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EVALUATION OF ANALGESIC PROPERTY OF TROLOX METHYL ESTER

Lalita Sharma*, Mayank Thakur and Anurita Rajput

Department of Pharmacology, Advance Institute of Biotech and Paramedical Sciences, Kanpur-209217, Uttar Pradesh, India.

***Corresponding Author: Lalita Sharma**

Department of Pharmacology, Advance Institute of Biotech and Paramedical Sciences, Kanpur-209217, Uttar Pradesh, India.

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ABSTRACT

Objectives: The purpose of this study was to investigate the analgesic effect of Trolox Methyl Ester on rats. **Materials and Methods:** Using Eddy's hot plate and tail immersion procedures, the current investigation was conducted to evaluate the analgesic characteristics of Trolox Methyl Ester, in rats. **Results:** When compared to the control (normal saline), Trolox Methyl Ester significantly reduced the amount of writhes and significantly prolonged the reaction time in the tail clip and Eddy's hot plate method, but less so when compared to regular aspirin. **Conclusion:** It was concluded that Trolox Methyl Ester had significant analgesic effects in rat models of heat, chemical, and mechanical pain.

KEYWORDS: Analgesic, Antioxidant, Eddy's hot plate, Tail immersion, Trolox Methyl Ester.

INTRODUCTION

Trolox is a vitamin E homologue that is water soluble (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). It is an antioxidant that, like vitamin E, is used in biological or chemical processes to reduce oxidative stress or damage. Vitamin E is naturally present in biochemical samples such as plasma and food samples such as fruits and vegetables.[1]

The chiral derivatizing agent trolox methyl ether, which is used to transform enantiomers into diastereoisomers, can be created from trolox by esterification. Trolox methyl ether can be used as a chiral derivatizing agent for the capillary gas chromatography (GC) and supercritical fluid chromatography (SFC) analysis of chiral alcohols as well as the enantiomeric separation of several benzodiazepines using GC, SFC, and subcritical fluid chromatography methods.[2]

Trolox has a significant antioxidant capacity in vitro. There is evidence that this drug can scavenge peroxy radicals eight times more efficiently than vitamin E in sodium dodecyl sulphate (SDS) micelles. The anti-inflammatory effects of trolox, however, have been extensively studied and documented.[4]

Trolox are well known for their anti-inflammatory[3], antioxidant[5], anti-cancer[6], etc properties. The effectiveness of trolox in treating neuropathic pain was also noted in other investigations.[7] Therefore its analogue, Trolox Methyl Ester was synthesized and through the use of the tail immersion and Eddy's hot plate procedures, the current study sought to investigate the analgesic potential of Trolox Methyl Ester.

EXPERIMENTAL

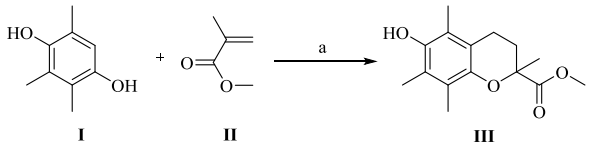
Materials and Methods

The study was conducted after getting approval from Institution Animal Ethical Committee (IAEC). CPSEA approval number from IAEC of: 1122/PO/Re/S/2007/CPCSEA.

All of the chemicals were bought in bulk from Spectrochem Pvt. Ltd. and Sigma-Aldrich. On E Merck silica gel GF-254 prefabrication plates, the reaction's progress was monitored using thin-layer chromatography (TLC), and the visualisation was carried out using a charring solution spray. The melting points of open glass capillaries were measured. On the JEOL ECX-500 spectrometer, NMR spectra in CDCl3 at 400 MHz were captured. Chemical shifts () in relation to TMS were calculated in parts per million (ppm). The IR spectra were captured using a Perkin Elmer spectrophotometer, version 10.6.1, and are expressed in KBr.

Chemistry

The reaction involved the synthesis of a racemic mixture of Trolox (III) from commercial-free acid using the Hetro-Diels Alder reaction. In the presence of acetic acid, trimethylhydroquinone (I), methyl methacrylate (II), paraformaldehyde, and dibutyl amine were refluxed (Scheme 1).[8]



Scheme 1: Synthetic pathway for Trolox methyl ester. (a) (HCHO)n, $[CH_3(CH_2)_3]_2NH$, CH_3COOH, reflux, 20 h.

Synthesis of Compounds

Synthesis of 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid methyl ester (III)

In an RB flask, 1.59 ml (15 mmol) of methyl methacrylate, 0.048 g (0.36 mmol) of dibutyl amine, 0.09 g (3 mmol) of paraformaldehyde, and 0.45 ml of glacial acetic acid were refluxed. At rt, the reaction mass was stirred for 20 minutes before adding 0.456 g (3 mmol) of trimethylhydroquinone. The reaction mass was then stirred under reflux for another 15 hours. The result was a dark brown mixture. It was brought down to room temperature. The solid was then filtered and washed twice with cold methanol to yield 0.307 g of off-white solid. The obtained solid was recrystallized with methanol to produce white prism crystals. Yield: White solid **(**45%); M.P. 161°C; IR (KBr, ν, cm^{-1}): 3524 (-OH), 2990, 2927, 1736 (>CO), 1190, 1104; ^{1}HNMR (400MHz, $CDCl_3$, ppm): 5.65 (1H, br S, OH), 3.67 (s, 3H), 2.65–2.72 (m, 1H), 2.41–2.55 (m, 2H), 2.18 (s, 3H), 2.16 (s, 3H), 2.06 (s, 3H), 1.89–1.82 (m, 1H), 1.59 (s, 3H).

EXPERIMENTAL

Animals

Generally weighing 30–50 g, albino mice of both sexes were used in the experiments. Prior (1962) to testing, animals spent at least an hour becoming used to the lab setting. Paget and Barnes claim that the drug dosages were calculated using a human daily dose and converting it to a mouse dosage.

1. Eddy's hot plate method

Chemicals: Tween80 (CDH) and Aspirin

Equipment: Eddy's hot plate

Treatment Protocol: The animals were given numbers and weights before being divided into 4 groups of six each as follows:

Group I: Served as vehicle control, 2% Tween 80 (10 ml/kg body weight, I.P) was administered.

Group II: Aspirin (200 mg/kg body weight, I.P) was given as a reference drug.

Group III: Trolox methyl ester (40 mg/kg body weight, I.P) was given.

Procedure

24 rats were placed on Eddy's hot plate, which was kept at 55 0.5 °C. The animal's reaction time to heat stimuli was measured as the time between its introduction to the plate and its first lick of its limbs or jump. The drugs were administered intravenously to the appropriate groups (as specified in treatment protocol). The reaction times of the control and treated animals were recorded at 0, 15, 30, and 45 minutes after treatment.[9]

2. Tail Immersion Method

Material & Methods Chemicals: Tween80 (CDH) and Aspirin.

Equipment: Analgesiometer

Treatment Protocol: The animals were given numbers and weights before being divided into 4 groups of six each as follows:

Group I: Served as vehicle control, 2% Tween 80 (10 ml/kg body weight, I.P) was administered.

Group II: Aspirin (200 mg/kg body weight, I.P) was given as a reference drug.

Group III: Trolox methyl ester (40 mg/kg body weight, I.P) was given.

Procedure

24 Rats were restrained in the proper manner, their tails held out. The tail was then immersed in water that was 55 +/- 0.5°C, up to a length of 5 cm. The amount of time it took to visibly draw the tail out of the water was used to calculate the reaction time. Drugs were intravenously administered to the proper groups (as specified in treatment protocol). The animals were treated and the reaction times in the control group were tested at 0, 15, 30, and 45 minutes after the treatment.[10]

RESULTS AND DISCUSSION

Eddy's hot plate method

24 Rats were restrained in the proper manner, their tails held out. The tail was then immersed in water that was 55 +/- 0.5°C, up to a length of 5 cm. The amount of time it took to visibly draw the tail out of the water was used to calculate the reaction time. Drugs were intravenously administered to the proper groups (as specified in treatment protocol). The animals were treated and the reaction times in the control group were tested at 0, 15, 30, and 45 minutes after the treatment.

Table 1: The analgesic activity of Trolox Methyl Ester and standard in thermal pain model-Eddy's hot plate.

S.N.	TREATMENT	DOSE (MG/KG)	MEAN REACTIONTIME (MIN)			
			0 min	15 min	30 min	45 min
1	CONTROL	----	3.56±0.08	3.76±0.06	3.62±0.12	3.52±0.10
2	STD (I.P)	200	3.84±0.04*	5.98±0.20**	6.94±0.13**	7.82±0.88**
3	Trolox Methyl Ester	40	4.01±0.05*	4.63±0.10**	5.15±0.8**	5.65±0.16**

Variation in Reaction Times. All values are expressed as mean± S.E.M (n=6). Statistically analyzed by t-test where *p<0.05, **p<0.01, ***p<0.001 compared with the Control group.

2. Tail Immersion Method

The tail immersion test is another method to measure analgesic activity. The study's findings demonstrated that the trolox methyl ester lengthened the reaction time of the tail immersion procedure. According to Table 4.2, compared to vehicle control, pretreatment with trolox methyl ester and conventional aspirin dramatically raised pain threshold. The effects peaked at 45 minutes at doses of 200 mg/kg for conventional aspirin and 50 mg/kg for trolox methyl ester. Table illustrates the drug's strong analgesic activity, which was noticeably below average.

Table 2: The analgesic activity of trolox methyl ester and standard in thermal pain model- Tail Immersion.

SL.NO	TREATMENT	DOSE (mg/kg)	REACTION TIME (IN SECONDS)			
			0 min	15 min	30 min	45 min
1	CONTROL	---	2.03±0.12	2.40±0.45	2.85±0.13	2.20±0.57
2	STD (I.P)	200	2.76 ±0.19	3.68±0.35*	6.20±0.40**	7.04±0.54**
3	Trolox Methyl Ester	50	2.16±0.18	2.56±0.37*	5.45±0.00**	6.21±0.33**

Variation in Reaction Times. All values are expressed as mean± S.E.M (n=6). Statistically analyzed by t-test where *p<0.05, **p<0.01, ***p<0.001 compared with the Control group.

Animal models of pain that rely on polysynaptic responses that are started at the spinal level and controlled from supraspinal areas use the hot plate and tail immersion approaches. The hot plate and tail immersion techniques use thermal heat and radiant heat, respectively. Jumping and licking the paws are signs of a pain reflex in response to a hot plate, but dragging the tail away from boiling water during a tail immersion exhibits the same behaviour. Both types of stimulation cause pain through tissue damage brought on by heat and inflammation, which releases peripheral mediators. Both are models for acute pain. The tail immersion treatment for acute pain uses spinal and bulbospinal channels, whereas the hot plate method additionally incorporates extra supraspinal modulation.[11]

Trolox methyl ester at 50 mg/kg significantly raised the threshold of pain at all stages of acute pain compared to the normal saline (control) group (hot plate and tail immersion). Trolox methyl ester's effects peaked after 30 minutes in both models of acute pain. Compared to conventional aspirin, the onset of action of trolox methyl ester was delayed.

STATISTICAL ANALYSIS

The latency period of trolox methyl ester was significantly better (P 0.05) than the control's between 30 and 120 minutes, whereas the standard's latency period was more significant (P 0.05) than trolox methyl ester's overall.

CONCLUSION

Trolox Methyl Ester demonstrated significant analgesic activity when compared to the control in both pain models: hot plate and tail immersion. The analgesic activity peaked at 45 minutes. As a result, trolox methyl ester was found to be an effective analgesic agent in acute pain mouse models. In the experiment, it was found to be non-inferior to standard Aspirin. Although more preclinical and clinical research is needed to determine its long-term efficacy and safety.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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