ARTICLE

PALLIATIVE EFFECT OF CATECHIN FLAVONOIDS (GREEN TEA) ON ACETIC ACIDDERIVED PAIN IN RATS

Seyyed Hamid Bathaei [1*], Hossein Kargar Jahromi [2] ,Hooman Khodamoradi[3]

[1]*Institution of Supreme Education and Industry of Maragheh, IRAN*

[2]*Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom, IRAN*

[3]*Urmia University, Faculty of International Pardis, Urmia, IRAN*

ABSTRACT

This research was designed to study the effects of catechins (green tea flavonoids) on acetic acidderived visceral pain in rats. The study is based on a completely randomized experiment with two groups, and two factors were studied. Mean values were compared using GLM procedure of SAS software (9.3). Duncan test was used for comparing mean values in each test, and Dunnett method was used for comparing mean values of each experiment for the control group. Injection of catechin, activated GABAergic system (in visceral pain), reducing acid pain, and the difference between the mean values in this study was statistically significant ($P<0.05$). then the role of GAB_B in this phenomenon was emphasized. It can be concluded that there is a relationship between visceral pain, opioid system, and GABAergic system, which is slightly proven in the present study.

KEY WORDS
catechin, GABAergic system, rats and visceral pain

Published: 15 September 2016

***Corresponding Author**
Email:
hamidbathaee@gmail.com
Tel.:+*989141781292*

INTRODUCTION

Pain is one of the most complex and extraordinary senses, this sense is the most common symptom of diseases, and it's the most common reason for physician visits. Pain does not only include stimulating of the nerve fibers terminals and transferring it to the CNS, but also its quantity and quality is affected, and could be changed by a wide variety of experiences. This matter shows a complex neural mechanism for intervention in pain experience and the psychological response dedicated to that [1]. Among them, visceral pain is a complex pain, which is created by stimulation of pain receptors by a variety of pathophysiological reasons in different organs, such as colon, bladder and stomach, and obtained data is transmitted to the central nervous system by means of afferent pathways. According to various studies, neurotransmitters participating in the analysis of pain are divided into two "opioid" and "non-opioid" categories. These two systems can work together closely, to regulate the pain mechanisms. Despite various commonly used methods of pain relief and treatment, Researchers are still looking for new and better methods of treatment for this physiological phenomenon. Despite the advanced pharmaceutical and chemical drugs for pain relief, lack of ability to succeed the treatment, and widespread side effects of treatment is affecting patients [2]. Use of herbal medicine is quickly developing for treatment of a wide range of diseases, and special attention is paid to the protective effects of antioxidants (with natural origin) against poisoning caused by chemical agents [3]. Today, tea is considered as a beneficial source of pharmacological and biological activities to human health. therapeutic properties of tea extract and its catechin polyphenols, has leaded to conducting scientific studies on prevention and treatment of various diseases by this extract [4]. In the past, extensive studies have been performed in tea and its flavonoid compounds. Flavonoids are derived from plants' secondary metabolism, which are widely found in plants territory. Flavonoids can be divided into six groups based on the structure and position of the heterocyclic oxygen ring, namely flavones, flavanons, isoflavones, flavonols, flavanols, and anthocyanins. The most important flavonoids, present in tea, are flavonols, or to be more precise, catechins [5]. Catechins are categorized into four main ingredients of ECG, EGC, EC and EGCG and four sub- ingredients of GC, CG, C and GCG, which are epimers of the four original above materials [6]. Catechin (C), epi-catechin (EC), epi-galo-catechin (EGC), epi-catechin -gallate (ECG), epi-galo-catechin-gallate (EGCG), galo-catechin-gallate (GCG) are six catechins in tea which are responsible for the biological characteristics of tea, such as antioxidant [7], antimicrobial [7, 8], anti-cancer [7, 9] and anti-mutagenicity [7] activities. Catechins consist more than 30% of tea dry matter (7). Therefore, the objective of this study was to investigate the effect of catechin flavonoids on visceral pain derived by acetic acid in rats.

MATERIALS AND METHODS

In this study, adult male Wistar rats weighing 200-250 g were used, Mice were stored as groups of six rats in polyethylene cages in a room with ambient conditions and the optimum temperature about 23±2 ° C and 12 hours of light period, and the animals were fed with commercial pellet food while food and water was freely available for them.All tests were performed within 8 am to 3 pm and each rat was used once for the test. All the principles of laboratory animal care standards, such as laboratory temperature and humidity, were taken into consideration. Bought from Sigma company, Sterile normal saline solution, acetic acid 1 percent (this solution was diluted from pure acetic acid), catechin, and baclofen was used in the study. The study was performed in 10 tests, and 16 groups with 6 rats in each group, and a total of 96 rats. to create and study visceral pain, Writhing test (one of the standard tests to create and study visceral pain) was performed in the study. Intra peritoneal injection of acetic acid (1%) was used to create visceral pain in Writhing test method. Before beginning the test, animals were put into a glass container for 30

minutes with dimensions of 20 × 30 × 40 cm to avoid stress, and also for the laboratory animals to get used to the condition (this amount of time is called Adaptation). After the adaptation period, the animal was brought out of the enclosure glass slowly, and after constraining and injecting the study drug, one ml of acetic acid (one percent) was injected into the peritoneal area, and immediately after, animals were placed into the glass chamber and latency time, the first abdominal contractions' time, and the number of abdominal contractions after injection of acetic acid was recorded in special forms for an hour with an interval of five minutes. In order to investigate visceral pain, Mirror of pain device was used, which is made of a wooden framework, a cubical glass box in dimensions 20 × 30 × 40 cm, and a mirror in dimensions of 30 × 40 cm which is placed in 45 degrees inside the wooden frame, which this mirror makes the observer able to see abdomen part of the rat easily during the time of creating and recording abdominal contractions.

Statistical analysis method

Eachcompletely randomized experiment was performed with six replications; which statistical model is as follows:

$Y_{ij}=\mu+Ti+€ij$

μ =Average of total population

Ti =The effect of the i-th treatment

€ij =The effect of random error with zero mean and Q^2 variance

GLM procedure of SAS (9.3) software was used for comparison of mean values. Duncan test was used for comparing mean values in each test, and Dunnett method was used for comparing mean values of each experiment to control group.

RESULTS

injection of 5 mg Catechin per kg of body weight, took 1091/17 seconds for first contraction to start from latency period, and it caused 1/6178 full contractions of the abdominal wall.

The results showed that 5 mg of catechin per kilogram of body weight, had a significant effect on the onset time of first abdominal wall contraction from latency period (P<0.05), And reduced acetic acid (1%) derived visceral pain, but it didn't have a significant effect on the number of full contractions in the abdominal wall, although a reduction in number was recorded [Table 1]. Also the main effects of acetic acid are shown in [Table 2]. The results showed that intra peritoneal injection of catechin, had significantly reduced onset time of first abdominal wall contraction from latency period, but it didn't have a significant effect on the number of full contractions in the abdominal wall.

Table 1: Effect of catechin injection

Writhing test (NO)	Latency time (sec)	
4/ 1594 [b]	761/ 21 [b]	Acid acetic +Cathechin0 $^{mg}/_{kg}$ %1
1/ 6178 [b]	1091/[a]17	Acid acetic + Cathechin5 $^{mg}/_{kg}$ %1
0/381	35/0271	SEM
<0/0001	<0/0001	P-value

Table 2: Main effects of acetic acid

Writhing test (NO)	Latency time (sec)	%	
035 [b]/ 2 375 [a]/ 12 208 [a]/ 9	0 [a] /1478 0 [b]/ 466 8 [b]/ 386	5/0 1 2	Acid acetic Acid acetic Acid acetic
615/1	812/79	SEM	
0013/0	<0/0001	P-value	

Table 3: Interactions between the effects of catechin and acetic acid

Writhing test (NO)	Latency time (sec)	
4/ 1594 [b]	761/ 21 [b]	Acid acetic +Cathechin0 $^{mg}/_{kg}$ %1
1/ 6178 [b]	1091/[a]17	Acid acetic + cathechin 5 $^{mg}/_{kg}$ %1
0/381	35/0271	SEM
<0/0001	<0/0001	P-value

DISCUSSION AND CONCLUSION

The results of the study indicate that the catechin (green tea flavonoid), has analgesic effects on acetic acid-induced pain. Recently, the effect of flavonoids on pain and inflammation caused by pain reactions have been studied, and their anti-inflammatory and analgesic effects have been proven. Studies on a new herbal flavonoid, called the Hypolytin8-glucoside (obtained from the plant Hypericum) in rats, have shown that it has anti-inflammatory effect on acute inflammatory phase, and has no effect on the chronic or long-term phase [10]. oral administration of catechins in amounts of 60 to 120 mg per kilogram of body weight, reduced arthritis-derived secondary inflammation in young rats. [11]. Possible mediators in the inflammatory pain, induced by acetic acid, are still not well recognized. It is reported that bradykinin, neuro quinine and prostanoids are involved in sensory fibers activation after intraperitoneal injection of propionic acid, lactic acid and acetic acid.

Catechin (C), epi-catechin (EC), epi-galo-catechin (EGC), epi-catechin -gallate (ECG), epi-galo-catechin-gallate (EGCG), galo-catechin-gallate (GCG) are six catechins in tea which are responsible for the biological characteristics of tea, such as antioxidant [7], antimicrobial [7, 8], anti-cancer [7, 9] and anti-mutagenicity [7] activities. Catechins consist more than 30% of tea dry matter [7]. According to different studies performed on catechins, the effects of catechin on pain, in particular visceral pain, has not been studied yet. Therefore, the recent findings and results from this study, indicate that the catechins have visceral analgesic effects on acetic acid derived pain. In conclusion, the results of this study showed that, catechin induces analgesia through the mechanism of ..... in acetic acid derived visceral pain.

CONFLICT OF INTEREST
There is no conflict of interest.

ACKNOWLEDGEMENTS
None

FINANCIAL DISCLOSURE
None

REFERENCES

[1] Cervero F, Laird JM. [1996]Mechanisms of touch-evoked pain (allodynia): a new model. Pain.68(1):13-23.
[2] Pollard H, Moreau J, Arrang J, Schwartz J.[1993] A detailed autoradiographic mapping of histamine H 3 receptors in rat brain areas. Neuroscience. 52 (1): 169-189.
[3] Frei B, Higdon JV. [2003] Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. The Journal of nutrition. 133(10):3275S-84S.
[4] Mandel S, Weinreb O, Reznichenko L, Kalfon L, Amit T. [2006] Green tea catechins as brain-permeable, non toxic iron chelators to “iron out iron” from the brain. Oxidative Stress and Neuroprotection: Springer; p. 249-257.
[5] Labbé D, Tremblay A, Bazinet L.[2006] Effect of brewing temperature and duration on green tea catechin solubilization: Basis for production of EGC and EGCG-enriched fractions. Separation and Purification Technology. 49(1):1-9.
[6] Mohamadin A, El-Beshbishy H, El-Mahdy M.[2005] Green tea extract attenuates cyclosporine A-induced oxidative stress in rats. Pharmacological research. 51 (1): 51-57.
[7] Hara Y. [2001]Green tea: health benefits and applications: CRC press;.
[8] Kumamoto M, Sonda T.[1998] Evaluation of the antioxidative activity of tea by an oxygen electrode method. Bioscience, biotechnology, and biochemistry. 62(1):175-177.
[9] You H-J, Mørch CD, Chen J, Arendt-Nielsen L. [2003]Role of central NMDA versus non-NMDA receptor in spinal withdrawal reflex in spinal anesthetized rats under normal and hyperexcitable conditions. Brain research. 981(1):12-22.
[10] Matías L, Gómez-Aparicio L, Zamora R, Castro J. [2011] Effects of resource availability on plant recruitment at the community level in a Mediterranean mountain ecosystem. Perspectives in Plant Ecology, Evolution and Systematics. 13(4):277-285.
[11] Tang L-Q, Wei W, Wang X-Y.[2007] Effects and mechanisms of catechin for adjuvant arthritis in rats. Advances in therapy. 24(3):679-690.
[12] Ikeda Y, Ueno A, Naraba H, Oh-ishi S. [2001]Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. Life sciences. 69(24):2911-2919.