



## Surveying the effect of hydroalcoholic tarooneh (spathe of phoenix dactylifera) extract on anesthesia and EEG brain waves

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### ABSTRACT

No single anesthetic drugs could provide all the characteristics of an ideal general anesthesia, but a combination of intravenous drugs given produced the desired balanced anesthetic. Tarooneh (spathe of phoenix dactylifera) is a hard covering of date palm pollen that torn and open in pollination season, in south and west of Iran since a long time people take tarooneh's extract as a sedative and calmative substance, according to its proved effect on pain and its usage it is examined on anesthesia and EEG brain waves. Pharmacological maceration technique has been used for the tarooneh extract and the Solvent was 70% ethanol. Animals divided to 4 groups for anesthesia analysis and 4 groups for EEG analysis, in anesthesia analysis one of the groups just injected by chloral hydrate and 3 other groups received doses 62.5, 125 and 250 mg/kg tarooneh extract after chloral hydrate. 4 other groups of EEG injected by these three doses of extract and saline for control group after anesthetizing. The mean anesthesia time in the ch+250 group (the dose 250 mg/kg) was more than the other groups. In fact we can say time of anesthesia in this 4 group is significantly different. By doing a posttest it has shown that ch+250 and ch+125 groups are significantly different from 2 other groups. This result in EEG brain wave is similar and in 125 and 250 mg/kg significant difference was observed. This study showed the efficacy of tarooneh in anesthetized rats that increased anesthesia time so its sedative affect can be helpful in sleeping and anesthesia.

**Key words:** Anesthesia, EEG, Brain wave, spathe of phoenix dactylifera

### INTRODUCTION

Phoenix dactylifera (date palm) is a diploid and member of monocot family arecaceae (1) which cultivates more in countries around Persian Gulf and date palm is completely known in these zones because since a long time it had used for food, appliances, transferring appliances and home making instrument also for a long time different parts of date palm have used for various disorders in traditional medicine (2). In traditional medicine till 50 years ago extract usage has a great place (3) so today we should consider herbal remedy in performing new drugs as it can provide good therapeutic agents for modern and traditional medicine (4). Tarooneh (spathe of phoenix dactylifera) is a hard covering of date palm pollen that torn and open in pollination season and remains like an extra shale (5). Chemically tarooneh's components are fat, reduction and unreductional sugar, kafur component, 1-2 dimethoxyl 4 benzen, wood ash, moisture, herbal sterol and 3 coumarin subtype (6). In south and west of Iran since a long time people take tarooneh's extract as a sedative and calmative substance also several articles examined tarooneh to show its nature and property, Hamed and coworker showed it's different fractions are steroid, teriterpen steroid, oils and flavonoids in different amount and oil compound that identified by GC\_MS analysis, oxygen containing monoterpens were the main class of components (73%), carvacrol (37%), linalool (24%) and thymol (10%) as the most constituent of the oil. In the ancient Iranian Gharabaddin Kabir book it is written that tarooneh could relief headache and diarrhea (7). Researcher who worked on tarooneh showed; its repellent activity against the yellow fever mosquito (8), anti diarrheal effect (9), impression on concentration of LH, FSH and testosterone (10), reducing chronic pain (11) and therapeutic nature of tarooneh include reducing blood fat, , increase breast milk in nurse feeding mothres, calmative

and nerves relife, sedative, joint pain relife and remedy for rheumatic diseases (12) also containing fibers can help preventing cancer, heart safe keeping, diuretic and reducing cholesterol(13).

Anesthesia is defined as loss of sensation and usually of consciousness without loss of vital functions, artificially produced by administration of one or more agents (14). In this field we had to implement drugs with the least supervision (15) so having great knowledge's about drugs, drug effects and combination is necessary. One of the greatest anesthesiologist Lundy JS introduced the concept of balance anesthesia by emphasizing on use of multiple drugs to produce unconsciousness and antinociception, provide skeletal muscle relaxation, and obliterate reflex response (16). No single anesthetic drugs could provide all the characteristics of an ideal general anesthesia, but a combination of intravenous analgesics, neuromuscular blocking drugs, and hypnotics given produced the desired balanced anesthetic. Lower doses of each drug could be used since the different drugs tended to act synergistically (17). So nowadays we can face with more drug combination for general anesthesia, for example combination of sufentanil having quick onset and short duration of action with neostigmine having slower onset and longer duration of action is benefit and result in reducing side effect (18). In this research according to use of this extract in Iran as sedative and calmative and its proved effect on pain we are going to survey the effect of hydro alcoholic tarooneh extract on anesthesia and anesthesia waves.

## EXPERIMENTAL SECTION

### Plants extract

Spathes of phoenix dactylifera were collected from Queshm island of Iran in February 2015. It cleaned and dried in room temperature then cut into small pieces and grinded in order to 70% alcohol will be added to the created powder. All action had implemented in a place out of direct sunshine to avoid chemical changes that can cause reactions of sun flam on herbal materials.

In this research pharmacological maceration technique had been used to reach the extract. Each 100 g of the powder was mixed with 400 ml 70% ethanol then the bottles valve fastened to prevent alcohol evaporation. After solving for two times fixed on shaker to be percolated. The obtained extract condensed by rotary in 40 °C and same temperature in bain-marie (water bath) for 4-8 hours followed by freeze drying of the supernatant. The achieved extract had diluted in normal saline by DMSO to made three 62.5, 125 and 250 doses that had implemented in the experiment.

### Animals

Adult Wistar male rat weighing  $270 \pm 20$  from Isfahan medical university in Iran were used. They were housed in lucid cages in the animal house at  $24 \pm 2$  °C and relative humidity 44–56%, light and dark cycles of 12 and their cycles were reversed, from 19 pm until 7 am the light automatically turned on and off respectively, for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet and water was allowed ad libitum.

### Protocol

We checked sedative effect of extract by enhancing or reducing chloral hydrate anesthesia time and then checked the extract effects on brain wave by recording electroencephalography (EEG). All these procedure separately help us to gather information about the effects of this extract on anesthesia in different aspects. Protocols of working with animal were according to medical university of Isfahan and international rules. All experimental groups had 5 member and injections were intra peritoneal (IP). Light, temperature and conditions were same for all groups of each protocol.

### Sedative experiment

Animals were anesthetized by 450 mg/kg chloral hydrate first and injected by extract, increasing the anesthesia time by extract can show its sedative efficacy and decreasing anesthesia time can shows adverse effect on sedativity. Animals divided to 4 groups each group had 5 members, one just injected by chloral hydrate and 3 other groups received doses 62.5, 125 and 250 mg/Kg of extract after chloral hydrate. After being anesthetized, rats placed uphill into the box and placed in sleep system to check out the anesthesia and the recovery time. System depicted a flat line as rat was anesthetize and a swing line in rats recovery, rat changes the uphill position while its recovering at the first so it can shows recovery time, so total anesthesia time differences between groups can help us to underestimate the sedative effect of extract.

### Brain wave

For this protocol rats were divided in 4 groups of saline and 62.5, 125 and 250 doses, each rats first anesthetized by urethane and fixed on stereotaxic and surgeries on the skull to make two holes. Cranial holes were drilled 1mm anterior to bregma and 1 mm lateral to midline and, contralaterally 2mm posterior to bregma and 2mm lateral to

midline. EEG electrodes placed subcranially over the dura. After surgery rats putted in EEG system, animals kept warm with a pad in lower part and coving during experiment. When brain waves stabilized recording started for 3 part of 20 minute recording. First 20 minute is control and no injection was done also this group called before part, before second 20 minute of record, injection occurred and this record is test record and called after1 part, third record was like the second and is the post test of experiment and we called it after2. The resulted data compared to before part and are a percent of before part resulted activity.

## RESULTS

To assess significant difference in 4 groups of chloral hydrate and three extract dose we used the methods of one way ANOVA analysis and for analyzing EEG data one way ANOVA and repeated measure were implemented. After data had analyzed post hock TUKEY test used to show the difference between groups. At the first data normalization checked by Kolmogorov-Smirnov, According to test result p-value is 0.659 that is more than 0.05. It shows that null thesis of Kolmogorov – Smirnov test about following the data in normal distribution could be accepted, and data is coincide with normal distribution. At Levene's test for homogeneity of variances the resulted amount of p-value from Homogeneity of variances test is more than 0.05 so thesis of homogeneity accepted.

The mean anesthesia time in the ch+250 and ch+125 groups (the dose 250 mg/kg) were more than the other groups; in the following chart it has been shown.

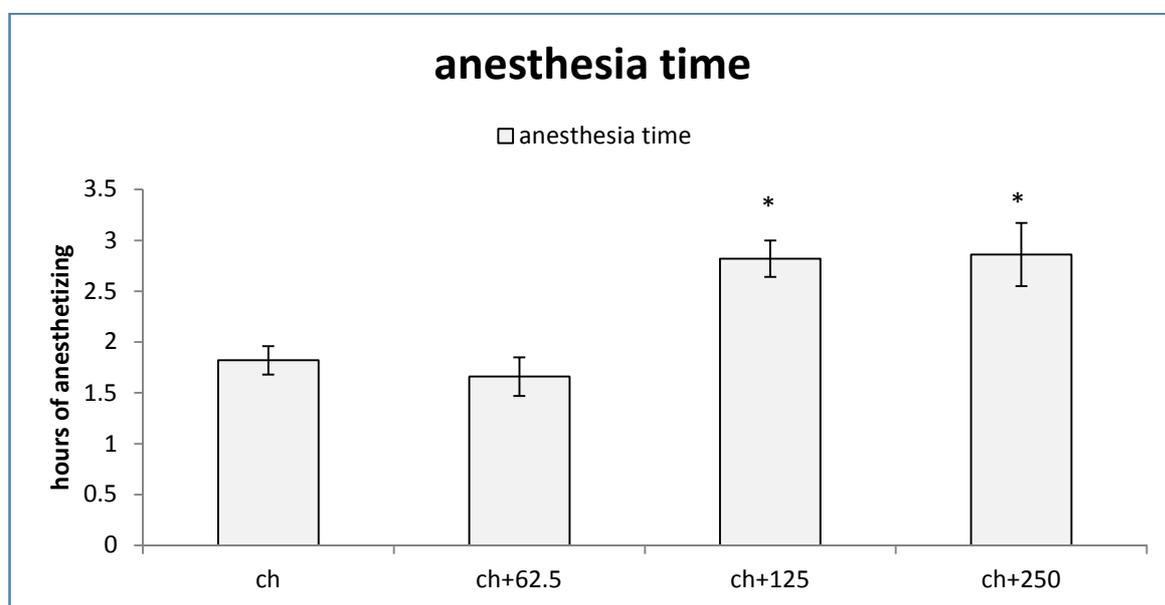


Chart 1. hours of anesthesia by chloral hydrate injection and chloral hydrate plus doses 62.5, 125 and 250 mg/kg of taroon extract injection

One way ANOVA analysis:

According to p-value amount in the table as p-value is less than 0.05, test thesis of same mean anesthesia time in 4 groups is refused. In fact we can say time of anesthesia in these 4 groups are significantly different. By doing TUKEY post test it shows that ch+250 and ch+125 groups are significantly different from 2 other group that this finding is clear in the chart.

As it's shown in the baseline charts there are equal waves among saline control groups and saline waves of alpha, beta, delta and theta all are around baseline of 100% but its different in extract groups. In dose 62.5 of extract alpha wave is similar to control group and did not change by extract injection but there is a small decrease in beta after2 even it is not meaningful and also a small increase in theta and delta that these are also not significant.

The baseline chart of 125mg/kg extract shows huge difference to saline and 62.5 mg/kg, it is clear that after1 and after2 waves had changed in contrast to before wave. In theta wave there is a significant increase in theta after2 in compare with before wave ( $p=0.042$ ) and in compare with theta after1 wave showing a little increasing sleepy wave in this dose of extract but this one is not significant ( $p= 0.052$ ).

At alpha activity in dose 250 mg/kg alpha after1 decreased in contrast to alpha before ( $p=0.011$ ), beta after1 reduced in contrast to beta before ( $p= 0.050$ ) and beta after 2 had same effect in compare with beta before and is also reduced ( $p=0.018$ )

In the EEG results we can see that among implemented doses, the 125 mg/kg of extract had more effects on all 4 waves but increased theta more. In dose of 250 mg/kg we can see in chart that extract reduced alpha and beta waves and shows its sedative effect more by reducing these two waves.

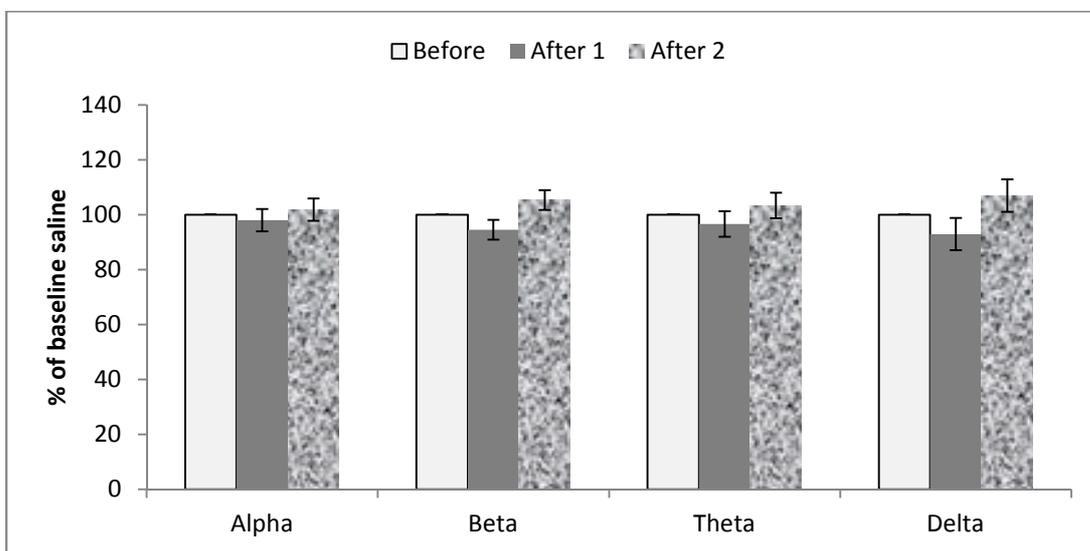


Chart 2. saline injection for surveying EEG brain waves

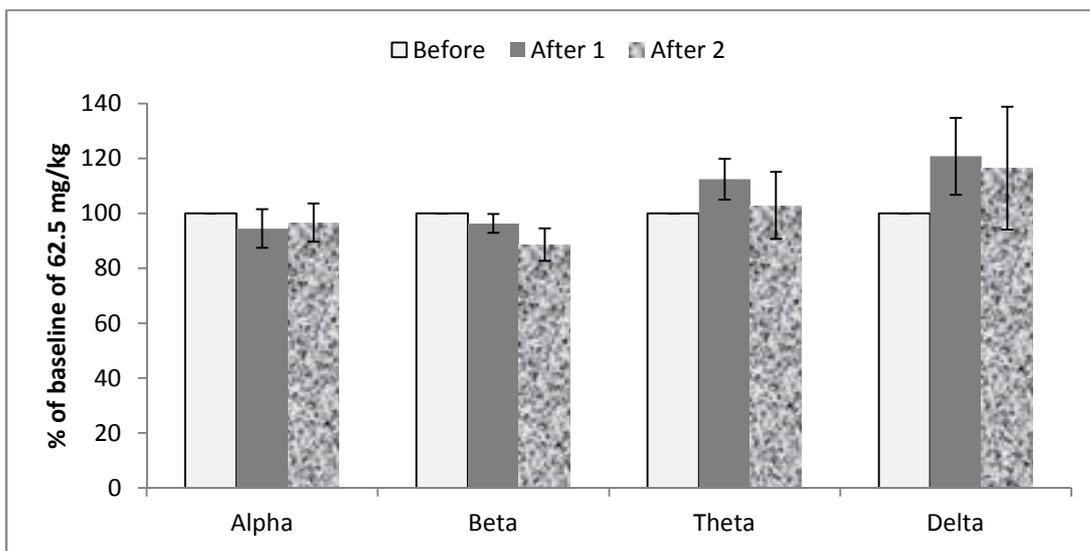


Chart 3. Injected dose 62.5 mg/kg of taroon extract for surveying EEG brain waves

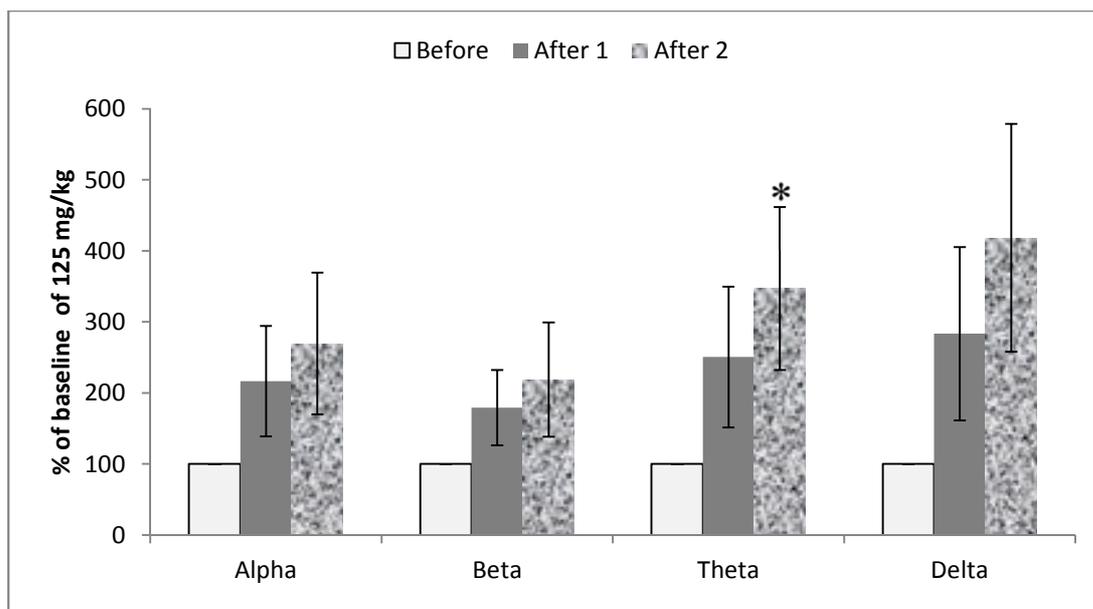


Chart 4. Injected dose 125 mg/kg of taroon extract for surveying EEG brain waves. Significant increase in theta after 2(p=0.042)

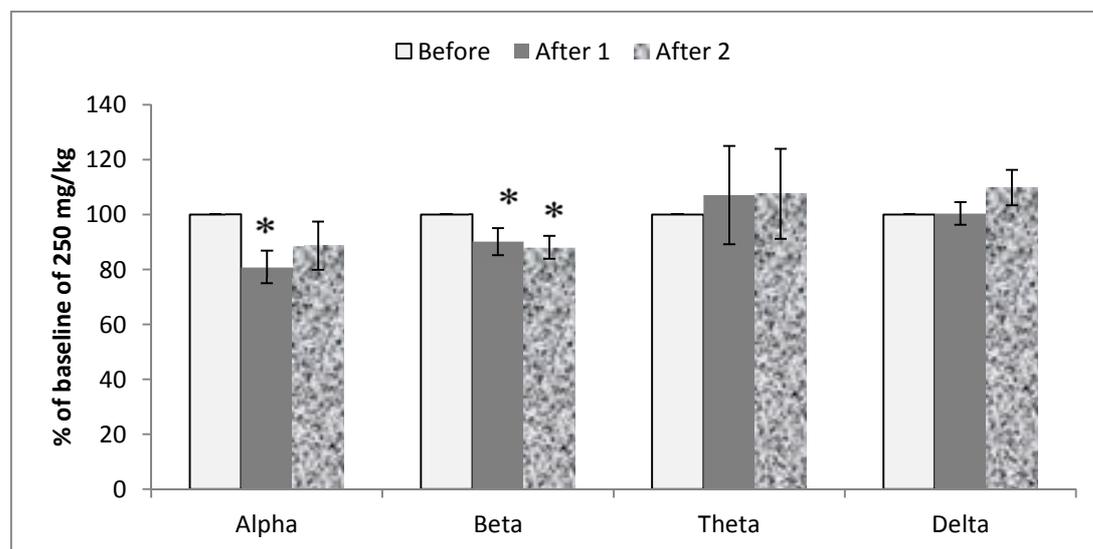


Chart 5. Injected dose 250 mg/kg of taroon extract for surveying EEG brain waves. Alpha after1 (0.011),beta after1 (0.050) and beta after2 (0.018) were significant

### CONCLUSION

Effect of extract by examining in anesthesia showed difference in doses 125 and 250 mg/kg more than control group of chloral hydrate injection and the 62.5 mg/kg dose, it shows two 125 and 250 mg/kg doses are effective in anesthesia elongation and according to increase in chloral hydrate anesthesia time these two doses can be mentioned as sedative doses of extract (19). In the EEG analyze results is similar and just these 2 doses were effective but impression of 125 mg/kg dose was sharp and there is point that it also increase alpha and beta wave that are not very deep wave of anesthesia even though increase of alpha and beta is less than delta and theta and are not significant. In 250 mg/kg dose there are decreases in less deep wave of alpha and awareness wave of beta and this dose may shows its efficacy by decreasing less deep wave of alpha and beta.

In this century EEG usage in extending as it can helps in exploring new windows to the brain wave, it usually used for physiological identification of consciousness along with mental processing (20). In EEG brain wave there's 4 regular frequency bands, these waves are delta (0-4 Hz), theta (4-8 Hz), alpha (8-13 Hz) and beta (13-20 Hz) (21), one of these can be dominated in one part of anesthesia or sleep so could be reliance indicators. Scientist observed

delta activity in sleep state and theta activity in less deep sleep state also alpha activity shows calmness and beta activity is a good indicator of awareness (22)

It's possible that flavonoid agents in extract can be responsible for sedativity (23) which observed in 125 and 250 mg/kg dose of tarooneh.

These results showed more efficacy of extract in 125 and 250 doses even in anesthesia or EEG brain wave activity suggesting continuing research around these doses and also continuing investigation more about anesthesia quality and more detail of EEG effects in this way.

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