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Original article

Evidence for antinociceptive effects of combined administration of vitamin E and celecoxib in tail-flick and formalin test in male rats

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ABSTRACT

Background: The aim of this study was to evaluate the effect of vitamin E co-administration with celecoxib in thermal and inflammatory pain in two model of pain assessment including thermal tail flick test of acute pain and formalin induced inflammatory model in adult male rats.
Methods: Seventy two male Wistar rats were divided into a vehicle received intraperitoneally olive oil, indomethacin (20 mg/kg), vitamin E (100, 200 and 400 mg/kg), celecoxib (3, 10, 30 and 60 mg/kg) groups, and combination groups received the combination of vitamin E (100 and 200 mg/kg) and celecoxib (3, 10 and 30 mg/kg). All drugs were dissolved in olive oil. Antinociceptive effect in tail-flick was measured using Area Under Curve (AUC) of responses and Maximum Possible Effect (%MPE) and pain score was used for antinociceptive response in formalin test.
Results: Vitamin E and celecoxib changed time course of pain scores in a dose related manner in formalin test but not in tail-flick test. Vitamin E (200 mg/kg) had no effect and merely 60 mg/kg of celecoxib increased %MPE and AUC in tail-flick. The combination of vitamin E (100 or 200 mg/kg) with celecoxib (3 or 10 mg/kg) decreased pain scores compared to vehicle in both phases of formalin test, while in chronic phase (II) the pain scores of combination groups were also decreased compared to vitamin E and celecoxib. However, in tail-flick test the combination of ineffective doses of vitamin E (200 mg/kg) and celecoxib (10 and 30 mg/kg) increased %MPE and AUC compared to vehicle but not compared to celecoxib or vitamin E.
Conclusions: Vitamin E and celecoxib showed a dose related antinociceptive effect in inflammatory but not in thermal model of acute pain. However the co-administration of vitamin E with celecoxib caused a significant increase in the antinociceptive effect which was similar to indomethacin, as a standard anti-inflammatory drug. So we suggest the concomitant use of vitamin E with celecoxib and other NSAIDs for potentiation of both anti- inflammatory and analgesic response, as well as the reduction of cardiovascular side effects of celecoxib.

Introduction

Not steroidal anti-inflammatory drugs (NSAIDs) are widely used in treatment of pain symptoms. The NSAIDs prevent prostaglandins formation inhibiting cyclooxygenase (COX) enzymes (COX1 and COX2) but their prolonged use as analgesics and anti-inflammatory drugs is generally associated with the development of gastrointestinal (GI) symptoms ranging from simple dyspepsia to life threatening GI bleeding and perforations [1,2]. So selective NSAIDs were proposed in direction to prevent inflammatory process with low damage to stomach [3]. The approach was to find NSAIDs that inhibit selectively inducible COX2 involved in inflammatory process, without affecting constitutive COX1 [4]. In a systematic review COX2 inhibitors showed less risk of GI events [5].

Despite the lower risk of GI adverse effects, however, COXIBs were associated with an increased risk of serious cardiovascular (CV) and thromboembolic events, including the higher risk of myocardial infarction, and worsening of congestive heart failure [6,7]. In this regard celecoxib was considered less dangerous than other COXIBs [4]. At present celecoxib is prescribed for several conditions, including rheumatoid arthritis, osteoarthritis, and acute musculoskeletal pain, considering the drug's label warning its CV adverse effects [8,9].

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Drive benefit of additive or synergistic effect of combination therapy, is a current approach in development of a better anti-inflammatory drugs. For example the use of proton pomp inhibitors with aspirin [10] or misoprostol as a protective stomach prostaglandin with diclofenac [11] are combinations with concomitant benefit of increasing efficacy and decreasing side effects.

Vitamin E showed antinociceptive effect in different models of pain. In our recent study, it improved neuropathic pain after sciatic nerve constriction in rats [12,17]. Vitamin E decreased biphasic pain behavior in rat model of formalin test [13] and also reduces the mechanical allodynia and hyperalgesia manifested in streptozocin-induced diabetic in rats [14]. From 1970s it was known that vitamin E decreases mortality due to cardiovascular disease because of its antioxidant effects [15]. Then several cardioprotective effect has been detected for tocotrienols and vitamin E such as anti-atherosclerotic, platelet aggregation inhibition and preventing smooth muscle proliferation [16].

Since cardiovascular adverse effect of celecoxib is still one of its main limiting side effects, so strategies for reduction of adverse drug reactions is desirable in clinical practice. Since vitamin E has shown antioxidant and beneficial effects on cardiovascular system, as well as antinociceptive and anti-inflammatory properties, so this study was performed to investigate the antinociceptive effect of combination of vitamin E with celecoxib in two models of pain assessment; including tail-flick test, as the response to thermal pain, and formalin test as inflammatory pain response to chemical stimulus.

Material and methods

Drugs

Celecoxib, pure powder (Hetero drugs limited, India). Indomethacin, pure powder gifted of Behdashtkar Pharmaceutical Company, Iran. Vitamin E (DL-α-tocopheryl acetate) purchased from Osvah Pharmaceutical Company, Iran. All drugs were dissolved freshly in olive oil and stirred properly before intraperitoneal (*ip*) injection in a volume of 3–4 ml. The doses of drug used were based on previous research studies [12,13,17–20].

Animals

Male adult rats (n = 72) weighing 180–200 g were provided from Kerman Medical School Animal House and divided into subsequent nine groups, each group consisted of 8 rats.

- Vehicle group (Veh) which received olive oil as solvent of all drugs.
- Indomethacin group (Indo) which received 20 mg/kg of indomethacin.
- Vitamin E groups (E100, E200, E400), which received vitamin E at doses 100, 200 and 400 mg/kg.
- Celecoxib groups (Cel3, Cel10, Cel30, Cel60) which received celecoxib in at doses of 3, 10, 30 and 60 mg/kg.
- Combination groups used in formalin test which received celecoxib 3 mg/kg plus vitamin E 200 mg/kg (Cel3+E200) or celecoxib 10 mg/kg plus vitamin E 100 mg/kg (Cel10+E100) and combination groups used in tail-flick test; celecoxib 10 mg/kg plus vitamin E 200 mg/kg (Cel10+E200) or celecoxib 30 mg/kg plus vitamin E 200 mg/kg (Cel30+E200).

All animals were kept in 15 × 30 × 40 cm cages in room temperature of 23 ± 2° C with a 12:12 h light/dark cycle and unlimited access to food and water. Drugs were injected thirty minutes before antinociceptive tests. Antinociceptive tests; tail-flick and formalin test, were performed between 9 AM to 16 PM. All experimental data were recorded by an observer who was not aware of the treatment protocol. The animals were acclimated for an hour in a low illuminated sound proof room. The protocol was ethically approved by Animal Experimental Committee of Kerman University of Medical Sciences at 18 Nov 2012 (Ethical code = EC/95/1/KNRC) in accordance with principles for laboratory animal use and care, as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Formalin test

To assess inflammatory pain 50 μl of 2.5% formalin was injected subcutaneously (*sc*) into dorsal right hind paw using a 30-G needle. Then the rats were placed immediately in open plexiglass chamber (25 × 25 × 40 cm) with a 45°mirror located under it and observed for 60 min. Quantification of pain behavior was based on the animal behaviors (flinches or shaking of injected paw) every minutes during a period of 60 min using manually a stopwatch. According to Dubuisson and Dennis, 1977 behavioral categories were recorded as 0), the injected paw is comparable to the contralateral paw and is used normally by the animal; (1), the injected paw has little or no weight placed on it; (2), the injected paw is elevated and is not in contact with any surface; and (3), the injected paw is licked, bitten or shaken. So the maximum number was assigned in each minute. Phase I formalin test considered as acute pain response and phase II considered as inflammatory phase.

Phase I (acute pain) was considered 0–5 min, following an interphase (6–14 min), phase II (inflammatory) continued from 14thto 60th min after injection. Pain score of each phase was the mean of behavioral category assigned of 8 rats weighing the category assigned according to following formula; Pain score = (1T1 + 2T2 + 3T3)/4.

1T1=behavioral category (1)×1, 2T2=behavioral category (2)×2 and, 3T3=behavioral category (3)×3

Tail-Flick test

The test was used to measure nociceptive pain based on latency time, time required to avoid thermal stimulus applied by a radiant heat on 5–6 cm from the tip of rat tail. The apparatus was PANLAB 7160 (Spain). The intensity and focus of the light was adjusted to obtain a base line from 2 to 4 s. To avoid tissue damage the irradiation was automatically stop after 10 s (cut-off = 10 s). The baseline was recorded three times before any experiment and those animals with outranged latency times were excluded from study [12].

Latency times (LT) were recorded in 30th, 60th and 90th min immediately after injection to obtain time course of drug effect. Antinociceptive effect in tail-flick was measured using Area Under Curve (AUC) of responses and Maximum Possible Effect (%MPE). AUC was calculated using Trapezoid rule: AUC = 30×LT[(min30)+(min60)+(min90)/2]. And %MPE in 90th min was calculated based on following formula. The %MPE= [(LT min90 - T0)/(T2 - T0)] × 100. T0 was the base line and LT min 90 was the latencies time 90 min after drug administration, and T2 was cut-off time (10 s).

Statistical analysis

One way and repeated measure ANOVA models were used to assess the effects of time and drug. In formalin test the dependent variable was pain score while in tail-flick, it was latency time. As *post-hoc* Tukey's or Student-Newman-Keuls (S-N-K) were used for comparison between groups as independent variable. One-way analysis of variance (ANOVA) was used to evaluate the significance

difference in pain scores, %MPE and AUC. Statistical analysis was performed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered significant. Data were expressed as Mean ± SEM of at least six rats.

Results

The effect of vitamin E and celecoxib and on pain score in formalin test

The effect of vitamin E (100, 200, and 400 mg/kg *ip*) on time response of pain scores in phase I was shown in Fig. 1. Vitamin E reduced dose-dependently pain scores in formalin test. The repeated ANOVA model showed that temporal variation was significantly different among groups ($F_{3,66} = 26.7$, $p = 0.000$). No interaction has been observed between group and time response. But the time responses of vehicle group showed a significant increase while those of indomethacin showed a significant decrease compared to all groups. Following acute phase an interphase 5–14 min was occurred for all groups. In chronic phase of formalin test (15–60 min), temporal variation showed significant difference among groups ($F_{13,320} = 35.8$, $p = 0.000$). Also the interaction of time response with groups showed significant difference ($F_{53,320} = 1.8$, $p = 0.000$) with a linear relationship. Student- Newman-Keuls (S-N-K) *post-hoc* showed significant decrease time response curve in order of groups Indo (46%), E400 (35%), E200 (19%), E100 (11%) with vehicle (Fig. 1-A).

In phase I the mean pain score except E100 was significantly decreased (8%) compared to vehicle group ($p < 0.05$). A dose dependent relationship have been observed using S-N-K *post-hoc*. In chronic phase pain score of all groups was significantly decreased compared to vehicle group (Indo; 42%, E400; 33, E200; 19%, E100; 14%) ($p < 0.05$). Obviously mean pain score of 20 mg/kg of indomethacin was significantly diminished compared to all other groups (Fig. 1-B).

The time response of pain scores of celecoxib was shown in Fig. 2. Celecoxib 3, 10 and 30 mg/kg *ip* reduced dose-dependently pain scores in both phases of inflammatory pain. In acute phase the *ip* injection of 3, 10 and 30 mg/kg of celecoxib produced a significant temporal variation ($F_{2,25} = 11.0$, $p = 0.000$). Also time response between groups was significantly different; the curve of Indo20 group was significantly decreased compared to all other groups while that of vehicle was significantly increased compared to all groups despite Cel3 ($p < 0.05$) (Fig. 2-A). After interphase, chronic phase started from 15th to 60th min. The repeated model

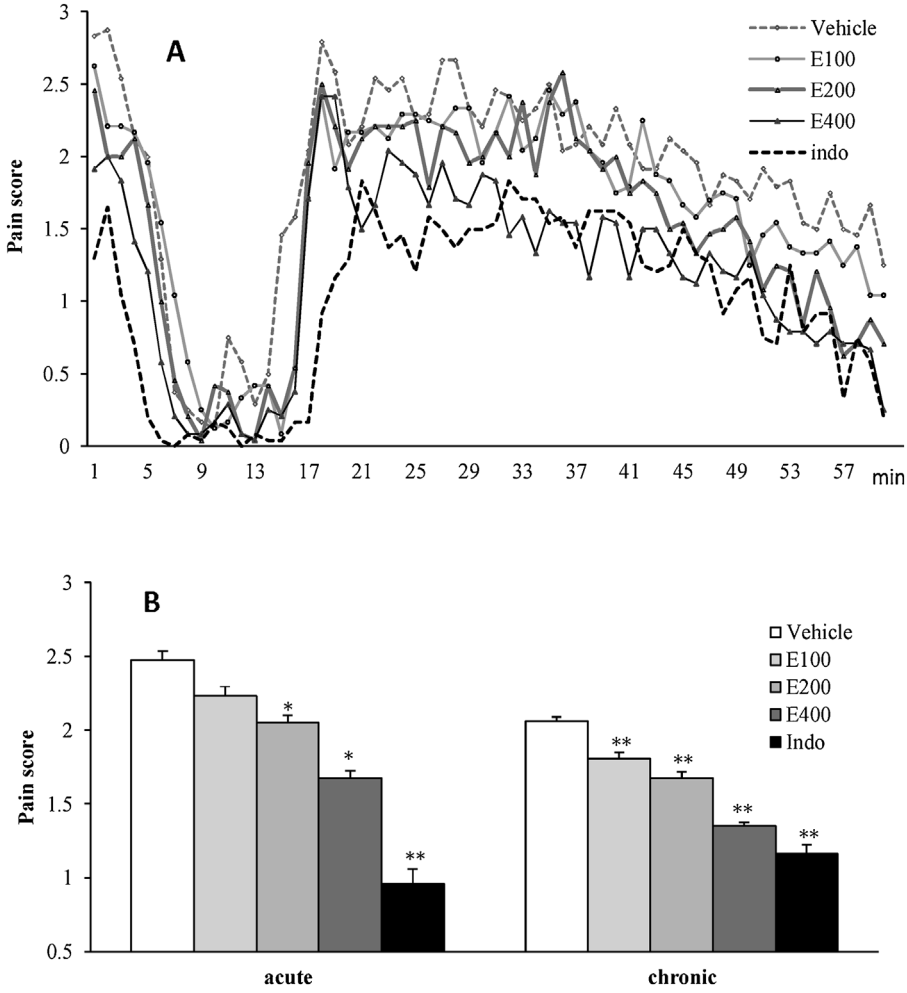

Fig. 1. The antinociceptive effect of vitamin E in formalin test. Animals received olive oil, 100, 200 and 400 mg/kg of vitamin E and 20 mg/kg of indomethacin thirty min before test in respective Vehicle, E100, E200, E400 and Indo groups. (A) The time course of pain scores; In acute phase pain scores increased in phase I (acute) during first 5 min followed by a decrease in interphase and increase from 15th minute (phase II or chronic). Repeated measure of ANOVA model showed the interaction of time responses between indomethacin and vehicle compared to vitamin E groups in both acute and chronic phase. (B) Mean pain scores in acute and chronic phase. Mean score of Indo group was significantly decreased compared to all groups ($p < 0.005$). In acute phase pain scores of E200 and E400 were decreased, while in chronic phase those of all E100, E200 and E400 groups were decreased compared to vehicle group. S-N-K *post-hoc* showed a dose-dependent antinociception of vitamin E in both phases of formalin test. Data were expressed as mean ± SEM of 8 rats. Significancy * and ** $p < 0.05$ and $p < 0.005$ compared to vehicle.

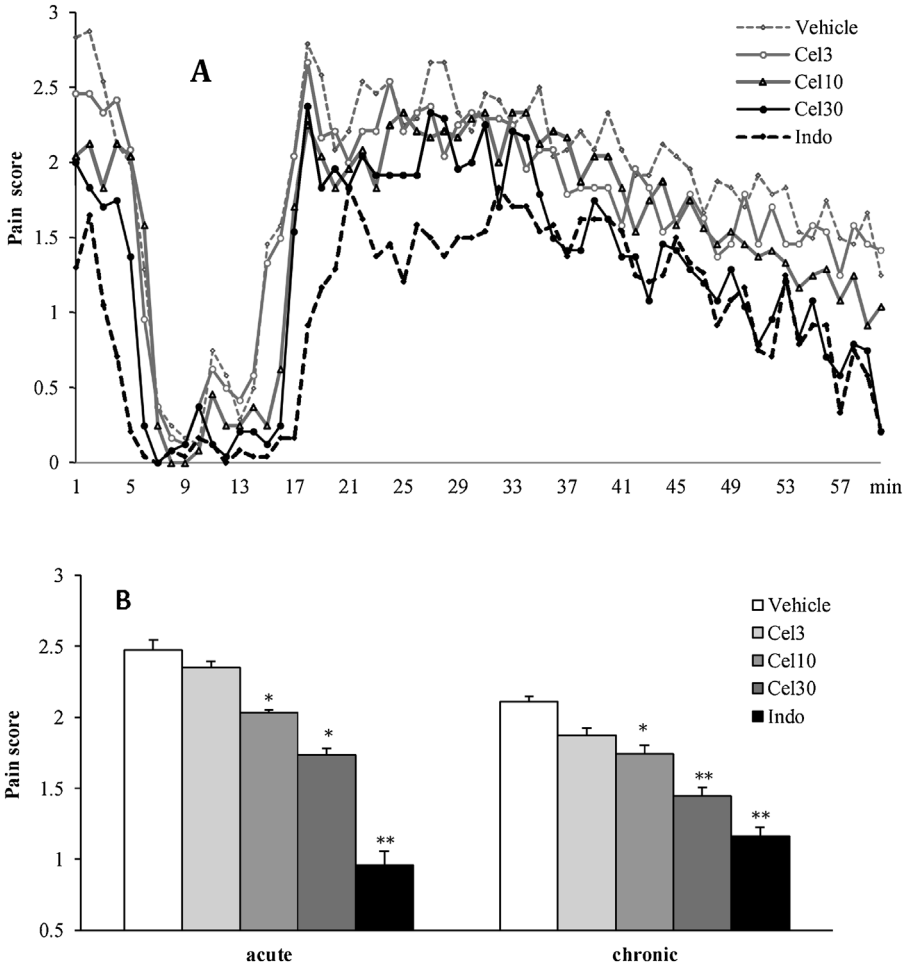

Fig. 2. The antinociceptive effect of celecoxib in formalin test. Animals received olive oil, 3, 10, and 30 mg/kg of celecoxib and 20 mg/kg of indomethacin thirty min before test in respective Vehicle, Cel3, Cel10, Cel30 and Indo groups. (A) The time course of pain scores: In acute phase pain scores increased in phase I during first 5 min of formalin test followed by a decrease in interphase and increase from 15th minute (phase II or chronic), where time response and temporal variation showed significant difference among groups ($p < 0.05$). (B) Mean pain scores in acute and chronic phase. Mean score of Indo group was significantly decreased compared to all groups. Mean pain scores of Cel10 and Cel30 were decreased compared to vehicle group. S-*N*-K *post-hoc* showed a dose-dependent antinociception of celecoxib in both acute and chronic phases of formalin test. Data were expressed as mean ± SEM of 8 rats. Significancy * and ** $p < 0.05$ and $p < 0.001$ compared to vehicle.

of ANOVA showed significant difference of temporal variation ($F_{4,25} = 1.6$, $p = 0.018$). Time response of pain scores of indomethacin 20 and all other groups were decreased compared to vehicle ($p < 0.005$) Cel3 (8%), Cel10 (16%), Cel30 (31%), Indo (46%) (Fig. 2-A).

The mean of pain scores of all celecoxib groups were increased compared to vehicle group, except that of Cel3, in both phases of inflammatory pain (Fig. 2-B). Also the mean pain score of Indo group was significantly different compared to all groups ($p < 0.05$).

The effect of combination of vitamin E and celecoxib and on pain score in formalin test

In acute phase the combination of 100 mg/kg of vitamin E with 10 mg/kg of celecoxib (Cel10+E100) decreased significantly (16%) pain score compared to vehicle group ($p < 0.001$), while in phase II this combination decreased significantly pain score compared to E100 (14.5%) ($p < 0.005$), Cel10 (11%) ($p<0.05$) and vehicle groups (24%) ($p < 0.001$) (Fig. 3). In acute phase the combination of 200 mg/kg of vitamin E with 3 mg/kg of celecoxib (Cel30+E200) decreased pain score compared to Cel3 (20%) and vehicle groups (24%) ($p < 0.001$). In phase II, pain scores of E200+Cel3 group decreased significantly compared to both Cel3 (20%) ($p < 0.001$), E200 (11%) ($p < 0.05$) and also compared to vehicle group (26%) ($p < 0.001$) (Fig. 3).

The antinociceptive effect of vitamin E and celecoxib in tail-flick

The antinociceptive effect after injection of 10 (Cel10), 30 (Cel30) and 60 mg/kg of celecoxib (Cel60) and 200 mg/kg of vitamin E (E200) in tail-flick test was illustrated in Fig. 4. In The latency time-response curve of different groups (Fig. 4-A), it was seen that temporal variation was significantly different among groups ($F_{3,90} = 207.2$, $p = 0.000$). Time response curve was different based on drug injected; as those of Indo and Cel60 groups were significantly ($p < 0.05$) increased 50% compared to vehicle group (Fig. 4-A).

%MPE of 10 and 30 mg/kg doses of celecoxib (24.3 ± 8.4 and 27.7 ± 5.1) and vitamin E 200 mg/kg (34.9 ± 8.9) groups was similar to that of vehicle, but %MPE of Indo and Cel60 (55.7 ± 13.9 and 46.5 ± 7.9) was increased significantly compared to vehicle ($p < 0.05$) (Fig. 4-B). The comparison of AUC in Fig. 4-C showed a significant augmentation in Cel60 and Indo groups compared to vehicle ($p < 0.05$) (Fig. 4).

The antinociceptive effect of combination vitamin E and celecoxib in tail-flick

%MPE and AUC of combination groups Cel10+E100 and Cel30 +E200 with corresponding component were shown in Fig. 5. %MPE

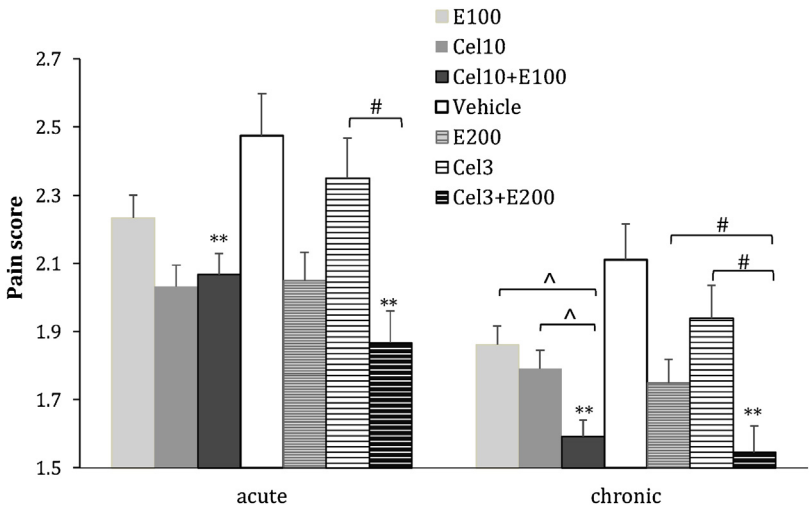

Fig. 3. The effect of combination of vitamin and celecoxib on pain score in formalin test. Animals received olive oil (vehicle), celecoxib 10 mg/kg (Cel10), vitamin E 100 mg/kg (E100) or both of them (Cel10+E100) and celecoxib 3 mg/kg (Cel3), vitamin E 200 mg/kg (E200) or both of them (Cel3+E200) thirty min before test. The co-administration of vitamin E with celecoxib decreased pain scores significantly in combination groups of (Cel3+E200) and (Cel30+E100) compared to vehicle. In acute phase pain score of (Cel3) was significantly increased compared to (Cel3+E200). In chronic phase pain score of both (Cel3+E200) and (Cel10+E100) were decreased compared to respective component (Cel3, E200, Cel10 and E100). Data were expressed as mean ± SEM of 8 rats. $^{**}p < 0.005$ compared to vehicle; $^{\wedge}p < 0.05$ compared to (Cel10+E100) group; $\#p < 0.0$ compared to (Cel3+E200) group.

of combination groups Cel10+E200 and Cel30+E200 (56.9 ± 6.9 and 85.4 ± 9.8) were increased compared to vehicle ($p < 0.005$) as did 20 mg/kg of indomethacin and 60 mg/kg of celecoxib previously (Figs. 4 and 5). Same results were seen for AUC of combination groups. Both AUC and %MPE of Cel30+E200 groups was significantly increased compare to E200 and Cel30, and also compared to indomethacin ($p < 0.05$) (Fig. 5).

Discussion

The results of this study indicated that celecoxib showed antinociceptive and anti-inflammatory response in a dose dependent manner, but vitamin E caused a significant antinociception in a dose dependent manner in a rat model of formalin-induced hyperalgesia, but not in thermal model of acute pain assessment. However, the antinociceptive and anti-inflammatory effects of both vitamin E and celecoxib were significantly lower than indomethacin as a standard drug.

The results are in agreement with previous report indicating that intraperitoneal injection of vitamin E caused a significant antinociception in a rat model of formalin-induced hyperalgesia [13], but not in tail-flick model of pain assessment [12]. Also the dose-dependent (10–30 mg/kg) antinociceptive effect of celecoxib in formalin test has been previously shown in rats and more clearly in inflammatory phase (II) in mice [19,20]. In accordance to our study, even maximal dose of celecoxib showed low effect in tail-flick test [24] neither topical use of celecoxib succeeded to decrease significantly latency times in tail-flick [17]. The precise mechanism of vitamin E induced analgesia is not known, but it may be mediated through the inhibition of COX-2 activity or reducing central sensitization in spinal dorsal horn neurons [21,22].

The combination of vitamin E and celecoxib produced antinociceptive effect in formalin-induced hyperalgesia and thermal model of acute pain assessment, i.e. the combination of both effective dose of celecoxib (10 mg/kg) with the ineffective dose of vitamin E (100 mg/kg) and ineffective dose of celecoxib (3 mg/kg) with lowest effective dose of vitamin E (200 mg/kg) cause a significant decrease in pain score and a significant increase in % MPE (and AUC) compared to vehicle group. Moreover the combination of vitamin E with celecoxib increased antinociceptive effect in both tests. In formalin test, the combination of ineffective dose of celecoxib (3 mg/kg) with lowest effective dose of vitamin E (200 mg/kg) showed more antinociceptive effects compared to each component alone. Also in tail-flick test, the combination of celecoxib (30 mg/kg) and vitamin E (200 mg/kg) increased antinociceptive response compared to each component alone. The precise type of pharmacological interaction between antinociceptive and anti-inflammatory effects of celecoxib and vitamin E combination is not determined in this study and needs a isobolographic study, however, since the antinociceptive effects of celecoxib and vitamin E combination in both thermal model of acute pain and formalin-induced hyperalgesia was significantly higher than each drug alone, so we suggest an additive effect for their combination use.

In agreement with our results, Abate et al. showed the synergistic effects of the combination of vitamin E and aspirin on the inhibition of COX-2 expression by unknown mechanisms [23]. Antinociceptive effects of vitamin E in chronic (inflammatory) phase of formalin test may be mediated through its anti-inflammatory and antioxidant properties [24]. Also in agreement with our results, Kim et al. showed that neither vitamin E nor gabapentin alone had antinociceptive effect in the early phase of formalin test, but their combinations produced analgesic effects in early phase of formalin test and moreover in the inflammatory phase, the combination revealed synergistic effects of formalin model of inflammatory pain in rats [25]. Same results have been reported for the combination of vitamin E and pregabalin in partial sciatic nerve ligation induced neuropathic pain in rats [12]. Vitamin E showed potentials to reduce inflammatory process in induced neuropathy [26]. In patients suffering of knee osteoarthritis vitamin E decreased pain sensation [27], as did diclofenac and after 21 day of treatment no significant difference have been observed between vitamin E and diclofenac [28]. Also it is reported that co-administration of vitamin E and C caused a significant reduction of inflammation and improved insulin action in older persons with impaired fasting glucose [29]. Riffel et al. reported that in rats with chronic constriction injury of the sciatic nerve, both vitamin E and vitamin C (*po* or *ip*) possesses antinociceptive activity and their combinations showed synergistic effects in increasing mechanical and thermal thresholds [30].

Previous studies showed the antinociceptive property of vitamin E in neuropathic or inflammatory, but not acute pains.

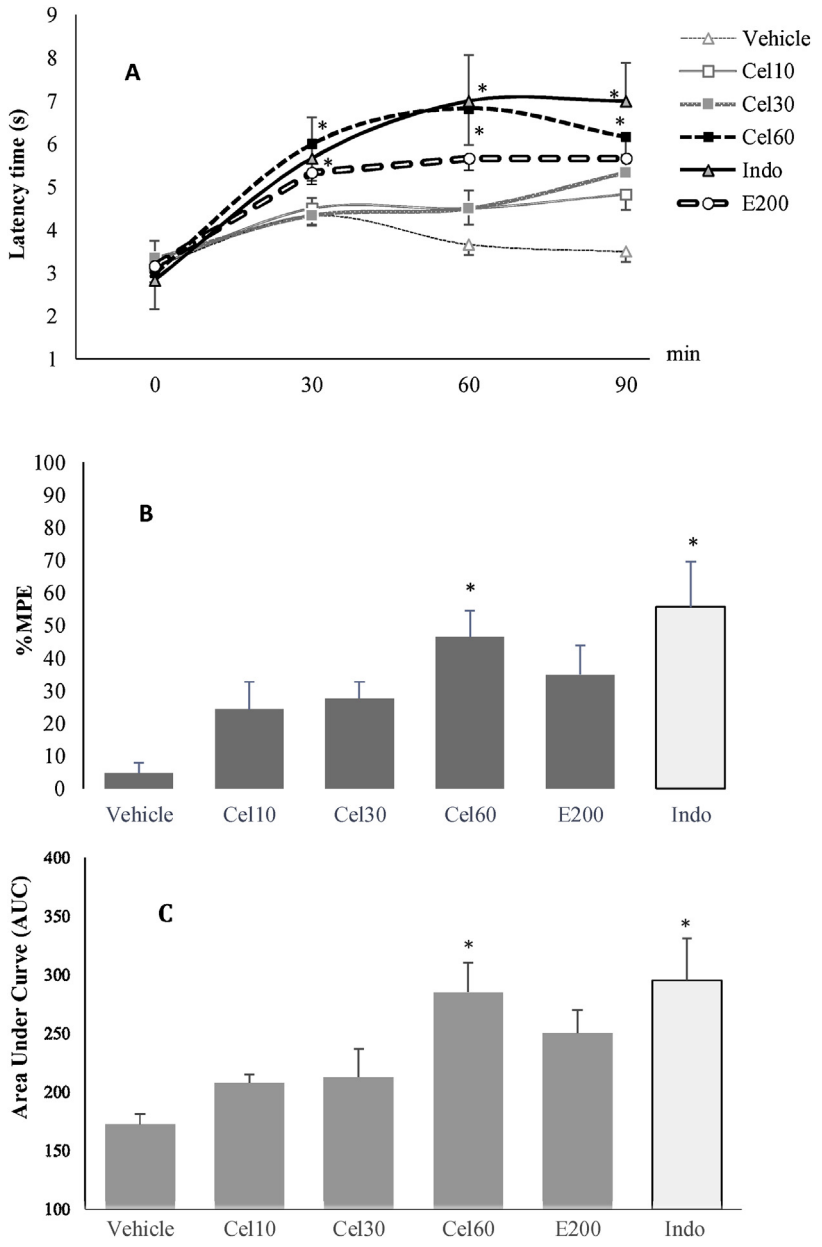

Fig. 4. The antinociceptive effect of celecoxib and vitamin E in tail flick test. Animals received olive oil (vehicle), celecoxib 10 mg/kg (Cel10), celecoxib 30 mg/kg (Cel30), celecoxib 60 mg/kg (Cel60) and vitamin E 200 mg/kg (E200) thirty min before test. (A) The time course of latency times of drugs. Time response curve of during 90 min of tail flick showed significant temporal variation among groups, time responses of Cel60 and Indo were increased compared to other groups. (B) Maximum Possible Effect percent (%MPE) of groups. The %MPE of Cel60 and Indo were significantly increased compared to vehicle group. (C) The Area Under Curve of groups. The AUC of Cel60 and Indo were significantly increased compared to vehicle group. Data were expressed as mean ± SEM of 8 rats.*$p < 0.05$ compared to vehicle.

The results showed that the combination of vitamin E with non-effective dose of celecoxib in tail-flick model of acute pain assessment produced more antinociception (%MPE = 60%–85%) than 20 mg/kg of indomethacin (%MPE = 56%) as the standard NSAIDs. Many authors reported that co-administration of vitamin E with NSAIDs or gabapentinoides showed either additive or synergistic effects on antinociception [12,25,29,31].

Cyclooxygenase and prostaglandins mediate the spread of acute pain at the spinal cord level and the inflammatory pain is caused by release of eicosanoids including arachidonic acid through the activation and sensitization of peripheral nociceptors, possibly through the activation of glutamate, an excitatory amino acids [32]. The precise mechanism(s) underlying the vitamin E antinociceptive and anti-inflammatory effects is not completely determined yet. This probable additive effect could be due to the combination of mechanisms of action of both substances, vitamin E could potentiate the antinociceptive and anti-inflammatory responses through affecting different pathways including antioxidant property or the inhibition of COX2 or reducing central sensitization [21,22,33]. Reported evidences indicates that in persistent pain, reactive oxygen species (ROS) are contributed to pain hypersensitivity, therefore vitamin E, as a ROS scavenger neutralizes the harmful effect of ROS [13]. The anti-inflammatory effects of vitamin E could be also attributed to the inhibition of COX- and 5-lipoxygenase-catalyzed eicosanoids, and the suppression of pro-inflammatory signaling such as NF-κB (a protein complex that controls transcription of DNA, cytokine production and cell survival) and Signal Transducer and Activator of Transcription 3 (STAT3) [34]. It was seen that in alcohol neuropathy vitamin E increased pain threshold through oxidative stress mediated pro-inflammatory cytokines TNFα and IL-1β [35].

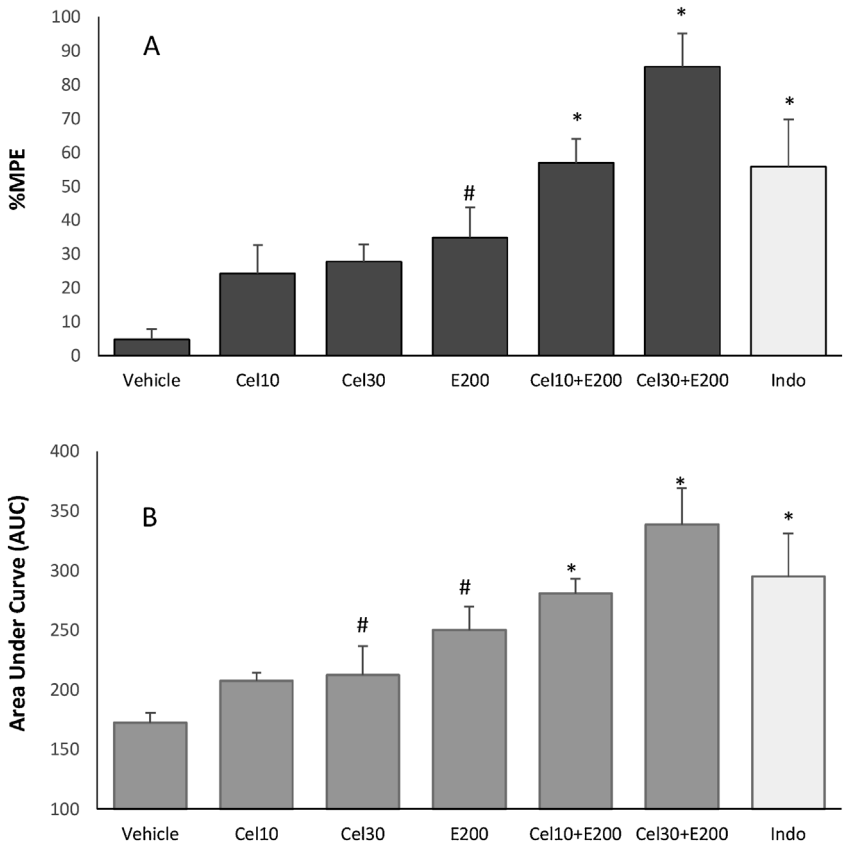

Fig. 5. The antinociceptive effect of combination of celecoxib and vitamin E in tail flick test. Animals received olive oil (vehicle), celecoxib 10 mg/kg,(Cel10) celecoxib 30 mg/kg (Cel30), vitamin E 200 mg/kg (E200) or both of them Cel10+E200 and Cel30+E200. (A) Maximum Possible Effect percent (%MPE).The %MPE of combinational groups were increased compared to vehicle. Vitamin E increased the antinociception of celecoxib. (B) The Area Under Curve. The AUC of combinational groups were increased compared to vehicle and that of Cel30+E200 was increased compare to both Cel30 and E200. Data were expressed as mean ± SEM of 8 rats; $^{*}p < 0.05$ compared to vehicle, $\#p < 0.05$ compared to (Cel30+E200) group.

Vitamin E and tocopherols inhibit the COX-2 activity, resulting in reduction of the synthesis of prostaglandin E2 as an important mediator of inflammation in cancer and cardiovascular disease [22]. Another cardioprotective mechanism of vitamin E might be the reduction of monocyte-endothelial cell adhesion through inhibiting HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis [36].

Results of this study showed that co-administration of vitamin E with celecoxib increased the antinociceptive effect. The probable additive effects of combination of vitamin E and celecoxib will provide benefits in the treatment of inflammatory and chronic pain states and reduces adverse drug reactions in risk population including elderly and patients at risk of cardiovascular diseases [15,16,36]. Vitamin E improves cardiac function by enhancing ERK1/2 activity, Bcl2 expression and reducing JNK [37]. Interestingly, vitamin E as well as tocopherol, showed beneficial effects in aspirin-induced gastric ulcers, probably through their antioxidant activity and reducing lipid peroxidation which is involved in gastric lesions [31]. This beneficial effects of vitamin E has important therapeutic implications in prevention or amelioration of NSAIDs induced gastrointestinal side effects in patients using such drugs for long term. The results showed lower efficacy of vitamin E in acute model of pain than neuropathic or inflammatory pain (formalin test) which is in agreement with the Bruunsgaard et al. study which indicated that long-term combined supplementations of therapeutic doses of α-tocopherol and vitamin C had no significant systemic anti-inflammatory effects in a healthy population of men [38].

In summary, the results showed that both celecoxib and vitamin E possess antinociceptive effects in a dose dependent manner in formalin-induced hyperalgesia, but vitamin E unlike celecoxib showed no antinociceptive effects in thermal model of acute pain. However, the combination of vitamin E and celecoxib was associated with additive antinociceptive effects in both model of acute and inflammatory pain in adult male rats. The underlying mechanism(s) involved in either antinociceptive and anti-inflammatory response of vitamin E is not determined but it could probably be mediated through different pathways including reduction in ROS synthesis, inhibition of COX- and 5-lipoxygenase-catalyzed eicosanoids which result in decrease in synthesis of inflammatory mediators such as prostaglandin E2 and suppression of pro-inflammatory signaling. We suggest that the beneficial additive antinociceptive effects of vitamin E in combination with celecoxib may be due to simultaneous action of both agents by different mechanisms. Beside their cardioprotective effects, vitamin E supplements appear safe for most adults across a broad range of intakes [39], so we recommend the concomitant use of vitamin E with celecoxib and other NSAIDs for the palliative treatment of pain in debilitating diseases.

Conflict of interests

The Authors have no conflict of interest.

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