



Evaluation of The Analgesic Activity of Coenzyme Q10 Ointment in Mice

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ABSTRACT

The aim of this study is to examine analgesic effect of Coenzyme Q10 ointment in mice. Fifteen healthy male albino mice were selected for this study. The animals were divided into three groups of five animals for each group. The pain reaction time was recorded pretreatment for each animal and was taken as a basal threshold(1). Group 1 served as a control and was applied Vaseline ointment topically on fore and hind limb. Group 2 and 3 were applied topically Coenzyme Q10 ointment 4%, 8% respectively on fore and hind limb. The onset and duration of analgesic effect of Coenzyme Q10 ointment were evaluated in mice by utilizing a Hot-Plate test at 55±1°C. Latency reaction time was recorded after 3min. and (10- 60 min) after ointment applied. The prolongation of latency times compared with the values of the control was used to express about analgesic effects of Coenzyme Q10 ointment and the percentage of antinociceptive ,Maximal Possible Effect (MPE) was calculated. The Coenzyme Q10 ointment at concentrations (4%, 8%) produced analgesic effect in mice after 3 min, respectively in comparison with control. The percentage of maximum possible effect (MPE) was significantly increased in group treated with (4%,8%) Coenzyme Q10. It can be concluded that, the Coenzyme Q10 ointment have a good analgesic activity in mice after 3 minutes of topical application and prolong the duration of analgesia more than 40 minutes depending on concentration of Coenzyme Q10 ointment.

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INTRODUCTION

Analgesics drugs are commonly prescribed for pain management (Curtero, *et al.*, 2006). The analgesic drugs can be defined as a drug that relief , manages and treats pain (Dahl and Reader, 2000). Systemic administration of analgesic drug produces many side effects (Therefore it (of paramount importance) to develop non-oral dosage forms of analgesic drug to minimize the gastric pain , to persist for a long period at the site of application and to minimize the disadvantages of oral administration (Alarcon, 2002). Topical application of drug is considered the best route of administration because by passing through the skin, it has a large area for drug penetration with fast exposure to circulating and the

route is noninvasive (Daniels and Knie, 2002). Coenzyme Q10 is a fat-soluble antioxidant compound which acts as a key element of the electron transport chain for cellular adenosine triphosphate (ATP) production (DiNicolantonio, *et al.*, 2015) , in addition to that it has anti-inflammatory effect (Zhai, *et al.*, 2017). The biosynthesis of CoQ10 takes place in the mitochondria of the liver, heart, kidneys and muscles, where they require a greater quantity of energy for their multiple biological roles (Acosta, *et al.*, 2016).

The chemical structure of CoQ10 is similar to that of vitamin K, CoQ10 is not considered a vitamin because it is the only lipid-soluble antioxidant that animal cells synthesize de novo in the body (Bhagavan and Chopra, 2006). Coenzyme Q10 is known to be a redox-active lipophilic antioxidant, scavenging reactive

oxygen species (ROS) and inhibiting lipid peroxidation in the body (Niklowitz, *et al.*, 2004) .

The capability of CoQ10 to scavenge ROS is lot greater than vitamin E and it was also identified to facilitate the absorption of lipid soluble antioxidant vitamins such as vitamin A and E (Shekelle, *et al.*, 2003). The research about analgesic activity of Coenzyme Q10 was very little. The aim of the present study is to prepare different concentration of Coenzyme Q10 ointment to examine the analgesic effects of topical application in mice by using a hot plate test.

MATERIALS AND METHODS

Animals

Fifteen healthy male albino mice weight about 20-30 gm with equal age were selected to use for this study. Mice were obtained from animal care housed in Dentistry Collage of Mosul University in Iraq. The animals were housed in plastic cages under 12h light\12h dark cycle at 22±2°C and access to fed with standard diet and tap water.

Preparation of Coenzyme Q10 ointment

Coenzyme Q10 ointment were prepared in two different concentration by mixing (4, 8) g from Coenzyme Q10 powder in 100 gm Vaseline respectively to give final concentration (4%, 8%) W\W with continuous mixing using a glass rode until homogenous ointment were formed. The ointment were kept in plastic containers and stored in refrigerator at 4°C until used.

Experiment On Animals

The mice were divided into 3 groups; each group consisted of five animals. The pain reaction time was recorded pretreatment for each animal and was taken as a basal threshold. Group 1 served as a control and was applied vasaline ointment topically on fore and hind limb. However group 2 and 3 were applied Coenzyme Q10 ointment 4%, 8% respectively topically on fore and hind limb. The onset and duration of analgesic effect of Coenzyme Q10 ointment were evaluated in mice by utilizing a Hot-Plate test. Mice were placed on a hotplate maintained at 55±1°C. The reaction time is that between placing the animals on the hot-plate and holding, jumping, licking of the fore or hind paws.

A cut off time of 30 seconds is followed to avoid any thermal injury to the paws (Ghosh, 1984). Latency reaction time was recorded after 3 min. and (10, 20, 30,40, 50, 60 min.) following topically applied of Coenzyme Q10 ointment in treated group and ointment only in control group by using hotplate test to assess the onset and duration of treated ointment. The prolongation of latency times compared with the values of the control was used to express about antinociceptive effects of Coenzyme Q10 ointment. The percentage of antinociceptive Maximal Possible Effect (MPE) was calculated from the formula: (Giusti, 1997)

$$\%MPE = (\text{Test latency} - \text{predrug latency} / \text{cut off time} - \text{predrug latency}) \times 100$$

MPE means Percentage of antinociception maximal possible effect.

Test latency means Second after drug treatment.

Predrug latency means Second before drug treatment at zero time.

Cut off time means 30 second.

Statistical Analysis

The data were expressed as mean ± SD, difference between three experimental groups were statistically analyzed by one way analysis of variance (ANOVA) followed by the Duncan . The level of significance was at $p < 0.05$.

RESULTS

In the present study, the Coenzyme Q10 drug was evaluated for its antinociceptive activity against pain induced by hot-plate in mice. Topical application of Coenzyme Q10 drug at (4%,8%) create good analgesic effects in comparison with control treated with Vaseline only .The results for increasing the pain reaction time of the Coenzyme Q10 drug at (4%) and (8%) showed that the drug has a highly significant difference after (3 and 10, 20, 30, 40,50, 60) min. The maximum pain reaction time at 30 second after drug application (Table 1).

Reaction time (antinociceptive) was augmented from 0 % in control group after 3 min. to (5.08, 15.87)% respectively according to the concentration of Coenzyme Q10 at (4% and 8%). In the hot plate test, the percentage of reaction time (antinociceptive) in concentration of Coenzyme Q10 drug at 4% increased according to the time (Table 2 and Fig. 1).

Table 1: The pain reaction time of Coenzyme Q10(4%, 8%) topically in mice.

Group	+3min	+10min	+20min	+30min	+40min	+50min
Control	4.3±0.5 ^a	4.3±0.5 ^a	4.3± 0.5 ^a	4.3± 0.5 ^a	4.3±0.5 ^a	4.3±0.5 ^a
Coenzyme Q10 ointment (4%)	5.0 ± 0.0 ^a	5.3±0.5 ^a	7.0 ± 1.0 ^b	8.0±1.0 ^b	6.0 ± 1.0 ^a	4.6±1.1 ^a
Coenzyme Q10 ointment (8%)	7.0 ±1.0 ^b	8.3±0.5 ^b	11±1.7 ^c	12.0 ±2.0 ^c	9.0 ± 1.0 ^b	8.3±0.5 ^b

^{Abc}: The horizontal different letters means significant difference by Duncan at p<0.05.

Table 2: Effect of Coenzyme Q10 (4%, 8%) topically on antinociceptive maximum possible effect (MPE%) in hot plate test in mice.

Group	3min	10min	20min	30min	40min	50min
Control	0	0	0	0	0	0
Coenzyme Q10 ointment (4%)	5.08	5.08	12.68	16.47	8.88	3.79
Coenzyme Q10 ointment (8%)	15.87	19.53	30.50	34.16	23.18	20.73

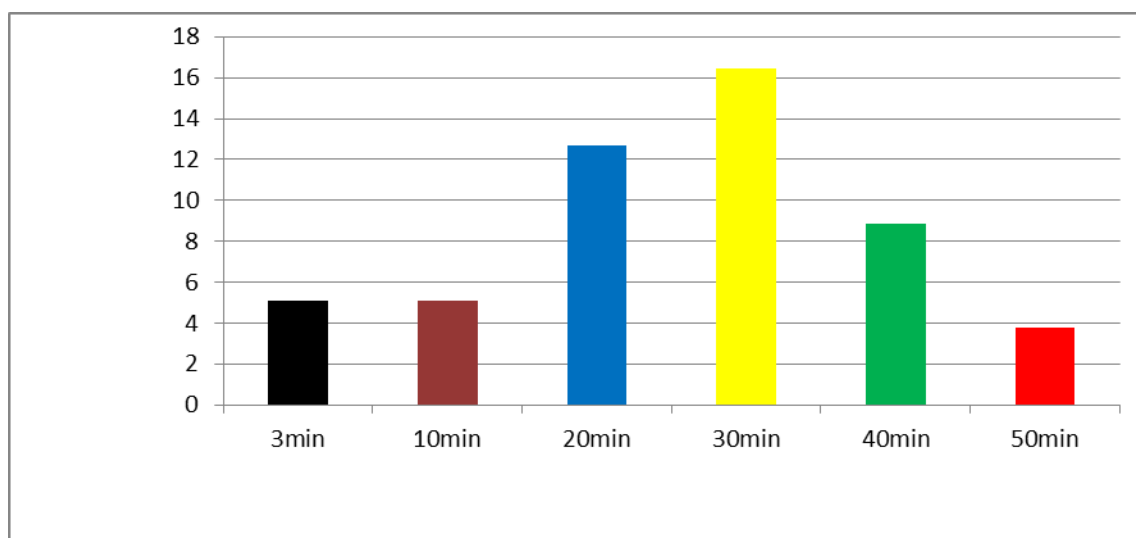


Fig. 1: Effect of Coenzyme Q10 4% topically on antinociceptive maximum possible effect (MPE) in hot plate test in mice.

In concentration of Coenzyme Q10 drug at 8% the (MPE) increased to (15.87, 19.53, 30.50, 34.16, 23.18, 20.73) respectively according to time (Figure 2). While the maximum effect (best time) action of Coenzyme Q10 drug always happen at 30 min. in different concentration (4%,8%) as shown in Table 2and Figure 3.

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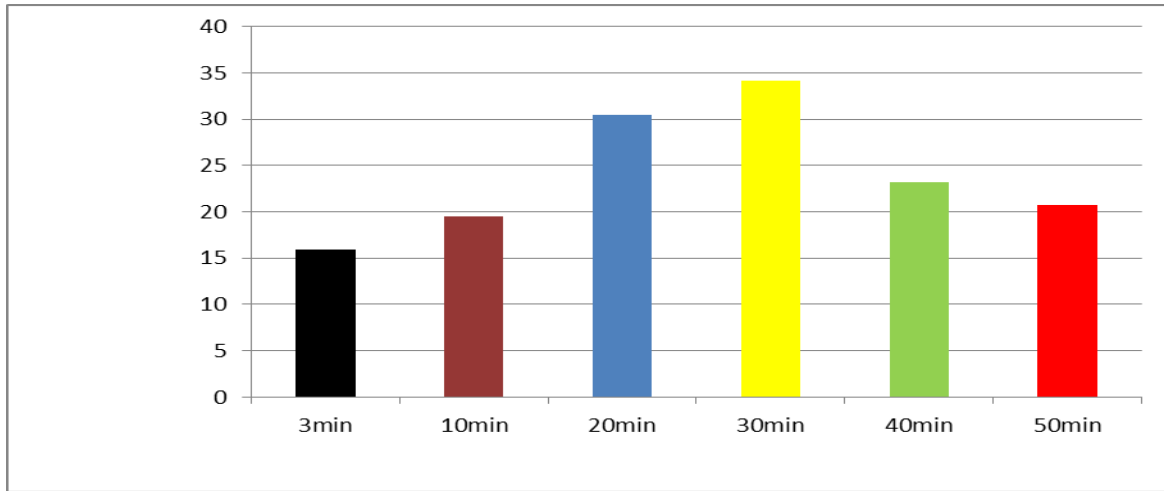


Fig. 2: Effect of Coenzyme Q10 8% topically on antinociceptive maximum possible effect (MPE) in hot plate test in mice.

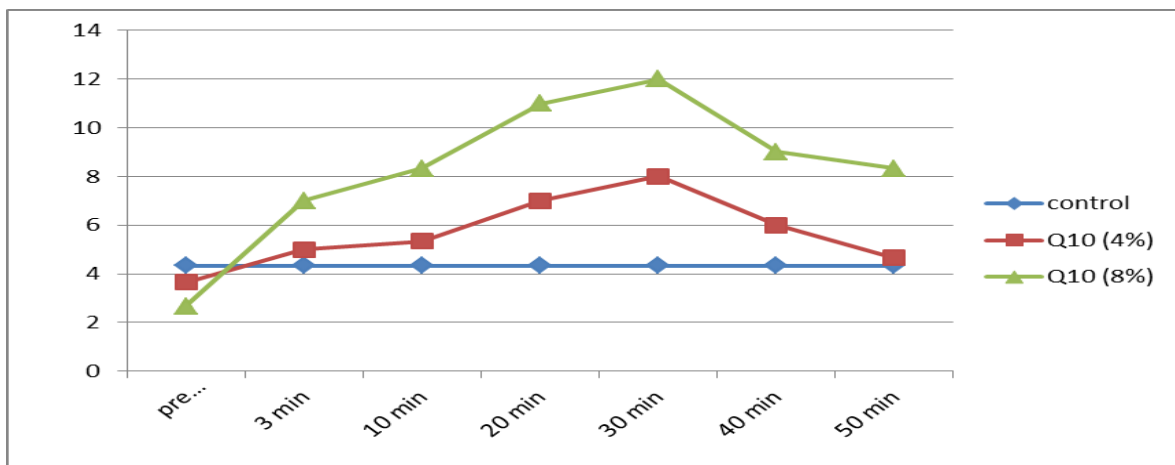


Fig. 3: Duration of analgesic activity of Coenzyme Q10 (4% and 8%) after topical application.

DISCUSSION

Topical analgesics drugs application have many advantages such as the ability to provide good analgesia with reduced systemic drug levels (Kreitler and Niv, 2007), remains for long period at the application site and enough drug penetration and high effective are other advantages (Illum, *et al.*, 1994; Wael, 2011; Ghada, 2014).

In present study we found that Q10 have analgesic activity after using as a topical application at (4%,8%) ointment. The analgesic activity of Q10 in the present study may be return to the effect of Coenzyme Q10 on reducing inflammatory mediator (Nesami, *et al.*, 2015). Pain generating pro-inflammatory cytokines including 1L-1 β and IL-6 (Kapoor, *et al.*, 2011). IL-1 β was found to increase the production of substance P and prostaglandin E₂ (PGE₂) in a number of neuronal

and glial cells (Jeanjean, *et al.*, 1995; Schweizer, *et al.*, 1988). IL-6 contributes to the development of neuropathic pain behavior following a peripheral nerve injury (DeLeo and Colburn, 1995; Ramer, 1998). TNF- α can enhance the sensitivity of sensory neurons to the excitation produced by capsaicin and this enhancement likely is mediated by the neuronal production of prostaglandins (Nicol, *et al.*, 1997).

The anti-inflammatory function of CoQ10 However, it remains unclear what is their mechanism of action (Lee, *et al.*, 2012). Several potential mechanisms might partially explain it. It seemed that CoQ10 might play a potential role in decreasing the production of pro-inflammatory cytokines by inhibiting NF- κ B gene expression (Schmelzer, *et al.*, 2008), attenuating miR-146a and IL-1 receptor associated kinase modulation (Olivieri, *et al.*, 2013), and reducing the secretion of macrophage

inflammatory protein-1 alpha and regulated upon activation normal T-cell expressed and secreted factors (Schmelzer, *et al.*, 2007). In addition, several studies observed a negative relationship between adiponectin and inflammatory factors (TNF- α , IL-6, hs-CRP) (Engeli, *et al.*, 2003; Zhou, *et al.*, 2011).

Zhou *et al.* (2011) reported that adiponectin can inhibit stimulated monocytes secreting TNF- α (Zhou, *et al.*, 2011), so it is likely to that increasing in adiponectin which obtained by using of coenzyme Q10 (Farsi, *et al.*, 2016; Nesami, *et al.*, 2015) can indirectly bring about a plunge in inflammatory factors such as TNF- α . Tsai *et al.* represented that CoQ10 suppresses iNOS and protect endothelial cells from oxidative stress-induced injury (Tsai, *et al.*, 2012). Therefore CoQ10 reduced the pain (Jennifer, *et al.*, 2013). Co enzyme Q10 is a potent antioxidant and reduces oxidative stress by reduction of the reactive oxygen species (ROS) production (Ernster L, Dallner, 1995; Ferrante, *et al.*, 2002).

The reactive oxygen species (ROS) are also involved in NMDA receptor phosphorylation, which leads to activation of NMDA receptors in nerve injury models (Siniscalco, *et al.*, 2007; Muscoli, *et al.*, 2004). Activation of NMDA receptors is another mechanism for induction of hyperalgesia seen after nerve injury (Wang, *et al.*, 2004). Activation of NMDA receptors following excessive production of ROS could also lead to decrease in the antioxidant activity (Coyle and Puttfarcken, 1993). the role of CoQ10 is more emphasized.

CONCLUSION

Topical Coenzyme Q10 is an alternative dosage form, it is easy to apply, safe and not irritant. Because of the Coenzyme Q10 having all these above mechanisms, our study showed that Coenzyme Q10 have a good analgesic effect on the pain stimulated by using the hot-plate test in mice.

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