an injection of thiopental to induce sleep prior to administration of the agents that are necessary for maintaining anesthesia during the surgical procedure. Thiopental sodium remains the standard for comparison with new agents and the main advantages of that anesthesia are pleasant, smooth, rapid induction, smooth and rapid recovery. However, thiopental sodium produces unconsciousness, poor muscle relaxant, local and systemic reactions, weak analgesic activity and must be used with other agents (5 & 6). Medicinal herbs may participate in the basic triad of anesthesia: analgesia, amnesia, and microrelaxation, added to autonomic stability maintenance [7].

Citrus aurantium L. (*C. aurantium*), commonly known as bitter orange, belongs to the order Geraniales and the family Rutaceae [8].Several bioactive compounds have been extracted from *C. aurantium*, such as alkaloids [9], limonoids and phytosterols [10], flavonoids [11], dietary fibers and essential oils with various therapeutic effects(12). Among them, *C. aurantium* oils, including neroli from flowers, have great economic, medicinal and nutritional values. *C. aurantium* oils have been generally recognized as safe because of their wide-spectrum biological activities, such as antimicrobial activity [13],antifungal activity(14),antioxidant activity(15),anti-inflammatory activity, anxiolytic effects [16], treatment of mild depression, insomnia, muscle relaxant [17, 18],and as an anticonvulsant, suggesting depressive action

upon the central nervous system (CNS) [19, 200]. Therefore, the aim of this study was to comparison of anaesthesia with thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil in male rat.

MATERIAL AND METHODS

Preparation of Sample and essential oil

Citrus aurantium flowers (blossoms) were collected from Shiraz (south of Iran). Samples were collected in spring 2013 and dried in shadow in room temperature. The sample were ground into a coarse powder with a household grinder and stored in capped bottles in refrigerator before preparation. For the extraction of the essential oils by ordinary HD method, 40 g of the dried sample was weighted and transferred into a 500 ml round bottom flask. Then, 250 ml double distilled water was added and the mixture was boiled in a Clevenger-type apparatus for 90 min. After cooling, the essential oil was collected and dried on sodium sulphate. Finally; the essential oil was stored in refrigerator at 4 °C.

Animals

Twenty four male adult Wistar rats (250-300g) were obtained from Urmia University of Medical Science (Urmia, Iran). The animals were housed five to a cage with food and water available ad libitum and were maintained on a normal 12-h light/dark cycle (lights on at 7:00 AM). This study was performed in accordance with guidelines of Urmia University of Medical Science.

Pilot study

In this section, different doses of CA essential oil were studied to access appropriate dose. At least four rats were used for each drug dose.

Experimental design and drug grouping

Induction times (time to loss of righting reflex), duration of surgical anesthesia (SA) (the period of loss of pedal withdrawal reflex) and walking time (duration from loss of righting reflex until ability to walk) were recorded. Following loss of righting reflex, rats were laid in dorsal recumbency. Respiratory rate (counted by observing thoracic movement) and heart rate (counted by ECG recording: Lead II, using hypodermic needle electrode, paper speed 50 mm/sec) at the 5, 10, 15, 20, 25, 30, 40, 50 and 60 min post injection intervals until the righting reflex returned. Rectal temperature was monitored using digital thermometer inserted at least 3 cm into the rectum at 5 min post injection intervals. Body temperature was maintained at 37-38°C during anesthesia by using a heat lamp (21).

The depth of anesthesia was monitored by use of the withdrawal reflexes (pedal withdrawal, lip and tail pinch reflexes). The withdrawal reflexes were assessed by pinching the interdigital of the hind-foot, the lower lip (with an atraumatic plastic forceps), or distal part of the tail (with the thumb and index finger) response were scored on a scale of 0-4, with complete reflex absent scoring 0 and strong withdrawal response scoring 4. All reflex tests were carried out and assessed by the same operator (22). The animals were grouped into 3 different groups, each containing 8 animals as follows:

Group 1: thiopental sodium alone (50 mg/kg, i.p.).

Group 2: *Citrus aurantium* L. essential oil alone (500 mg/kg, i.p.)

Group 3: thiopental sodium (50 mg/kg, i.p.) combination with *Citrus aurantium* L. essential oil (500 mg/kg, i.p.).

Drugs

Thiopental sodium (Tritau, Germany) and *Citrus aurantium* L. essential oil were dissolved in distilled water and tween 80 respectively. A solution of 2.5 % thiopental was prepared. In present study, thiopental sodium and essential oil were injected intraperitoneally (i.p.) at a constant volume of 1ml/kg.

Statistical analysis

Statistical analysis of parametric data (induction and recovery times) was performed using one-way analysis of variance (ANOVA) followed by Duncan's test. A repeated measure analysis of variance with time and treatment as factor was used to compare heart and respiratory rates. Pain scores were analyzed using kruskal-wallis test. All results are expressed mean \pm SEM and differences were significant at $p < 0.05$.

RESULTS

The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on induction time, duration of surgical anaesthesia and walking time.

Results showed that the induction time of anesthesia was very fast with T group and very slow with CA group. Also, we firstly showed CA essential oil induced surgical anesthesia. In the other hand, all of groups induced SA and the duration of SA in CAT group longer than other groups. Walking time was the shortest with T group and longest with CAT group. But there was no significant difference between groups ($p > 0.05$) (Table 1).

Table 1: The effects of thiopental sodium alone and their combination with Citrus aurantium L. essential oil on induction time, duration of surgical anaesthesia and walking time.

Reflex Group	Induction time(min)	Duration of SA(min)	Walking time(min)
T	4.37±0.23 a	43±3.39 a	44.25±11.92 a
CA	7±0.81 a	44.25±11.92 a	48.25±11.92 a
CAT	5.25±1.18 a	60±4.42 a	78.60±17.32 a

Different letters in the same columns indicates significant different ($p < 0.05$) between the thiopental sodium (T), Citrus aurantium L. essential oil alone(CA) and thiopental sodium combination with Citrus aurantium L. essential oil(CAT) groups.

The effects of thiopental sodium alone and their combination with Citrus aurantium L. essential oil on heart rate (beats/min).

Table 2 showed that there was no significant difference between T and CAT groups on heart rate ($p > 0.05$). Although, T group increased heart rate. Heart rate was significantly lower in CA group compared T group ($p < 0.05$) (Fig 1, 2 and 3).

Table 2. The effects of thiopental sodium alone and their combination with Citrus aurantium L. essential oil on heart rate (beats/min).

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	337.5± 10.3 a	334± 9.44 a	340± 8.16 a	338.25± 6.94 a	337.5± 6.29 a	339± 9.53 a	329.25± 9.12 a	338.25± 8.35 a	332.5± 11.65 a	335± 5.68 a	332.75± 8.86 a	326.75± 9.91 a
CA	230± 12.9 a	238.5± 3.22 b	227.5± 11.08 b	235.25± 7.87 b	237.5± 13.14 b	233± 10.75 b	237.5± 3.96 b	229.5± 9.5 b	240.75± 6.32 b	247± 10.01 b	238.75± 8.4 b	236.5± 7.46 b
CAT	300± 48.3 a	294.5± 36.94 ab	315± 42.72 ab	314.75± 41.09 ab	305± 35.08 ab	296± 44.45 ab	286.5± 29.43 ab	310± 38.72 ab	311.25± 37.99 ab	307.5± 34.16 ab	289.25± 38.02 ab	282± 27.64 ab

Different letters in the same columns indicates significant different ($p < 0.05$) between the thiopental sodium (T), Citrus aurantium L. essential oil alone(CA) and thiopental sodium combination with Citrus aurantium L. essential oil(CAT) groups.

Figure 1: The ECG of Citrus aurantium L. essential oil alone; Paper speed 50 mm/sec.

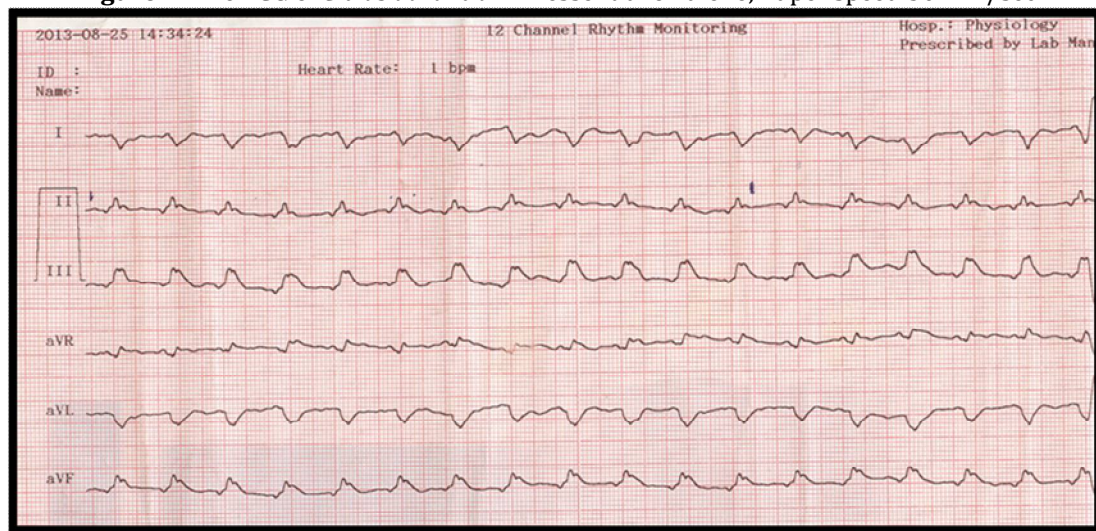
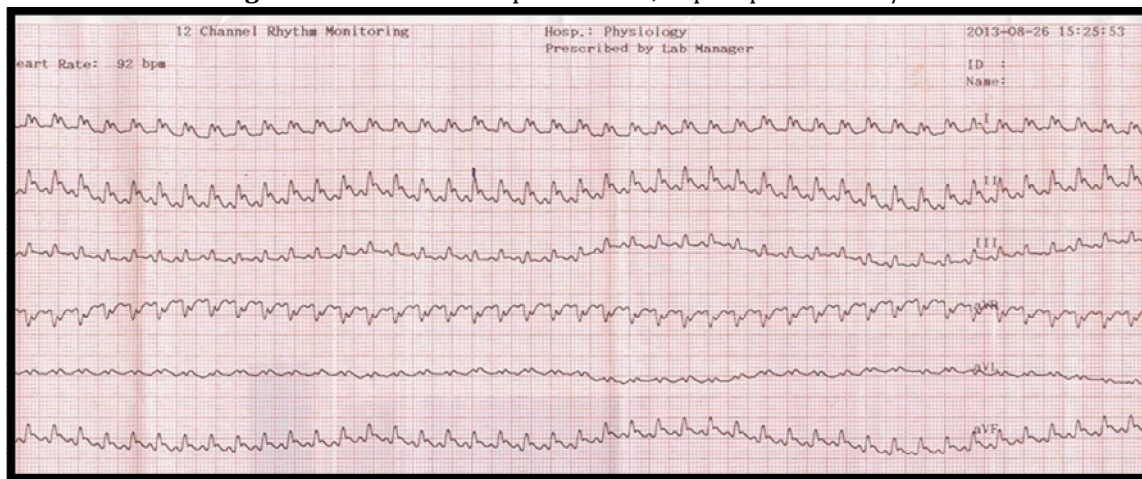
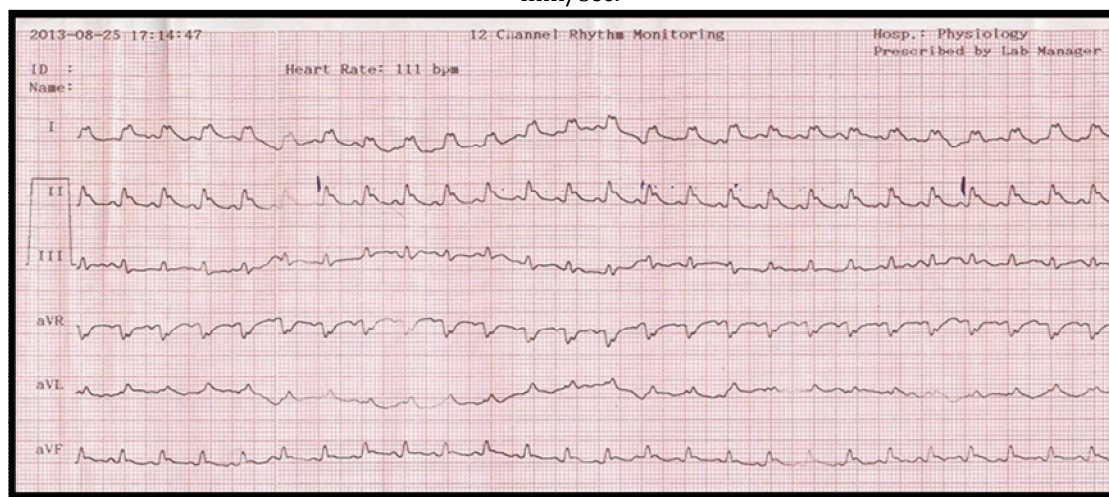


Figure 2: The ECG of thiopental alone; Paper speed 50 mm/sec.**Figure 3:** The ECG of thiopental combination with *Citrus aurantium* L. essential oil; Paper speed 50 mm/sec.

The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on respiratory rate (breath/min).

Table 3 indicated that there was no significant difference between T and CAT groups on respiratory rate ($p > 0.05$). But CA group compared to other groups significantly reduced respiratory rate (0-40min) ($p < 0.05$) (Table 3).

Table 3. The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on respiratory rate (breath/min).

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	73.5± 0.64 a	73± 0.91 a	70.75± 1.1 a	69.75± 0.75 a	67.5± 1.55 a	66.75± 1.37 a	65.5± 1.5 a	65± 1.77 a	64.75± 1.25 a	64.5± 1.32 a	65.5± 1.19 a	65± 1.47 a
CA	60± 4.89 b	59.5± 4.9 b	56.75± 4.95 b	55.75± 5.55 b	54.75± 5.26 b	55.5± 4.01 b	55.75± 4.4 b	56± 4.08 b	56.75± 3.85 b	58.75± 4.42 a	59.25± 5.26 a	60.75± 5.49 a
CAT	76± 2.73 a	75.75± 3.35 a	74.75± 2.75 a	73.25± 2.13 a	71.5± 1.84 a	71± 1.08 a	70.25± 1.7 a	70.25± 0.85 a	69.5± 1.19 a	69.25± 1.31 a	69.75± 2.17 a	71± 2.04 a

Different letters in the same columns indicates significant different ($p < 0.05$) between the thiopental sodium (T), *Citrus aurantium* L. essential oil alone (CA) and thiopental sodium combination with *Citrus aurantium* L. essential oil (CAT) groups.

The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on body temperature (centigrade)

Results showed that CA group compared T and CAT groups reduced body temperature but there was no significant difference between three group ($p>0.05$)(Table 4)

Table 4. The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on body temperature (centigrade)

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	37.6± 0.12 a	37.4± 0.3 a	37.02± 0.26 a	36.57± 0.21 a	36.42± 0.29 a	35.85± 0.35 a	35.45± 0.51 a	35.22± 0.46 a	35.2± 0.42 a	35.07± 0.5 a	34.87± 0.46 a	34.9± 0.41 a
CA	36.57± 0.47 a	36.47± 0.38 a	36.57± 0.3 a	35.3± 0.59 a	35.3± 0.55 a	35.4± 0.49 a	35.3± 0.61 a	35.05± 0.59 a	35.1± 0.61 a	35.35± 0.68 a	35.42± 0.74 a	35.5± 0.74 a
CAT	37.3± 0.23 a	37.3± 0.31 a	36.87± 0.44 a	36.62± 0.42 a	36.65± 0.49 a	36.35± 0.31 a	36.37± 0.33 a	36.15± 0.26 a	36.05± 0.21 a	35.97± 0.23 a	35.87± 0.28 a	35.55± 0.18 a

Different letters in the same columns indicates significant different ($p<0.05$) the thiopental sodium (T), *Citrus aurantium* L. essential oil alone(CA) and thiopental sodium combination with *Citrus aurantium* L. essential oil(CAT) groups.

The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on withdrawal lip reflex

Results showed that there was no significant difference between three group in inhibiting lip reflex except T group that showed lip reflex significantly high between 35 to 55min ($p<0.05$). (Table 5).

Table 5. The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on withdrawal lip reflex

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	3.25±0.75 a	1±0.4 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b
CA	3.25±0.47 a	0.25± 0.25 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
CAT	3.75±0.25 a	0.75± 0.47 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a

Different letters in the same columns indicates significant different ($p<0.05$) the thiopental sodium (T), *Citrus aurantium* L. essential oil alone(CA) and thiopental sodium combination with *Citrus aurantium* L. essential oil(CAT) groups.

The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on tail reflex

Table 6 showed that there was no significant difference between three group in inhibiting tail reflex except T group that showed lip reflex significantly high between 35 to 55min($p<0.05$)(Table 6).

Table 6. The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on tail reflex

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	3.25±0.75 a	1±0.4 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b
CA	3.5±0.5 a	0.25±0.25 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
CAT	3.75±0.25 a	1±0.7 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a

Different letters in the same columns indicates significant different ($p < 0.05$) between the thiopental sodium (T), Citrus aurantium L. essential oil alone (CA) and thiopental sodium combination with Citrus aurantium L. essential oil (CAT) groups.

The effects of thiopental sodium alone and their combination with Citrus aurantium L. essential oil on pedal reflex

Results showed that there was no significant difference between three group in inhibiting pedal reflex except T group that showed pedal reflex significantly high between 35 to 55min ($p < 0.05$) (Table 7).

Table 7. The effects of thiopental sodium alone and their combination with *Citrus aurantium* L. essential oil on pedal reflex

Time Group	0	5	10	15	20	25	30	35	40	45	50	55
T	3.25±0.75 a	1±0.4 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b	2±1.15 b
CA	3.75±0.25 a	0.25±0.25 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a
CAT	3.75±0.25 a	1±0.7 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a	0±0 a

Different letters in the same columns indicates significant different ($p < 0.05$) between the thiopental sodium (T), Citrus aurantium L. essential oil alone (CA) and thiopental sodium combination with Citrus aurantium L. essential oil (CAT) groups.

DISCUSSION AND CONCLUSION

The main aim of this study was to determine whether intraperitoneal injection of *Citrus aurantium* L. with thiopental compared with thiopental group maintained a surgical depth of anesthesia for at least 30 min. Our results showed that all of groups induced SA and CAT group longer than other groups induced surgical anesthesia. In agreement with our findings, T group faster than others induced SA [23]. In accordance with our findings, Carvalho-Freitas and Costa reported that *Citrus aurantium* L. essential oil [CA] at 0.5 g/kg showed a tendency to enhance, and at 1.0 g/kg was able to enhance, the sleeping-time duration induced by pentobarbital (24). It seems GABAergic pathways and subsequent impacts on GABAA receptors involved sedative/hypnotic activity of CA. Limonene, coumarin and flavonoids are compounds of CA which interact with GABAergic pathways (GABAA) by increasing their release (25&27). Moreover, Khosravi et al reported that *Citrus aurantium* L. essential oil via GABAergic system can reduce anxiety-related behaviors in male mice [28].

Our findings indicated that heart rate was not changed significantly between groups. In accordance with others, our results showed that T group increased heart rate (23). But, CA essential oil could decrease tachycardia induced by T group. In this regard, folklore medicine has used different parts of *C. aurantium* to treat many types of diseases, including tachycardia, and also either as a cardiac tonic or diuretic [29]. Khorrami et al also indicated the potential anti-arrhythmic effects of *C. aurantium* in treating supraventricular tachyarrhythmia. Based on our findings, respiratory rates were decreased in CA group compared to other two groups. Body temperature wasn't significantly changed between groups. Our results also indicated that there were no significant differences between lip, tail and pedal reflexes in three group. Therefore, these findings support essential oil's analgesic activity. In line with our findings, Gürkan et al reported *C. aurantium* had analgesic and anti-inflammatory effects. In this regard, they were reported that main components of the volatile fraction of CA essential oil are limonene, linalool, linalyl acetate, bergapten, citropten, bergamottin, gamma-terpinen, alpha-pinene and beta-pinene [30]. Many essential oils are found to exhibit varied biological properties, such as antinociceptive activity [31, 32]. Chemically, the oils are composed mainly of terpenoids and phenylpropanoids, including polyketides and very few alkaloids. The terpenoids from essential oils are often monoterpenes (e.g. Limonene and Linalool), which account for about 90% of the oils. Reported studies of monoterpenes pharmacological proprieties show their potential clinical use as analgesic drugs. For example, linalool, a major component of many plant essential oils acts on several receptors, including opioids, adenosine A1 and A2, cholinergic M2, and produces changes in K⁺ channels [33-39, 40]. They can penetrate the blood-brain barrier and act in the central nervous system.

In Conclusion, the results of present study showed that *C. aurantium* essential oil had anesthetic potential activity and CA combination with thiopental sodium potentiated their anesthetic properties, alleviates unwanted cardiovascular effects of thiopental and demonstrates anticonvulsive, analgesic and muscle relaxant effects. Therefore this study provided support to the traditional usage of CA in anesthesia. But, anesthesia with CAT combination prefers to long time of surgeries and CA group induced SA but it has slow

onset of action and recovery. It seems GABAergic pathways (GABAA) involved *C. aurantium* anesthetic activity. But, further studies needed for determine this exact mechanism of action.

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