ARTICLE

EFFECT OF SACLOFENAND CATECHIN FLAVONOIDS ON PAIN RELIEF IN RATS

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ABSTRACT

This research was designed to study the effects of saclofenand catechin on acetic derived visceral pain in rats. 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. Normal saline and acetic acid solution (1%) were used in the study. Visceral pain response was investigated before injecting catechin in amount of 5 mg per kg body weight, before saclofen 1 mg per kg body weight. In the control group which was consisted of 6 rats, visceral pain response was investigated by intra peritoneal injection of normal saline. Each experimentwas performed completely randomized with six replications. 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. Results obtained fromtwin injection of 5 mg catechin per kg body weight and 1 mg saclofen per kg body weight showed that it had a significant effect on onset time of first contraction of the abdominal wall from latency period ($p<0.05$) and reduced visceral pain caused by acetic acid (1%). It also had a significant effect on the number of abdominal wall full contractions and reduced its number. It can be said that catechin induces analgesia by saclofen dependent mechanism.

KEY WORDS
saclofen , catechin, rat

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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 complicated neural mechanism for experience interference and psychological response to pain[1,2]. Pain receptors are free nerve endings in the body, which Unlike other sensory receptors, do not have adaptation, or adaptation in them is extremely low;The mismatch allows pain receptors to warn the person about the damage making stimulus that causes pain as long as its present. pain receptors are sensitive to chemical, mechanical, and thermal stimulants, and followingthe tissue damage and release of chemical mediators such as Bradykinin, histamine, and prostaglandinare stimulated, and send the pain signals to the central nervous system by nerves, and eventually, pain is felt by stimulating the cerebral cortex [3]. 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. Examples of opioid system includes adrenergic, cholinergic, serotonergic, histaminergic, and GABAergic [4]. Adaptation in them is very low, and it lets the pain receptor to warn the person as long as the damaging stimulant is present. pain receptors are sensitive to chemical, mechanical, and thermal stimulants, and followingthe tissue damage and release of chemical mediators such as Bradykinin, histamine, and prostaglandin,they are stimulated, and send the pain signals to the central nervous system by nerves, and eventually, pain is felt by stimulating the cerebral cortex [3]. 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. Examples of opioid system includes adrenergic, cholinergic, serotonergic, histaminergic, and GABAergic (4). 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. Catechins are antioxidants, and they have beneficial effects for the body. 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 (5). Brain uses various neural mediators including Aminergic systems. Histaminergic system is one of mammals' aminergic systems which regulates many of the brain actions including grabbing of food, cardiac and respiratory functions, neuro endocrine responses, learning, and memory, by means of 4 types of receptors namely H_1 ،H_2 ،H_3, and H_4 [6,7].

Histamine is one of the aminergic neuro transmitters and it has an important role in regulation of a number of physiological and pathological events. In mammals, neural histamine is made in a few number of neurons gathered in Tuberomammillary core in posteriorhypothalamus. These neurons' appendixes

penetrate to most of brain's parts and interfere with many of the brain activities, such as sleeping and waking, hormones secretion, cardiovascular control, body temperature, food grabbing, and development of memories [8].

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. Herbal medicines are the best replaces for synthetic medicines, due to their less side effects, higher availability, and economical reasons. This study was performed to investigate effects of cathechin (green tea flavonoid) and saclofen as mediators, on acetic acid derived visceral pain in rat.

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. Sterile normal saline and acetic acid solution 1% (this solution was diluted from pure acetic acid), were used in the study. Cathechin and baclofen which was used, were bought from Sigma company. Control group, consisted of 6 rats, were investigated for visceral pain response after intra peritoneal injection of normal saline.

On this stage, visceral pain was created and investigated by intra peritoneal injection of acetic acid 0.5,1, and 2 percent in volume of 1 ml. then visceral pain response was studied after oral administration of cathechin in amounts of 5,10, and 20 mg per kg body weight.in this stage, visceral pain was studied after intra peritoneal injection of saclofen in amount of 2.5, 5, and 10 mg per kg body weight. Then visceral pain response after pre-injection of cathechin in amount of 5 mg per kg body weight before saclofen in amount of 2.5 mg per kg body weight was studied.

. 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.
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

Results obtained from twin injection of 5 mg Catechin per kg of body weight, and 5 mg saclofen per kg of body weight, showed significant effect on the onset time of first contraction of abdominal wall from latency period ($P<0.05$) and reduced acetic acid (1%) derived visceral pain.

Table1: Different percent of acetic acid's main effect

Writhing test (NO)	Latency time (sec)	%	
2/ 035 [b]	1478/ 0 [a]	0/5	Acetic acid
12/ 375 [a]	466/ 0 [b]	1	Acetic acid
9/ 208 [a]	386/ 8 [b]	2	Acetic acid

1/615	79/812	SEM
0/0013	<0/0001	P-value

Table 2: Twin main effect of saclofen and cathechin

Writhing test (NO)	Latency time (sec)	
6/ 715 [a]	673/ 417 [c]	Acetic acid+cathechin0 $^{mg}/_{kg}$+Saclofen0 $^{mg}/_{kg}$ %1
1/ 603 [b]	849/ 000 [bc]	Acetic acid+cathechin0 $^{mg}/_{kg}$+Saclofen1 $^{mg}/_{kg}$ %1
2/ 034 [b]	1178/ 333 [a]	Acetic acid+cathechin5 $^{mg}/_{kg}$+Saclofen0 $^{mg}/_{kg}$0 %1
1/ 201 [b]	1004/ 000 [ab]	Acetic acid+ cathechin5 $^{mg}/_{kg}$+Saclofen1 $^{mg}/_{kg}$ %1
0/538	49/536	SEM
0/0003	<0/0011	P-value

CONCLUSION

The results of the study indicate that the catechin (green tea flavonoid) and saclofen have analgesic effects on acetic acid-induced pain. Recently, the effects 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 [9]. oral administration of catechins in amounts of 60 to 120 mg per kilogram of body weight, reduced arthritis-derived secondary inflammation in young rats. [10]. Possible mediators in the inflammatory pain, induced by acetic acid, are still not well recognized, therefore, saclofen showed a mediator effect in the current study. 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, antimicrobial, anti-cancer, and anti-mutagenicity activities. Catechins consist more than 30% of tea dry matter (5). Recently, cathechins have attracted much attention in the scientific community and among the general public due to their beneficial effects on health, and their performance such as antioxidant, anti-mutagenic, anti-tumor and anti-cancer characteristics. However, not many studies have been done on their analgesic effects and since Gamma Amino Butyric Acid (GABA), is specified as an inhibitory neurotransmitter in the central nervous system of mammals, describing the relationship between catechin and GABAergic system in moderating visceral pain is important. It seems that each of opioid receptors, act separately and specifically. In opioid receptor knocked-out mice, μ receptors were affected in response to chemical, mechanical and thermal supraspinal pain. K receptors mediated thermal and chemical visceral pain spinally, and δ receptors mediated inflammatory and mechanism pains. Studies on oxidative stress induced by Chlorpyrifos in rats, showed that catechin and quercetin were able to prevent stress-related injuries. In another study, oral administration of catechin for 4 weeks improved Chlorpyrifos induced lung toxicity in rats but it did not prevent lung toxicity completely. Studies on herbal Polyphenols, such as catechin, Teaflavin, Malvidin and cyanidin showed that catechin by the amount of 35 mg per kg body weight has anti-inflammatory effect on intestinal damages caused by ketoprofen, and it effectively heals the gastric ulcers. Research has shown that certain flavonoid and cystosterol compounds, have anti-inflammatory and analgesic effects. Russian olive Leaves and fruits contain significant amounts of flavonoids compounds. Russian olive was introduced as an analgesic and anti-inflammatory fruit and it is used for treatment of rheumatoid arthritis. Studies on the fruits and leaves of the russian olive plant, approved its anti-inflammatory and analgesic effects. Despite the different studies on the cathechins, still catechin's effect on pain, in particular visceral pain has not been studied yet. Therefore, due to the recent findings, and results of this study, catechins, have a baclofen sensitive visceral analgesic effect on visceral pain induced by acetic acid. In conclusion, the results of this study showed that in visceral pain induced by acetic acid, the catechin induces analgesia through the saclofen affiliated mechanism.

CONFLICT OF INTEREST

There is no conflict of interest.

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