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Research Article

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The Effect of Flavonoids Catechin, Baclofen and Saclofen on Acetic Acid - Induced Visceral Pain by in Rat

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ABSTRACT

This study was conducted to investigate the combined effect of catechin (flavonoids in tea) , baclofen and saclofen on acetic acid induced visceral pain in rats. In this study, adult male Wistar rats with a weight range of 250-200 g purchased from Tehran School of Veterinary Medicine were employed. All experiments were performed in 8 to 15 intervals and each animal was used once. In this study, sterile normal saline solution, a 1% acetic acid solution was used. After pre-intraperitoneal injection, visceral pain response was studied in doses of 2/5 mg per kg of body weight and 1 mg saclofen. Each experiment was conducted based on completely randomized design. PROC GLM in SAS software (9.3) was used to compare the mean. In each test, Duncan and Dennett's tests were used to compare means in each test as well as for comparing means in control group.

Keywords: *Baclofen, GABAergic system, rats, pain reduction*

Introduction

Pain is one of the most complex and extraordinary senses in humans. It is the most common symptom of diseases and common reason for visiting the doctor. Nociceptive pain includes not only stimulate the nerve fibers and transfer it to the center console of pain, but also can be changed influencing by the quantity and quality of a wide range of experiences. This shows complex neural mechanisms for intervention and psychological response to pain experience (1, 2). Receptors are defined as free nerve endings that don't adapt unlike other sensory receptors in the body, or their adaptation is incredibly low. This non-adaptation allows the pain receptors to make aware person of damage stimulus that cause pain. The pain receptors are sensitive to chemical, mechanical, and thermal stimuli, and followed by tissue damage and release, chemical mediators such as bradykinin, histamine and prostaglandin are stimulated and send the pain signals by nerves to the central nervous system and eventually will feel pain by stimulating the cerebral cortex (3).

In addition, visceral pain is the complex pain that results from the stimulation of pain receptors in different organs of the body such as the colon, bladder and stomach in a variety of pathophysiological reasons and data is transmitted to the central nervous system by afferent pathways. According to various studies, neurotransmitters participating in opioid and non-opioid analysis of pain are divided into two categories. The two systems can closely work together to

regulate pain mechanisms. Non-opioid systems can be categorized as adrenergic, cholinergic, serotonergic, histaminergic and GABAergic systems (4). Flavonoids derived from secondary plant metabolites are widely found in the plant kingdom. Flavonoids can be divided into six groups of flavones, flavanols, isoflavones, aglycones, flavanol and anthocyanins based on the structure and position of the oxygen heterocyclic ring. The most important flavonoids found in tea are flavonovols, or to be more precise catechins. Catechin (C), epicatechin (EC), epigalocatechin (EGC), epicatechingalat (ECG), epigalocatechingallate (EGCG) and galocatechingallate (GCG) are six tea catechins in tea that have been accounted for a wide range of biological properties, such as antioxidant properties, anti-microbial, anti-cancer and anti-mutagenicity activities. Catechin in tea consists of more than 30% of dry matter (5). Their beneficial effects may be attributed to their health, and functional properties such as antioxidant, anti-mutagenic, anti-tumor and anti-cancer activities. To process information, the brain uses from various neurotransmitters including histaminergic system. Histaminergic system is one of the aminergic systems in mammalian's brain which acts through 4 receptors: H1, H2, H3 and H4 in the regulation of many brain functions such as food intake, cardiovascular and respiratory activities, neuroendocrine responses and learning and memory (the 6 , 7). Histamine is one of the aminergic neurotransmitters and plays an important role in the regulation of physiological and pathophysiological events. In the mammalian's brain, histamine is made in a limited number of neurons that are at the core of the Tuberomammillary posterior hypothalamus. Frills of these neurons penetrate in the most parts of the brain and are involved in many brain functions such as sleep and wakefulness, hormone secretion, cardiovascular control, regulating body temperature, food intake and memory formation (8). Despite the different methods that are commonly used pain relief and treatment, the researchers are still looking for new and better ways to treat this physiological phenomenon. Because of their cost-effectiveness, fewer side effects and easier accessibility, herbal medicines are the best alternative to synthetic drugs. The study was performed to investigate the combined effect of catechins (flavonoids in green tea), saclofen and baclofen on visceral pain induced by acetic acid in rats.

Materials and Methods

In this study, adult male wistar rats with a weight range of 250-200 g purchased from Tehran School of Veterinary Medicine were used. Rat were kept in groups of six in polyethylene cages, and a room with environmental conditions and standard temperature of 2 ± 23 ° C 12 hours in light and darkness. They were fed with a commercial pellet diet and had free access to food and water. All experiments were performed in the interval of 8 to 15 and each animal was used once. All the principles of laboratory animal care were taken into consideration in terms of standard laboratory temperature and humidity. In this study, sterile normal saline solution, a solution of 1% acetic acid was used: the solution was prepared of pure acetic acid and after dilution. Saclofen purchased from Sigma Company were used. In this study, Writhing Test as one of the standard tests was utilized to create and examine visceral pain. For visceral pain by Writhing method, acetic acid (1ml, 1%) was injected into the intraperitoneal area of the animal. Before the test, to avoid stress as well as to habituate the animals to laboratory conditions, animals were kept in a glass container with dimensions 20 × 30 × 40 cm for 30 minutes (which it is called the period of adaptation), so that the animal may be adapted to new conditions. After adaptation period, the animal was slowly brought out of the enclosure glass and after the drug's injection, the peritoneal injection of one ml acetic acid was carried out and immediately the animal was put in the chamber glass. Moreover, latency time (LatencyTime) and time to first abdominal cramps and number of contractions for an hour with an interval of five minutes of acetic acid infusion were recorded in special forms. A device called pain mirror was used to check visceral pain. The device has a wooden frame, a glass cube container with 20 × 30 × 40 cm and a mirror the size of 30 × 40 cm with an angle of 45 degrees inside it. While creating and recording of abdominal cramps in the animal, the mirror makes viewing more comfortable. After injection of saclofen for 5 mg per kg of body weight, visceral pain response was studied.

Statistical analysis method

Each experiment was conducted in a completely randomized design with six replications that its statistical model is as follows:

* = Average total population

Ti = effect of th treatment

€ij = effect of random error with zero mean and variance Q2

PROC GLM in SAS software (9.3) was used to compare the mean. For the statistical comparison of each test, Duncan and Dunnett' tests were used for comparing means of each experiment and control group, respectively.

Results

The results of pre-injection of saclofen in 1 mg per kg of body weight before the baclofen injection of 5.2 mg per kilogram of body weight suggest that saclofen prevented from pain effect induced by baclofen. P-value <0/05 (Table 2)

Table 1. The main effects of acetic acid

Writhing test (NO)	Latency time (sec)	%	
2/035 [b]	[a]/01478/0	0/5%	Acid acetic
12/375 [a]	[b]466/0	1	Acid acetic
9/208 [a]	[b]386/8	2	Acid acetic
1/615	79.812%	SEM	
0.0013%	00010/<	P-value	

Table 2. Effects of catechin, saclofen and baclofen

Writhing test(NO)	Latency time(sec)	
12/375 [a]	466/000 b	Saclofen mg/kg0 + baclofen mg/kg0 + catechin mg/kg 0 + 1% acetic acid
1/944 [b]	623/833 cd	
1/055 [b]	880/833 bc	Saclofen mg/kg 1 + baclofen mg/kg0 + catechin mg/kg 0 + 1% acetic acid
1/263 [b]	1074 /166 bc	Saclofen mg/kg0 + baclofen mg/kg0 + catechin mg/kg 0 + 1% acetic acid
3/20 8 [b]	1371/666 a	Saclofen mg/kg 1 + baclofen mg/kg 2.5 + catechin mg/kg 0 + 1% acetic acid
1/305 [b]	989/333 b	Saclofen mg/kg 0 + baclofen mg/kg 0 + catechin mg/kg 0 + 1% acetic acid
0/861 [b]	985/000 b	Saclofen mg/kg 1 + baclofen mg/kg 2.5 + catechin mg/kg 0 + 1% acetic acid
1/097 b	1018/667 [b]	Saclofen mg/kg 0 + baclofen mg/kg 2.5 + catechin mg/kg 5 + 1% acetic acid
		Saclofen mg/kg 1 + baclofen mg/kg 2.5 + catechin mg/kg 5 + 1% acetic acid
0.762	70/054	SEM
0.0003	0/0490	P-value

Discussion and conclusion

The results of recent study indicate that the catechins (flavonoids in green tea) caused analgesic effect on pain acceptance of acetic acid. Today, the effect of flavonoids on pain and inflammation causing pain reactions has been studied and their anti-inflammatory and analgesic effects have been shown. In studies on a new plant flavonoid called Hypoletin-8- glucoside obtained from the hypericum plant in rats, it has been shown that it has anti-

inflammatory effect on acute inflammatory phase and has no effect in the chronic or long-term phase (9). In a study, oral administration of 60 to 120 mg of catechins per one kilogram of body weight reduced secondary inflammation caused by arthritis in young rats (10).Possible mediators in the inflammatory pain induced by acetic acid has not well understood. It is reported that bradykinin, neurokinin A and prostanoids are involved in the activation of sensory fibers after intraperitoneal injection of propionic acid, lactic acid and acetic acid (11). Catechin (C), epicatechin (EC), epigalocatechin (EGC), epicatechingalat (ECG), epigalocatechingallate (EGCG) and galocatechingallate (GCG) are six tea catechins in tea that have been accounted for a wide range of biological properties, such as antioxidant properties, anti-microbial, anti-cancer and anti-mutagenicity activities. Catechin in tea consists of more than 30% of dry matter (5). Due to their beneficial effects on health, and functional properties such as antioxidant, anti-mutagenic, anti-tumor and anti-cancer activities, catechins have recently attracted much attention in the scientific community and among the general public. However, no studies have been done on the analgesic effect of gamma-amino butyric acid, and since GABA has been shown as an inhibitory neurotransmitter in the central nervous system in mammals, showing the relationship between catechin and GABAergic system in the modulation of visceral pain is important. It seems that each opioid receptor has a separate specific activity. In rat where their opioid receptors were knocked out, μ pain receptors were affected in response to chemical, mechanical and thermal pains in supraspinal part. K receptors in the spinal cord mediate visceral, thermal and chemical pain as well as δ receptors mediate mechanical and inflammation pains. Studies on oxidative stress induced by chlorpyrifos in rats showed that catechine and quercetin could prevent stress-related injuries. In another study, oral administration of catechin for 4 weeks improved lung toxicity produced by chlorpyrifos in rats, but completely failed to prevent lung poisoning. Studies on Polyphenols contained in plants, such as catechin, Theaflavin, Malvidin and cyanidin showed that catechin for 35 mg per one kilogram of body weight has anti-inflammatory effect on intestinal damage caused by Ketoprofen and effectively improve gastrointestinal ulcers. Research has shown that certain flavonoid and sistosterol compounds are qualified as anti- inflammatory and analgesic effects. Leaves and fruits in sea-buckthorn contain significant amounts of flavonoids. Sea-buckthorn is introduced as analgesics and anti-inflammatory compounds and is used in the treatment of rheumatoid arthritis. Studies on the sea-buckthorn plant and effects of anti- inflammatory and analgesic in fruit and leaves have been approved. According to different studies on the effect of catechin, no single study has been carried out about its effect on pain, particularly visceral pain. Therefore, recent findings and results of this study indicate that catechin has a visceral analgesic effect on visceral pain and is sensitive to baclofen induced by acetic acid.

In conclusion, the results of this study showed that in the visceral pain induced by acetic acid, catechin produces analgesia through the related mechanism of baclofen. Catechin also weakens the visceral pain induced by acetic acid through its GABAergic receptors (12)

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