

# The evaluation of analgesic effects of simvastatin, pravastatin and atorvastatin in hot plate test

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**Abstract. – Objectives:** Simvastatin, pravastatin and atorvastatin have been evaluated whether to have analgesic effects in mice in hot plate test.

**Materials and Methods:** Simvastatin (5, 10, 30 mg/kg), pravastatin (5, 10, 30 mg/kg) and atorvastatin (5, 10, 30 mg/kg) were administered acute and chronically by oral gavage in mice. Control (pretreatment value) and posttreatment (after drugs application) values in 60<sup>th</sup> and 120<sup>th</sup> minutes were measured in hot-plate test.

**Results:** All three drugs at 10, 30 mg/kg doses produced analgesic effects compared with their control values in 60<sup>th</sup> and 120<sup>th</sup> minutes on acute and chronic application in mice. The analgesic effects of drugs were evaluated after the application of L-nitro arginine methyl ester (L-NAME) (10 mg/kg) or naloxone (0.5 mg/kg). L-NAME (10 mg/kg) has no effect compared to the control value on both minutes. The analgesic effects of both atorvastatin (30 mg/kg) and simvastatin (30 mg/kg) in the presence of L-NAME (10 mg/kg) were not inhibited. However, the analgesic effect of pravastatin (30 mg/kg) in the presence of L-NAME (10 mg/kg) was inhibited significantly on both minutes ( $p < 0.05$ ). Naloxone (0.5 mg/kg) has no effect compared to the control value on both minutes. The analgesic effect of atorvastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was partially (43%) but significantly inhibited only on 60<sup>th</sup> minute ( $p < 0.05$ ). The analgesic effect of pravastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was partially (48-40%) but significantly inhibited on both minutes ( $p < 0.05$ ). However, the analgesic effect of simvastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was inhibited significantly on both minutes ( $p < 0.05$ ).

**Conclusions:** These finding indicated that the analgesic effect of pravastatin was related to nitrenergic systems and partially opioidergic system; analgesic effect of simvastatin was related to opioidergic system in hot plate test. However, the analgesic effect of atorvastatin was not directly related to both system.

*Key Words:*

Simvastatin, Pravastatin, Atorvastatin, Analgesia, Hot plate test.

## Introduction

The pain is an unpleasant sensory and an emotional experience occurs as a result of mechanical, thermal and chemical nociceptive stimuli induced tissue injury. Cytokines which are released from the damaged tissue cause the release of hyperalgesic neuromediators (such as prostaglandins, serotonin) and lead to pain and inflammation<sup>1,2</sup>. In the meantime, nitric oxide and opioid peptides, released from the damaged tissues, contribute to the anti-inflammatory and analgesic process<sup>3,4</sup>. It has been shown that as a result of reducing mast cell degranulation in the inflammatory tissues, nitric oxide causes antiinflammatory effects<sup>3</sup>. Also, opioid peptides that are released from the damaged tissue generate an analgesic effect via the opioidergic receptors located on peripheral nociceptors<sup>4-6</sup>.

Statins, 3-hydroxy-3 methylglutaryl coenzyme A (HMG-CoA) reductase enzyme inhibitors, are lipid-lowering drugs, often used in the treatment of cardiovascular diseases (hyperlipidemia, atherosclerosis). It has been shown that statins have antiinflammatory effects independent of their lipid-lowering effects and these antiinflammatory effects inhibit the inflammation and pain process in the inflammatory diseases (such as rheumatoid arthritis)<sup>7,8</sup>. The inhibition of releasing of cytokines is shown to play a role in anti-inflammatory effects of statins<sup>9,10</sup>.

In this study, we aimed to investigate the possible analgesic effects of three statins, two

lipophilic (atorvastatin, simvastatin) and one hydrophilic (pravastatin), in hot-plate test in mice by oral gavage.

## Material and Methods

Study was held on in the Karadeniz Technical University, School of Medicine, Behavioral Pharmacology Laboratory of Department of Pharmacology. Balb/c mice of either sex weighting 20-40 g were randomly used in the experiments. All of the animals were housed in cages with free access to food and water. The cages were placed in a quiet and temperature-humidity controlled room ( $22 \pm 2^\circ\text{C}$  and  $60 \pm 5\%$ , respectively) in which a 12:12-h light-dark cycle was maintained. Experiments were conducted between 9:00 and 17:00 h to minimize the diurnal variation. The experimental protocol was approved by the Local Ethics Committee of School of Medicine, Karadeniz Technical University. Hot plate test were used to analgesic method.

### Hot-Plate Test

The temperature of the hot plate was set to  $55 \pm 0.5^\circ\text{C}$ . The time of latency was defined as the time period between the zero point when the animal was placed on the hot plate surface and the time when the animal licked its back paw or jumped off to avoid thermal pain. Baseline latency (pretreatment value) was determined before drug application for each mouse. Post treatment latencies were determined after 60<sup>th</sup> and 120<sup>th</sup> minutes. Values were compared with pretreatment values. The cut-off time was set to 30 seconds in order to minimize skin injury.

### Drugs

Simvastatin (Merck Sharp & Dohme, Istanbul, Turkey) and atorvastatin (Pfizer, Istanbul, Turkey) were dissolved in phosphate buffered saline (PBS). Pravastatin (Bristol-Myers Squibb, Istanbul, Turkey) and L-NAME (L-nitro arginine methyl ester; nitric oxide synthase inhibitor) (Sigma: Saint Louis, MO, USA) were dissolved in saline. Naloxone (opioidergic receptor antagonist) (Abbott Laboratories, Abbott Park, IL, USA) was used in the form they are manufactured. Simvastatin, atorvastatin and pravastatin were administrated by oral gavage. L-NAME and naloxone were administrated intraperitoneally (i.p.) in a dose volume of no more than 10 ml/kg.

## Experimental protocols

Mice were divided into the following experimental groups:

### Group 1 (Acute application groups)

- Vehicle group (PBS) (n= 6)
- Atorvastatin (5 mg/kg) group [A(5)] (n= 6)
- Atorvastatin (10 mg/kg) group [A(10)] (n= 6)
- Atorvastatin (30 mg/kg) group [A(30)] (n= 6)
- Simvastatin (5 mg/kg) group [S(5)] (n= 6)
- Simvastatin (10 mg/kg) group [S(10)] (n= 6)
- Simvastatin (30 mg/kg) group [S(30)] (n= 6)
- Pravastatin (5 mg/kg) group [P(5)] (n= 6)
- Pravastatin (10 mg/kg) group [P(10)] (n= 6)
- Pravastatin (30 mg/kg) group [P(30)] (n= 6)

### Group 2 (Chronical application groups)

- Atorvastatin (5 mg/kg) group [A(5)] (n= 6)
- Atorvastatin (10 mg/kg) group [A(10)] (n= 6)
- Atorvastatin (30 mg/kg) group [A(30)] (n= 6)
- Simvastatin (5 mg/kg) group [S(5)] (n= 6)
- Simvastatin (10 mg/kg) group [S(10)] (n= 6)
- Simvastatin (30 mg/kg) group [S(30)] (n= 6)
- Pravastatin (5 mg/kg) group [P(5)] (n= 6)
- Pravastatin (10 mg/kg) group [P(10)] (n= 6)
- Pravastatin (30 mg/kg) group [P(30)] (n= 6)

### Group 3 (Investigation of mechanism groups)

- Naloxone (0,5 mg kg) group [N] (n= 6)
- L-NAME (10 mg/kg) group [L-NAME] (n= 6)
- Atorvastatin (30 mg/kg) +Naloxone (0.5 mg/kg) group [A(30)+N] (n= 6)
- Simvastatin (30 mg/kg) + Naloxone (0.5 mg/kg) group [S(30)+N] (n= 6)
- Pravastatin (30 mg/kg) +Naloxone (0.5 mg/kg) group [P(30)+N] (n= 6)
- Atorvastatin (30 mg/kg) + L-NAME (10 mg/kg) group [A(30)+L-NAME] (n= 6)
- Simvastatin (30 mg/kg) + L-NAME (10 mg/kg) group [S(30)+L-NAME] (n= 6)
- Pravastatin (30 mg/kg) + L-NAME (10 mg/kg) group [P(30)+L-NAME] (n= 6)

### Group 1 (Acute application groups)

Statins and vehicle were administered by oral gavage in mice. Control (pretreatment value) and post treatment (after drugs application) values in 60<sup>th</sup> and 120<sup>th</sup> minutes were measured in hot-plate test.

### Group 2 (Chronical application groups)

Statins were administered by oral gavage once daily for 3 days in mice. At the end of 3 days,

control (pretreatment value) and post treatment (after drugs application) values in 60<sup>th</sup> and 120<sup>th</sup> minutes were measured in hot-plate test.

### Group 3 (Investigation of the mechanism groups)

Firstly, L-NAME or naloxone have been evaluated whether to have an effect in hot plate test. In this context, naloxone (0.5 mg/kg) or L-NAME (10 mg/kg) was administered by intraperitoneal (i.p.) injection in mice. Control (pretreatment value) and post treatment values in 60<sup>th</sup> and 120<sup>th</sup> minutes after naloxone (0.5 mg/kg) or L-NAME (10 mg/kg) application were measured in hot-plate test.

Secondly, the analgesic effects of statins have been evaluated in the presence of L-NAME or naloxone. Before treatment with statins, pretreatment values were measured. Afterwards, statins were administered orally by gavage. After 30 minutes of statins application, naloxone (0.5 mg/kg) or L-NAME (10 mg/kg) was applied intraperitoneally (ip). Post treatment values in 60<sup>th</sup> and 120<sup>th</sup> minutes after statins application were measured in hot-plate test.

### Statistical Analysis

The data were expressed as the mean  $\pm$  S.E.M. Statistical analysis were carried out using Mann-

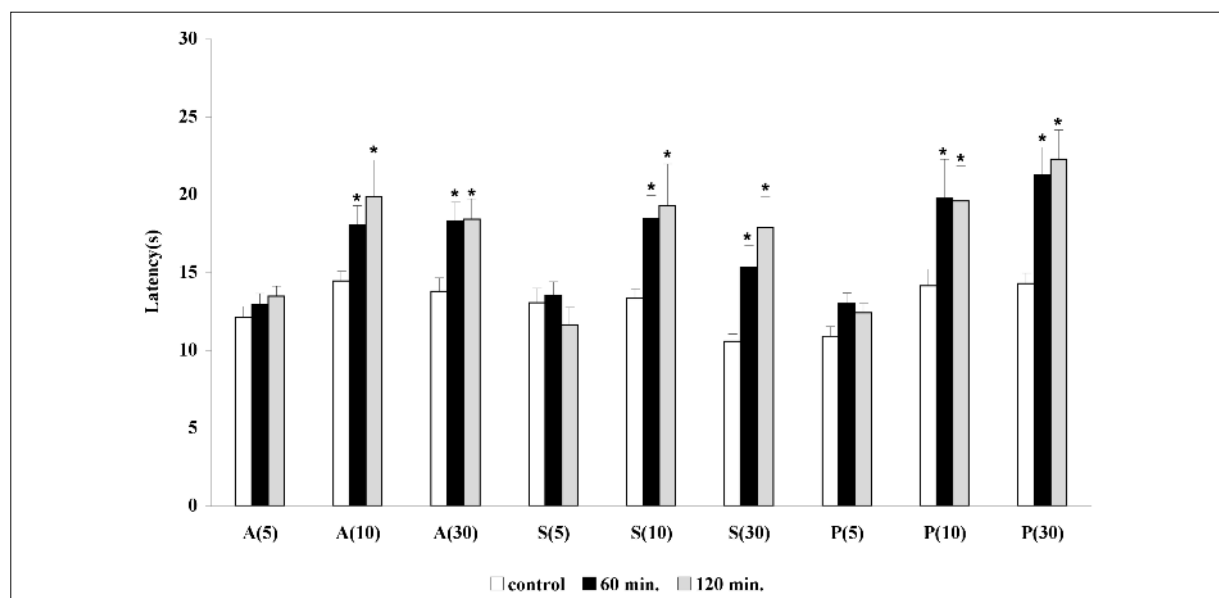
Whitney U test. The level of significance was set at  $p < 0.05$ .

## Results

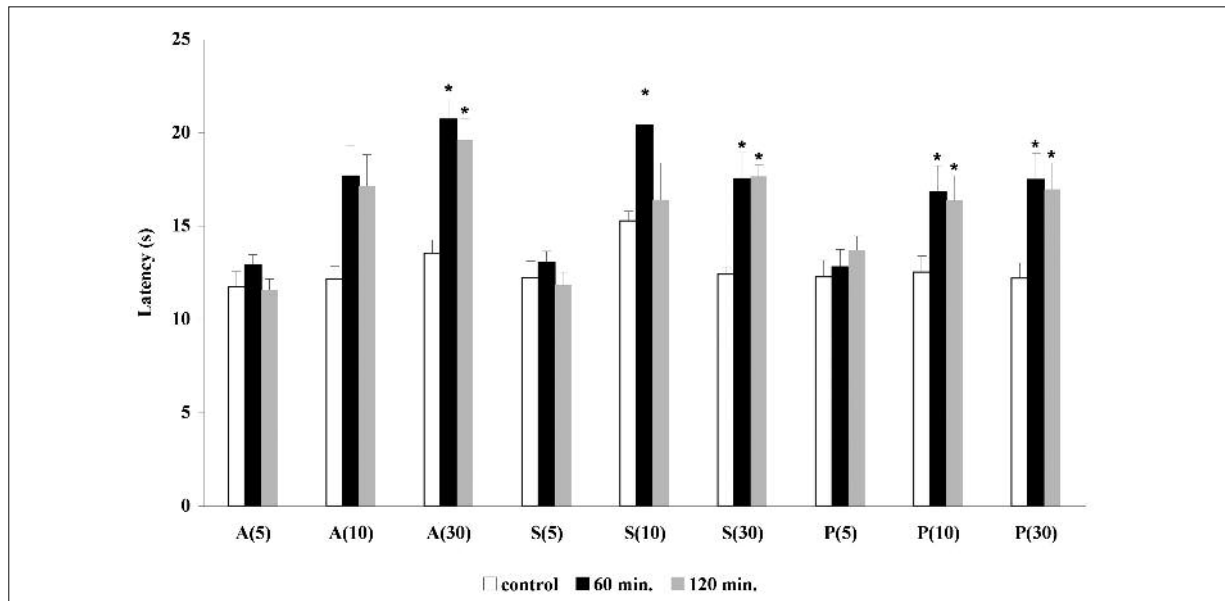
Atorvastatin (10, 30 mg/kg), simvastatin (10, 30 mg/kg) and pravastatin (10, 30 mg/kg) produced a statistically significant analgesic effect compared to their control values on 60<sup>th</sup> and 120<sup>th</sup> minutes in mice in group 1 (Figure 1) ( $p < 0.05$ ). However, atorvastatin (5 mg/kg), simvastatin (5 mg/kg) and pravastatin (5 mg/kg) did not produce anyone statistically significant analgesic effect compared to their controls on 60<sup>th</sup> and 120<sup>th</sup> minutes in mice in group 1 (Figure 1) ( $p > 0.05$ ).

Vehicle group did not produce a statistically significant effect compared to the control ( $p > 0.05$ ).

Atorvastatin (10, 30 mg/kg), simvastatin (30 mg/kg) and pravastatin (10, 30 mg/kg) produced a statistically significant analgesic effect compared to their control values on 60<sup>th</sup> and 120<sup>th</sup> minutes in mice in group 2 (Figure 2) ( $p < 0.05$ ). However, simvastatin (10 mg/kg) did not produce a statistically significant analgesic effect compared to their control on 120<sup>th</sup> minutes in mice in group 2 (Figure 2) ( $p > 0.05$ ). At doses of 5



**Figure 1.** Analgesic effect of atorvastatin, simvastatin and pravastatin after acute application in 60<sup>th</sup> and 120<sup>th</sup> minutes in hot-plate test. {Atorvastatin (5 mg/kg) group [A(5)], Atorvastatin (10 mg/kg) group [A(10)], Atorvastatin (30 mg/kg) group [A(30)], Simvastatin (5 mg/kg) group [S(5)], Simvastatin (10 mg/kg) group [S(10)], Simvastatin (30 mg/kg) group [S(30)], Pravastatin (5 mg/kg) group [P(5)], Pravastatin (10 mg/kg) group [P(10)], Pravastatin (30 mg/kg) group [P(30)]}. \*Compared with its own control value ( $p < 0.05$ ).



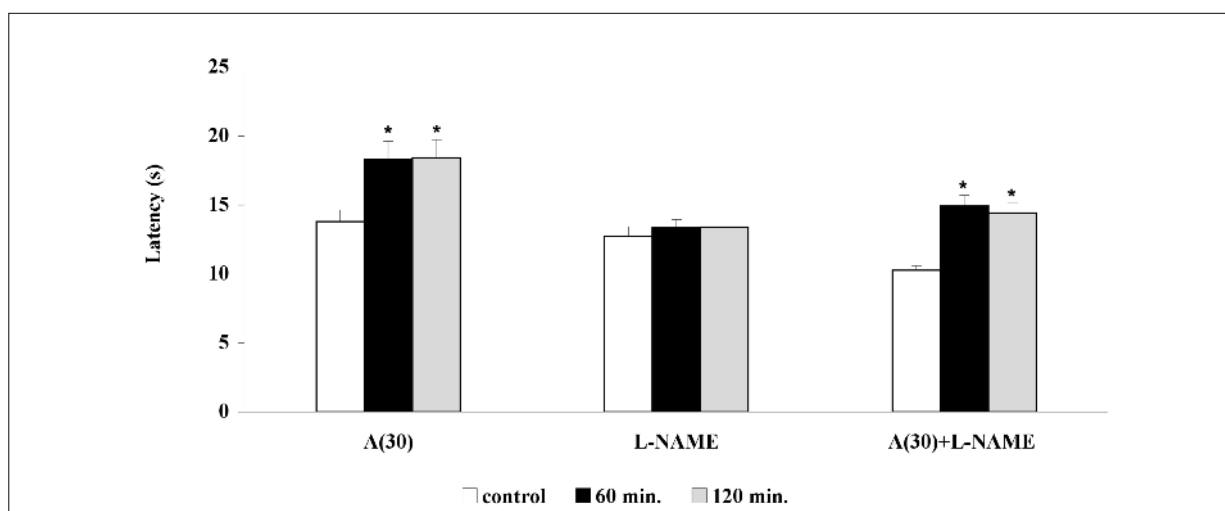
**Figure 2.** Analgesic effect of atorvastatin, simvastatin and pravastatin after chronically application in 60<sup>th</sup> and 120<sup>th</sup> minutes in hot-plate test. {Atorvastatin (5 mg/kg) group [A(5)], Atorvastatin (10 mg/kg) group [A(10)], Atorvastatin (30 mg/kg) group [A(30)], Simvastatin (5 mg/kg) group [S(5)], Simvastatin (10 mg/kg) group [S(10)], Simvastatin (30 mg/kg) group [S(30)], Pravastatin (5 mg/kg) group [P(5)], Pravastatin (10 mg/kg) group [P(10)], Pravastatin (30 mg/kg) group [P(30)]}. \*Compared with its own control value ( $p < 0.05$ ).

mg/kg in atorvastatin, simvastatin and pravastatin did not produce a statistically significant analgesic effect compared to their control values on 60<sup>th</sup> and 120<sup>th</sup> minutes in mice in group 2 (Figure 2) ( $p > 0.05$ ).

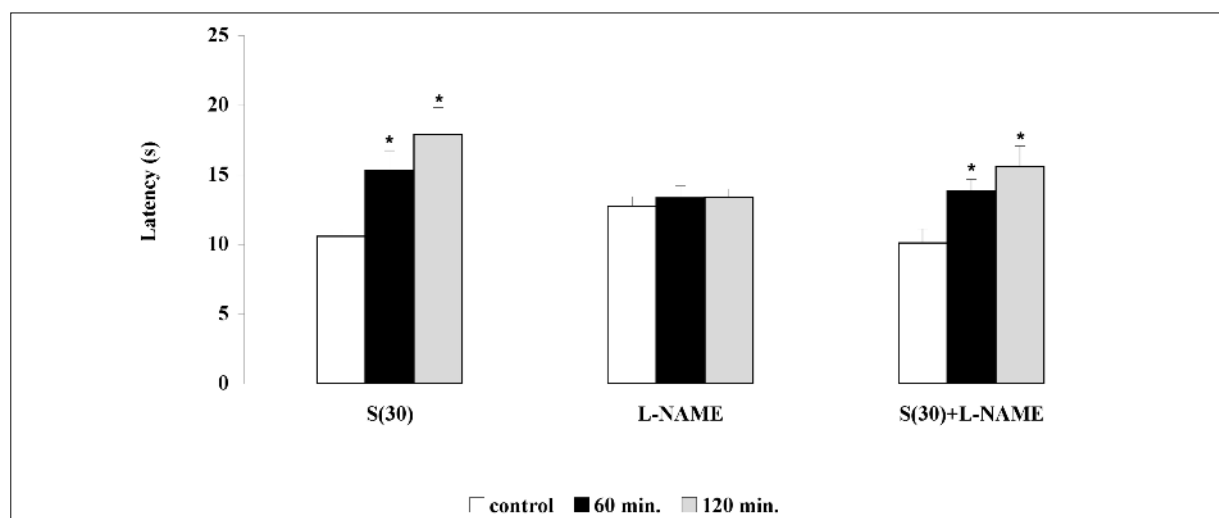
L-NAME (10 mg/kg) has no effect compared to the control value on both minutes. The analgesic effects of both atorvastatin (30 mg/kg) and

simvastatin (30 mg/kg) in the presence of L-NAME (10 mg/kg) were not inhibited (Figures 3 and 4). However, the analgesic effect of pravastatin (30 mg/kg) in the presence of L-NAME (10 mg/kg) was inhibited significantly on both minutes (Figure 5) ( $p < 0.05$ ).

Naloxone (0.5 mg/kg) has no effect compared to the control value on both minutes. The anal-



**Figure 3.** Effect of L-NAME (10 mg/kg) on the analgesia induced by atorvastatin (30 mg/kg) in hot plate test. {Atorvastatin (30 mg/kg) group [A(30)], L-NAME (10 mg/kg) group [L-NAME], Atorvastatin (30 mg/kg) + L-NAME (10 mg/kg) group [A(30)+L-NAME]}. \*Compared with its own control value ( $p < 0.05$ ).

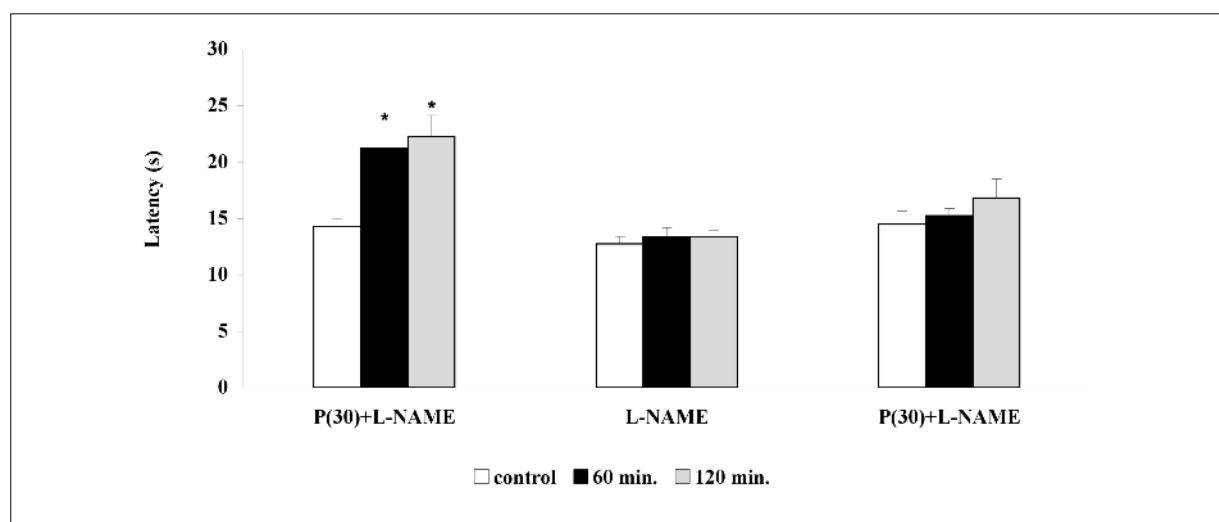


**Figure 4.** Effect of L-NAME (10 mg/kg) on the analgesia induced by simvastatin (30 mg/kg) in hot plate test. {Simvastatin (30 mg/kg) group [S(30)], L-NAME (10 mg/kg) group [L-NAME], Simvastatin (30 mg/kg) + L-NAME (10 mg/kg) group [S(30)+L-NAME]}. \*Compared with its own control value ( $p < 0.05$ ).

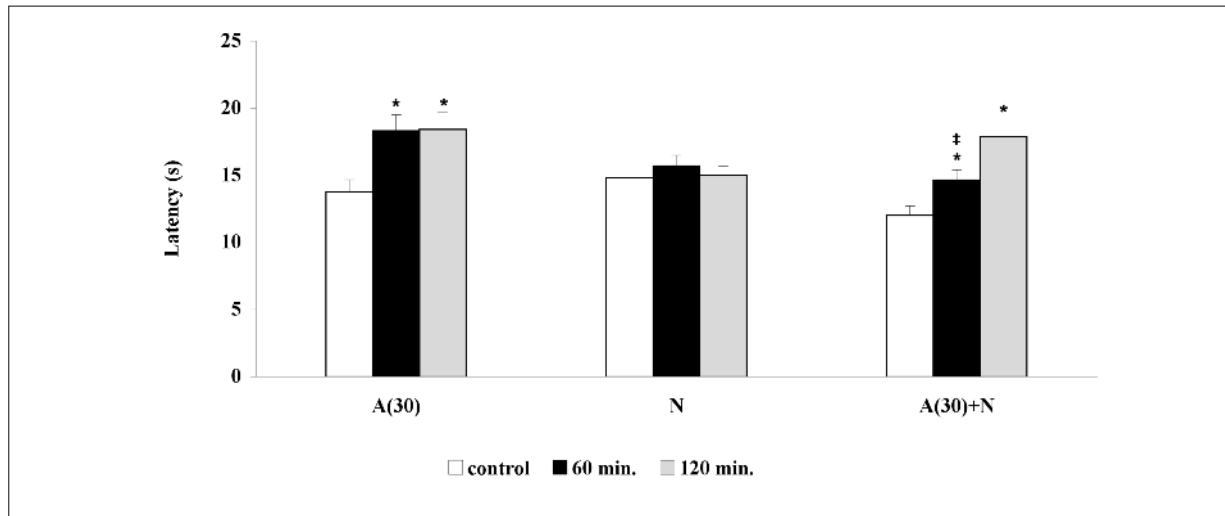
gesic effect of atorvastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was partially (43%) but significantly inhibited only on 60<sup>th</sup> minute (Figure 6) ( $p < 0.05$ ). The analgesic effect of pravastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was partially (48-40%) but significantly inhibited on both minutes (Figure 7) ( $p < 0.05$ ). However, the analgesic effect of simvastatin (30 mg/kg) in the presence of naloxone (0.5 mg/kg) was inhibited significantly on both minutes (Figure 8) ( $p < 0.05$ ).

## Discussion

Statins are widely used for the prevention of cardiovascular disease. Recent studies have focused on the antiinflammatory effects on the inflammatory disease. It has been shown that atorvastatin produced antiinflammatory and analgesic effects by preventing the release of inflammatory cytokines (such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6) in arthritis models in rats<sup>11-13</sup>. There are clinical studies showing that simvastatin and atorvastatin



**Figure 5.** Effect of L-NAME (10 mg/kg) on the analgesia induced by pravastatin (30 mg/kg) in hot plate test. {Pravastatin (30 mg/kg) group [A(30)], L-NAME (10 mg/kg) group [L-NAME], Pravastatin (30 mg/kg) + L-NAME (10 mg/kg) group [P(30)+L-NAME]}. \*Compared with its own control value ( $p < 0.05$ ).



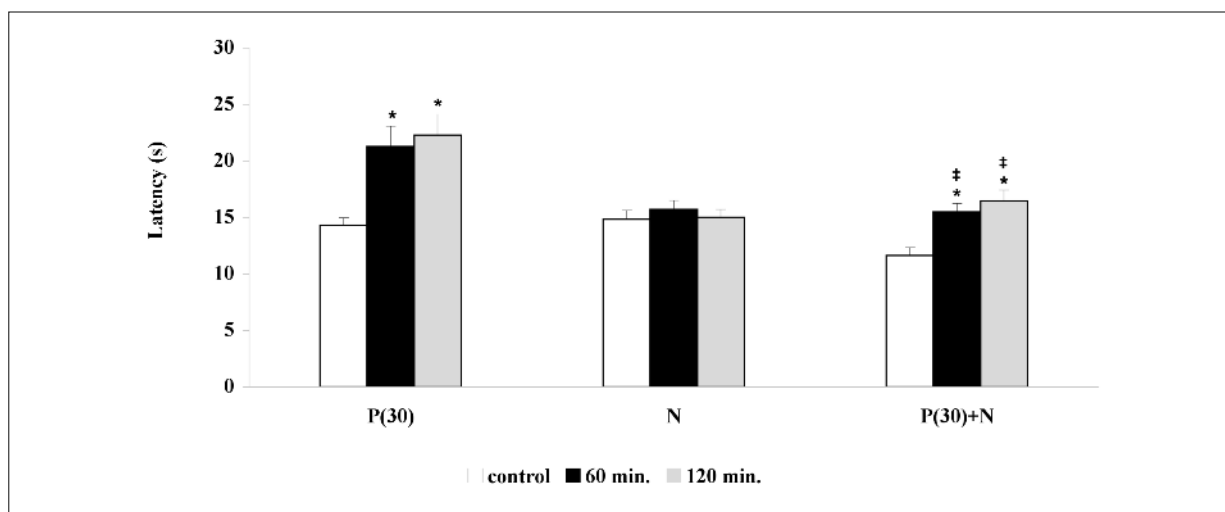
**Figure 6.** Effect of naloxone (0.5 mg/kg) on the analgesia induced by atorvastatin (30 mg/kg) in hot plate test {Atorvastatin (30 mg/kg) group [A(30)], Naloxone (0.5 mg/kg) group [N], Atorvastatin (30 mg/kg + Naloxone (0.5 mg/kg) group [A(30)+N]}. \*Compared with its own control value ( $p < 0.05$ ); ‡Compared with the % inhibition in its own corresponding value ( $p < 0.05$ ).

prevented the release of proinflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8) and reduced the level of C-reactive proteins (CRP) and on this way contributed to the antiinflammatory process in inflammatory diseases (RA, SLE, osteoporosis)<sup>8,14</sup>.

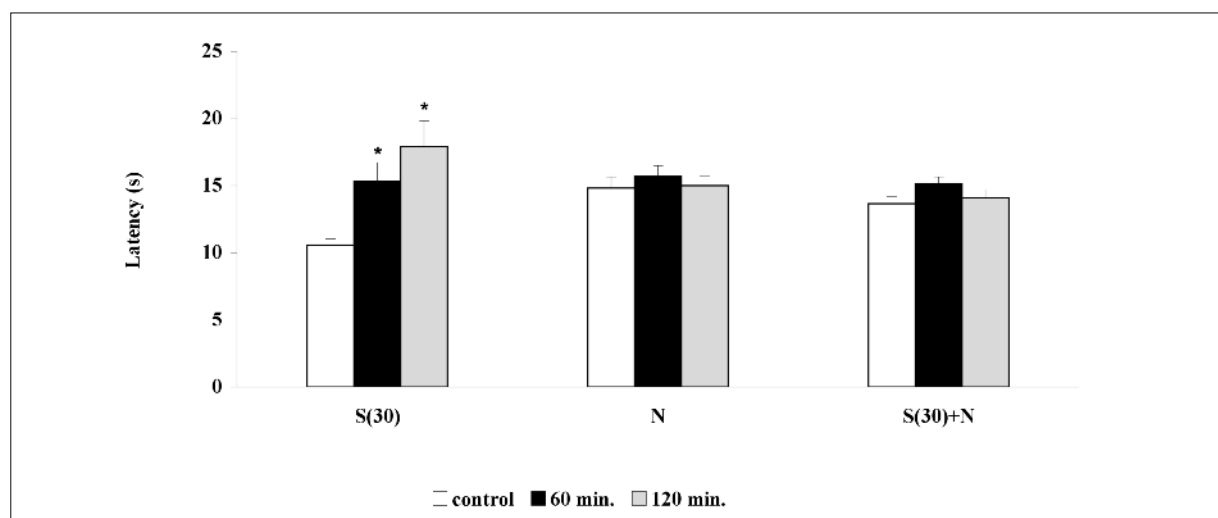
Although there are several studies reporting the antiinflammatory effect of statins, there are not enough data related to their analgesic effects. We have studied the analgesic effects of three statins, two lipophilic (atorvastatin, simvastatin) and one hydrophilic (pravastatin), in hot-plate test in

mice. In our study, atorvastatin, simvastatin and pravastatin produced analgesic effects on acute and chronically application in the hot-plate test.

The role of cytokines in pain is clearly known. Cytokines which are released from damaged tissue lead to pain and inflammation<sup>1,2</sup>. It is thought that statins produced antiinflammatory and analgesic effects by inhibiting the release of inflammatory cytokines. Also, nitric oxide and opioid peptides are released from damaged tissues and they contribute to anti-inflammatory and analgesic process<sup>3,4</sup>. Santodomingo-Garzón T et al<sup>10</sup>



**Figure 7.** Effect of naloxone (0.5 mg/kg) on the analgesia induced by pravastatin (30 mg/kg) in hot plate test {Pravastatin (30 mg/kg) group [P(30)], Naloxone (0.5 mg/kg) group [N], Pravastatin (30 mg/kg + Naloxone (0.5 mg/kg) group [P(30)+N]}. \*Compared with its own control value ( $p < 0.05$ ); ‡Compared with the % inhibition in its own corresponding value ( $p < 0.05$ ).



**Figure 8.** Effect of naloxone (0.5 mg/kg) on the analgesia induced by simvastatin (30 mg/kg) in hot plate test [Simvastatin (30 mg/kg) group (S30), Naloxone (0.5 mg/kg) group (N), Simvastatin (30 mg/kg) + Naloxone (0.5 mg/kg) group (S30+N)]. \*Compared with its own pretreatment value ( $p < 0.05$ ).

showed the analgesic and antiinflammatory effects of atorvastatin and the role of inhibition of cytokine release from tissues and the stimulation of the production of NO in rats in these effects.

In our investigation, while L-NAME inhibited the analgesic effect of pravastatin completely, it had no effect on the analgesic effects of atorvastatin and simvastatin. As a result of the findings related with the studies done by L-NAME, it can be said that the analgesic effects of pravastatin might be related with the stimulation of NO production. There are studies reporting that pravastatin increased the NO release and produced anti-inflammatory effects<sup>15-18</sup>. However, the analgesic effects of simvastatin and atorvastatin were not inhibited by L-NAME, so other factors in inflammation and pain process may have contributory role in the analgesic effects of simvastatin and atorvastatin.

Many investigations have shown that the peripheral opioid receptors underwent an expression during the tissue damage by thermal, mechanical or chemical stimulus and the stimulation of these receptors produced an analgesic effect<sup>4-6,18,19</sup>.

In our study, naloxone (0.5 mg/kg) inhibited the analgesic effects of atorvastatin and pravastatin partially. However, it inhibited the analgesic effect of simvastatin completely. These findings showed that the opioidergic system has a role in the analgesic effect of simvastatin but not of atorvastatin and pravastatin.

Atorvastatin and simvastatin used in this research have lipophilic characters; whereas pravastatin has hydrophilic character<sup>20</sup>.

When our findings are evaluated with these properties of statins, it could be said that there is a role of central and peripheral mechanisms in the analgesic effects of atorvastatin or simvastatin and only peripheral mechanisms in the analgesic effect of pravastatin.

In conclusion, it can be thought that there is a contribution of opioidergic system in the analgesic effect of simvastatin and pravastatin; contribution of nitrergic system in analgesic effect of pravastatin. However, in the analgesic effect of atorvastatin there can be other systems contributing to this effect in addition to opioidergic system rather than nitrergic system.

#### Acknowledgements

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