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# EFFECTS OF BLACK TEA EXTRACT AND ITS THEARUBIGINS ON WHOLE GUT TRANSIT TIME IN MICE: INVOLVEMENT OF 5-HT$_3$ RECEPTORS

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**Abstract**

Tea is the most popular beverage in the world. In the recent decades therapeutic effects of various type of tea drinking has been revealed in many studies. The purpose of this study was to evaluate the effects of the black tea extract (BTE) and its major polyphenolic pigments thearubigins (TRs) on whole gut transit time in mice by use of carmine marker. BTE of Iranian tea was prepared and its polyphenolic pigment TRs extracted by Liquid- liquid partition method. BTE (1.5%, 3%, 4.5%, 6%, and 10%) and extracted TRs (30 mg/kg, 40mg/kg, 50mg/kg, 60 mg/kg, 70mg/kg, and 100mg/kg) were gavaged to the fasted mice to measuring the whole gut transit time. Results showed BTE (3%, 4.5%, 6 %,) and TRs (40mg/kg, 50mg/kg, 60 mg/kg, 70mg/kg) significantly decreased the whole gut transit time dose dependant manner. For determination of serotonergic system involvement as a major neurotransmitter system in transit time alteration caused by BTE and TRs, ondansetron (3mg/kg, i.p) was used. Acquired data showed that 5-HT$_3$ antagonist blocked accelerating effects of BTE and TRs. Based on the results BTE and TRs could be regarded as gut accelerator movement dose dependant manner. Moreover, it was concluded that these effects at least partially involved with serotonergic system via 5-HT$_3$ receptors.

**Keywords:**

Black tea extracts (BTE), Thearubigins (TRs)**,** Whole gut transit time, 5-HT$_3$ receptors.

## Introduction

Apart from water, tea is the most popular beverage in the world (1). Tea beverage is processed in three forms from the leaves of *Camellia sinensis* (L) Ktze. green tea (none oxidized), Oolong tea (semi oxidized) black tea (totally oxidized)(2). At least 70% of tea consumers in the world drink the black tea (3). In Middle East and Iran whole consumption of tea confined to black tea. From the ancient time claims have been made about the beneficial effects of the tea drinking, nowadays therapeutic values of tea is the matter of many researches. Accumulating evidences suggest many benefits of tea drinking such as antioxidant properties, antimicrobial to anti diabetics effects (4). Especially in recent years some reports that black tea could be useful to alleviate some of digestive tract discomforts such

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as gastric ulcer, mucosal injury and diarrhea (5, 6).
Our aim in this study was to determine the effect of Iranian black tea extract (BTE) and its polyphenolic pigments (TRs) on the transit time of the whole length of gut and in propulsive motility of digestive tract. In previous studies the effects of black tea on the inhibitory neurotransmitters in gut to some extent has been investigated meanwhile effects of black tea and its thearubigins pigment on the excitatory neurotransmitters such as serotonin (5-hydroxytryptamine) has remained unclear (7), therefore we intended to examine the impact of the BTE and TRs on gut motility via 5-$HT_3$ receptors. Serotonin plays an important role in regulating gut movements; among serotonin receptors 5-$HT_3$ receptors have been described as main serotonin receptors in regulating gut movements which could be triggered by various agents (8).

## Materials and methods

*Black tea extract preparation*
For preparation of the water extract of Iranian black tea, we obtained clone – 100 tea bushes (*Camellia sinensis* .Var sinensis) leaves that are grown and processed in orthodox method in Iran Tea Research Institute (TRI). Black tea extract prepared by 1.5, 3, 4.5,6 and 10gr of dried black tea leaves in boiling water for 5 min then provided 1.5%, 3%, 4.5%, 6% and 10% solution of BTE (9).

*Extraction of TRs*
The Extraction of TRs was performed by Roberts method (10). Dried black tea leaves (10 g) were soaked in boiling distilled water (300 ml) and were boiled for 10 min then water extract (120 ml) was filtered and extracted continuously with chloroform to remove caffeine. Aqueous layer extracted continuously with ethyl acetate (2×40 ml) for removing the theaflavins (TFs).For extraction of TRs n-butanol (2×40 ml) was added to aqueous layer. Five individual fraction of TRs, TR1, TR2, TR3, TR4, TR5 were collected using rotary vacuum evaporator. Detection of TRs was done in UV-visible(11).

*Animals*
Male Balb/c mice were used (weighting 20-30g) bred in animal room in Physiology Department, Tehran University of Medical Sciences, in an air conditioned room (25 °C With 50% humidity) under 12 h light:12 h dark cycle prior to experiments.

*Experimental groups*
Groups that were treated with BTE: The mice were randomly divided into 11 groups that each of them consisting of eight mice. Control group received 0.3 ml saline and test groups comprise 5 groups were treated with 0.3ml BTE: 1.5%, 3%, 4.5%, 6% and 10% respectively.
Groups that were treated with TRs included 6 groups (n=8) and received 30mg/kg, 40mg/kg, 50mg/kg, 60mg/kg, 70mg/kg and 100mg/kg respectively. Groups that treated with BTE and TRs in the presence of 5HT-3 antagonist ondansetron consisting of 4.5%BTE + ondansetron 3mg/kg (i.p.) and 50mg/kg TRs + ondansetron 3mg/kg (i.p.)(12).

*Whole gut transit time measurement*
All mice were fasted over night (18 h) before experiments, animals treated with BTE and TRs (0.3 ml), 30 min later animals were gavaged by carmine marker. Then animals transferred to the cage that its bottom was covered with white sheet. First observation of defecated marker recorded as a whole gut transit time (WGTT) (13).

*Drugs*
5HT-$_3$ antagonist (ondansetron) purchased from sigma chemical co. Carmin and all the solutions in this study obtained from Merck.

*Statistics*

The data were statistically analyzed by ANOVA Dunnet t-test. Whole gut transit time was expressed as Mean ± S.E.M. A mean difference was significant at the 0.01 level.

## Results and Discussion

Results presented in Fig. 1 indicate that BTE in doses of 3 % ($p$<0.05) and 4.5 % ($p$<0.01) significantly reduced WGTT compared to the control group. Based on results, it seems that BTE in these concentrations work as a gastro kinetic agent, on the other hand in higher concentration, BTE (6% and 10%) failed to increase gastrointestinal movement. TRs 40, 50 and 60 mg/kg ($p$<0.01) significantly increased gastrointestinal motility and reduced WGTT (Fig. 2). It is assumed that TRs as main polyphenolic pigments of black tea fraction and BTE are responsible for gastro kinetic effect of this beverage. TRs in doses of (30 mg/kg, 70mg/kg and 100mg/kg) failed to increase the gastrointestinal movement (Fig. 2).

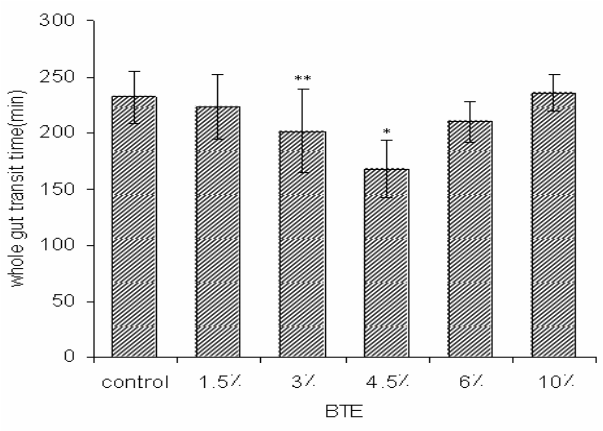


Figure.1- Effects of various concentration of Black Tea Extract (BTE) on whole gut transit time (percentage). Data are presented as Mean ± S.E.M, * p< 0.01

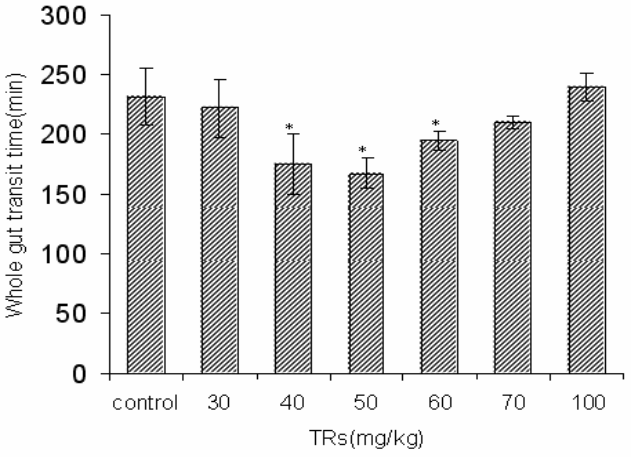


Figure.2- Effects of various concentrations of thearubigins (TRs) on whole gut transit time (percentage). Data are presented as Mean ± S.E.M, *p< 0.01

In groups that were treated with BTE (4.5%) and TRs (50mg/kg) use of ondansetron (3mg/kg) reversed prokinetic effects of BTE and TRs on gut ($p$<0.01) The (Fig.3 and Fig.4) that indicates gastrointestinal movement acceleration by BTE and TRs at least partially mediated via 5-$HT_3$ receptors. parasympathetic fiber which release acetylcholine, 5-$HT_3$ and 5-$HT_4$ are regarded as excitatory modulators in gut, but 5-$HT_3$ plays a crucial role in gut stimulation and movements(14). Prokinetic effect of BTE and TRs may introduce them as an efficient natural product in modulating gut movements (15). Also previous report indicates that inhibitory neurotransmitters are involved in the effect of BTE on gastrointestinal movement, our results confirm role of excitatory neurotransmitter that has already investigated in upper gut transit time (16). So we can consider BTE and TRs as 5-$HT_3$ receptors agonists.

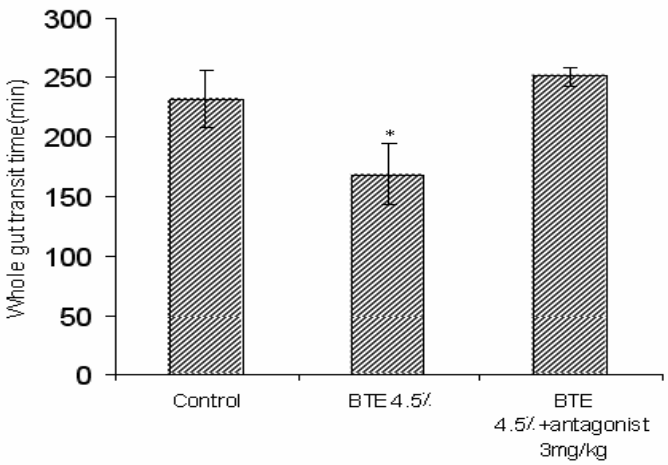


Figure.3 - Effect of Black Tea Extract (BTE) in the presence of 5HT-$_3$ antagonist on whole gut transit time (percentage). Data are presented as Mean ± S.E.M, *p< 0.01

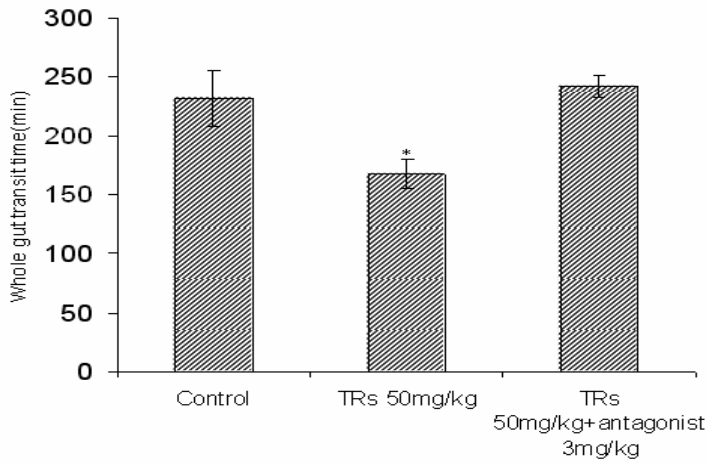


Figure.4 - Effect of thearubigins(TRs) on whole gut transit time in the presence of 5HT3 antagonist on whole gut transit time (percentage). Data are presented as Mean ± S.E.M, *p< 0.01

## Conclusion

The results of previous studies on prokinetic activity of black tea extract on gut motility via inhibitory neurotransmitters suggest a cholinergic involvement and a partial role of prostaglandin and nitric oxide in prokinetic effect of black tea extract and indicating a role of the opioid system in the antidiarrhoeal activity of the extract in the mechanism of action of black tea extract on gastrointestinal motility (7, 9). Based on previous results and Referring to use of natural products in alleviating of some of gut discomforts, the results of present work confirm that these natural products would be effective in acceleration of gut movements like some prokinetic drugs such as metoclopramide or cisapride. On the other hand in recent publication 5-$HT_3$ agonists were introduced as new agents.

To abate the symptoms of gastrointestinal disease like irritable bowel syndrome (IBS) and diarrhea (17), meanwhile the observations made here by us revealed agonistic role of BTE and TRs on 5-$HT_3$ receptors so it would be suggested that these natural products are able to relief gastrointestinal discomforts (18).

## References


1. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Anti-hyperglycemic effect of black tea (*Camellia sinensis*) in rat. J. Ethnopharmacol. 1995; 45: 223-26.
2. Wilson LC, Clifford MN. Tea cultivation to consumption.1st ed., Chapman & Hall. 1992.
3. Gupta S, Chaudhuri T, Ganguly DK, Giri AK.Anticlastogenic effect of black tea (world blend) and its two active polyphenols theaflavins in vitro in Swiss albino mice. Life Sci. 2001; 69: 2735-44.
4. Dufresne CJ, Farnworth ER. A review of latest research finding on the health promotion properties of tea. J. Nutr. Biochem. 2001; 12: 404-21.
5. Maity S, Vedasiromoni JR, Chaudhuri L, Ganguly DK. Role of glutathione in anti ulcer effect of hot water extract of black tea. Jpn. J. Pharmacol. 1998; 78: 285-92.
6. Maity S, Ukil A, Karmakar S, Datta N, Chaudhuri T, Vedasiromoni JR, Ganguly DK, Das PK. Thearubigins, the major polyphenol of black tea, ameliorates mucosal injury in trinitrobenzene sulfonic acid –induced colitis. Eur. J. Pharmacol. 2003; 470: 103-12.
7. Chaudhuri L, Basu S, Seth P, Chaudhuri T, Besra SE, Vedasiromoni JR, Ganguly DK. Prokinetic effect of black tea on gastrointestinal motility. Life Sci. 2000; 66(9): 847-54.
8. Hoyer D, Hannon JP, Martin GR. ,Hannon JP, Martin GR. Molecular pharmacological and functional diversity of 5HT receptors. Pharmacol. Biochem. Be. 2002; 71: 533-54.
9. Besra SE, Gomes A, Ganguly DK, Vedasiromoni JR. Antidiarrhoeal activity of hot water extract of black tea (*Camellia sinensis*). Phytother. Res. 2003; 17(4): 380-84.
10. Brown AG, Eyton WB, Holmes A, Ollis WD. The identification of the thearubigins as polymeric proanthocyanidins. Nature 1969; 221: 742 - 44.
11. Peterson J, Dwyer J, Jacques P, Rand W, Prior R, Chui K. Tea variety and brewing techniques influence flavonoid content of black tea. J. Food Consumption anal. 2004; 17: 397-405.
12. Pascual D, Alsasua A, Goicoechea C, Martín M.The involvement of 5-$HT_3$ and 5-$HT_4$ receptors in two models of gastrointestinal transit in mice. Neurosci. Lett. 2002; 326: 163-66.
13. Nagakura S, Nagakura Y, Naitoh Y, Kamato T, Yamano M, Miyata K. Compounds possessing 5$HT_3$ receptor antagonistic activity inhibit intestinal

propulsion in mice. Eur. J. Pharmacol. 1996; 311: 67-72.
14. Hansen MB. Neurohurmoral Control of Gastrointestinal Motility. Phys. Res. 2003; 52: 1-30.
15. Honnon J, Hoyer D. Serotonin receptors and system:endless diversity?.Acta boil. Szegedienssis. 2002; 71: 533-54.
16. Jafari K, Gharibzade S, Faghihi S, Karimian SM, Hamzehloo M, Keshavarz M. The effect of iranian black tea extract and its isolated thearubigins on intestinal transit time in mice. J. Kerman Uni. Med. Sci. 2006; 13: 37-42.
17. Yoshida S, Shiokawa S, Kawano K, Ito T, Murakami H, Suzuki H. Orally active benzoxazole derivative as a 5-$HT_3$ receptor partial agonist for treatment of diarrhea-predominant irritable bowel syndrome. J. Med. Chem. 2005; 48: 7075–79.
18. Jafari K, Faghihi M, Gharibzadeh S. Black tea extract and its major polyphenolic pigment may ameliorate the gastrointestinal disorder in irritable bowel syndrome. Medical Hypothesis. 2006; 67: 419.