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Analgesic Activity of Ascorbic Acid Verses Acetylsalicylic Acid

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ABSTRACT

This study was designed to explore antinociceptive potential of ascorbic acid using three different popular models of nociception. These models included tail flick method, hot plate method and writhing test. Ascorbic acid was administered orally at the dose of 300 mg per kg per oral and antinociceptive potential was noted at different time intervals using three models. Effects of ascorbic acid were also compared with standard drug acetylsalicylic acid. Results revealed that ascorbic acid has significant antinocicpetive effect in all three models showing potent analgesic potential of ascorbic acid. Increase in latency to withdraw tail in tail flick method showed that ascorbic has a central antinociception mechanism. Time to jump off from the hot plate was significantly increased that also suggest ascorbic acid's central mechanism of action. Moreover, highly significant decrease in number of writhes by ascorbic acid suggests action of ascorbic acid by inhibition of COX-II. Therefore, ascorbic acid can be suggested as a potential agent in managing different pain conditions.

Keywords: ascorbic acid, COX-II, nociception

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INTRODUCTION

Antioxidant plays a significant role in the cellular functions. It has also been implicated in the processes that are related to inflammatory damage and cancer (Barrita and Sánchez, 2013)[1].

Ascorbic acid is an antioxidant and plays its role in detoxification of toxic components in the liver. Moreover, it reduces symptoms of inflammation by interacting with peroxide and histamine as an antioxidant. Most of the animals and plants synthesize ascorbic acid for their own requirements. However, human-beings are not able to synthesize this vitamin due to lack of gulonolactone oxidase enzyme. Ascorbic acid is also required for carnitine, collagen and neurotransmitter synthesis (Zeraati et al., 2014)[2]. A variety of beneficial health related effects have been attributed to the use of ascorbic acid. Such beneficial effects include anticancer effects, immunomodulator effects and antioxidant effects (Naidu, 2003)[3]. The present study was designed to explore antinociceptive potential of ascorbic acid using three popular nociceptive models.

MATERIALS AND METHOD

Study Animals

For evaluation of analgesic activity, albino mice weighing 25±2g of either sex were used. The animals were placed in separate cages for 7 days before the experiment. Standard iron cages were used to keep the experimental animals in animal house of Department of Pharmacology, University of Karachi. Five animals per cage were housed at 25±1^{o}C on 12/12 h light and dark cycle and were allowed food and water *ad libitum*. Departmental Research Committee, Faculty of Pharmacy, University of Karachi approved this study.

Chemicals

Acetic Acid of lab grade was taken from the local supplier, ascorbic acid and acetylsalicylic acid were taken from the local market.

Protocol of the Experimental Study

Mice were divided into three groups (I, II and III) of five animals in each group. Group I and III were kept as control and standard groups and administered distilled water 1 ml per oral and acetylsalicylic acid (4.28 mg/kg) (Bryant and Knights, 2011)[4] respectively. Group II was set as treated group, ascorbic acid (300 mg/kg) (Zeraati et al., 2014)[2] was administered orally according to the weight of animal. The animals were subjected to following different tests to evaluate analgesic activity by three different methods.

ANALGESIC ACTIVITY

Anti-nociceptive activities of ascorbic acid were explored in albino mice by using tail flick method, hot plate method and acetic acid-induced writhing.

Tail Flick Method

This method was used to analyze the analgesic potential of ascorbic acid. Mice of group 1 and group 3 were orally treated with distilled water and acetylsalicylic acid respectively. Mice of group 2 were orally treated with ascorbic acid. For this experiment, tail of the selected mice was dipped in water bath maintained at 51±0.5 °C temperature. The latency time (in seconds) to withdraw the tail of mice was noted for each animal individually. Latency time was noted at different time intervals (0 min, 20 min, 30 min, 40 min, 50 min and 60 min) following oral administration of ascorbic acid, standard drug and distilled water (Ajeigbe et al., 2011)[5].

Hot Plate Method

To explore centrally related analgesic potential of ascorbic acid, hot plate method was employed. For this test each animal was individually placed on hot plate (temperature 55±0.5 °C) and nociceptive response was noted. Jumping or licking of paws by animal is considered as nociceptive response (Kumae et al., 2011)[6]. All the observations were made on different time intervals (0 min, 20 min, 30 min, 40 min, 50 min and 60 min) following administration of ascorbic acid, standard drug and distilled water.

Writhing Test

For peripheral analgesic potential of ascorbic acid, writhing test was used in this study (Kilic et al., 2012)[7]. Writhing is explained as abdominal contractions. After 30 minutes of treating animals with ascorbic acid, standard drug and distilled water, 10 ml per kg of 0.6% acetic acid was administered by intraperitoneal route to animals (Chiba et al., 2009)[8]. After the administration of acetic acid, animals were individually placed in a transparent box for observation of writhes. Number of writhes was observed for 5 minutes duration (Ajeigbe et al., 2011)[5].

Statistical Analysis

All the data is presented as mean ± standard deviation. Data was statistically evaluated using SPSS 20.0 version by one-way ANOVA followed by bonferroni post hoc multiple comparisons. P-value of <0.001 is considered as highly significant.

RESULTS AND DISCUSSION

Tail Flick Test

Table 1 shows effect of ascorbic acid verses acetylsalicylic acid in tail flick test. In tail flick test, latency to withdraw tail was more significantly increased after 20 minutes of oral treatment

($p<0.01$) while highly significant increased after 30, 40, 50 and 60 minutes of oral treatment ($p<0.001$) in comparison to control animal group. On the other hand, latency to withdraw tail by standard drug acetylsalicylic acid animal group was more significantly increased after 20 and 30 minutes of oral treatment in comparison to control ($p<0.01$). However, latency time was highly significantly increased after 40, 50 and 60 minutes time interval ($p<0.001$) in comparison to control animal group. Ascorbic acid animal group showed highly significant increase in latency to withdraw tail from water after 30 and 40 minutes of treatment ($p<0.001$) in comparison to standard drug showing better results of ascorbic acid than standard drug acetylsalicylic acid.

Table 1: Effect of Ascorbic Acid verses Acetylsalicylic Acid on Tail Flick Test

Tail Flicking Time (in seconds)						
Groups	**0 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
Control	1.04±0.089	0.94±0.089	0.96±0.089	0.98±0.044	1.02±0.27	0.98±0.044
Ascorbic Acid	0.95±0.088	2.0±0.707**	5.98±0.975***!!!	8.40±0.89***!!!	3.92±0.46***	3.60±0.54***
Standard	0.98±0.044	1.98±0.044**	2.8±0.447**	3.2±0.447***	3.6±0.89***	3.40±0.54***

Values are mean ± standard deviation N=5, p-value <0.01**,<0.001*** in comparison to control, p-value<0.001$^{!!!}$ in comparison to standard

Hot Plate Test

Table 2 shows effect of ascorbic acid verses acetylsalicylic acid in hot plate test. In hot plate test, ascorbic acid group showed highly significantly increase ($p<0.001$) in time to jump off the hot plate after 20 minutes of treatment with ascorbic acid. However, ascorbic acid animal group showed significantly ($p<0.05$) and more significantly ($p<0.01$) increase in time to jump off the hot plate at 30 and 40 minutes respectively in comparison to control animal group. Standard drug group showed more significantly ($p<0.01$) increase in time to jump off the hot plate at 20 minutes while highly significantly increase ($p<0.001$) in the time to jump off the hot plate at 30 and 40 minutes in comparison to control animal group. On the other hand, ascorbic acid showed highly significant increase ($p<0.001$) in time to jump off the hot plate in comparison to standard drug group at 20 minutes while highly significantly ($p<0.001$) and significantly ($p<0.05$) decrease at 30 and 40 minutes respectively.

Table 2: Effect of Ascorbic Acid verses Acetylsalicylic Acid on Hot Plate Test

Time of Lifting of Paws (in seconds)						
Groups	**0 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
Control	7.8±0.836	9.2±0.447	7.4±1.14	8.6±1.34	8.2±0.447	9.4±0.894
Ascorbic Acid	8.4±0.894	22.8±1.92***!!!	11.5±3.50*!!!	13.68±2.51**!	7.40±0.894	8.4±0.547
Standard	7.2±0.836	13.8±1.30**	23.2±0.83***	17.4±1.81***	7.0±0.707	8.2±0.447

Values are mean ± standard deviation, N=5, p-value <0.05*,<0.01**,<0.001*** in comparison to control, p-value<0.05[!],<0.001[!!!] in comparison to standard

Writhing Test

Table 3 shows effect of ascorbic acid verses acetylsalicylic acid in writhing test. In writhing test, ascorbic acid highly significantly decreased (p<0.001) number of writhes in comparison to control animal group. Standard animal group also showed highly significantly decreased (p<0.001) number of writhes in comparison to control animal group. The difference between ascorbic acid and standard drug in decreasing number of writhes was found statistically insignificant.

Table 3: Effect of Ascorbic Acid verses Acetylsalicylic Acid on Writhing Test

Groups	Number of Writhes 5 min
Control	28.0±1.73
Ascorbic Acid	16.2±2.86***
Standard	13.0±0.707***

Values are mean ± standard deviation, N=5, p-value <0.001*** in comparison to control

Pain is an unpleasant feeling. It is an unwanted psychological, physical and subjective experience (Savidge and Slade, 1997)[9]. Ascorbic acid was explored for its analgesic potential using different pain models. Central analgesic effect of ascorbic acid may be supported by results obtained by tail flick method. It is because tail flick method is highly selective method to screen opioid drugs acting centrally (Taherian et al., 2009)[10]. Increase in latency to flick tail by ascorbic acid group suggests its central mechanism of action. Hot plate method was conducted as this test indicates opioid receptor involvement (Turner, 1995)[11]. It has selectivity for opioid derived analgesics. Ascorbic acid showed significant antinociceptive effect on the acutely harmful thermal stimulation in hot plate method hence confirming central activity of ascorbic acid (Taherian et al., 2009)[10.] Writhes by acetic acid represents a peripheral nociception model (Wei et al., 1986)[14], however this is not considered a specific model. Writhing by acetic acid is explained to be originated from the pain of inflammation that is mediated by prostaglandins (PGs) (Zeraati et al., 2014)[2]. According to Fiebich et al (2003)[12] and Lopez-Lluch et al (2005)[13], ascorbic acid inhibits cyclooxygenase enzyme-II and acts in a synergistic manner with aspirin in inhibiting PGE_2.

CONCLUSION

Based on the above findings, it may be suggested that ascorbic acid is a potential antinociception component that can be employed in different pain conditions.

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