Research Article

Tert-Butylhydroquinone (TBHQ) Inhibits the Contractile Activity of the Small Intestinal Visceral Smooth Muscle in Male Albino Rats

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ABSTRACT

Tert-Butylhydroquinone (TBHQ), also known as E-319, is an extensively used food additive throughout the world. It is used as a preservative in a wide range of ultra-processed food stuffs, resulting in continuous exposure to human population through oral consumption. Small intestine, the organ mainly responsible for digestion and absorption of ingested stuffs, gets primarily exposed to TBHQ through consumption of TBHQ tainted food stuffs. The digestive and absorptive functions of the small intestine are achieved by the contractions of the smooth muscles located in the wall structure of small intestine, provides motility to it. TBHQ have already been studied and reported to be cyto-toxic, carcinogenic, mutagenic, genotoxic, teratogenic, endocrine disrupting and immune-modulatory. Till date no such studies have reported the effects of TBHQ on the contractile activity of small intestinal visceral smooth muscle (SiVSM). So, this study has been designed to examine the effects of graded doses of TBHQ on the contractile activity of SiVSM. TBHQ inhibits the contractions of small intestine (duodenum) *ex vivo*, by decreasing both the frequency and the amplitude of contractions in a dose-dependent manner. From the results it can be suggested that the TBHQ induced inhibition of the contraction of the SiVSM might be due to suppression of the activity of intrinsic cholinergic myenteric efferents and/or facilitation of the activity of nitrergic and/or adrenergic intrinsic myenteric efferents. Further, TBHQ decreases the percentage gastro-intestinal transit (GIT%) *in vivo*, assessed through charcoal meal test, which results due to inhibitions in the contractions of visceral smooth muscle of gastro-intestinal tract, as a result of TBHQ exposure in a dose dependent manner. In conclusion, it can be suggested that TBHQ inhibits the contractile activity of SiVSM in dose-dependent manner.

Keywords: Tert-Butylhydroquinone (TBHQ), small intestine, contractile activity, small intestinal visceral smooth muscle (SiVSM), gastro-intestinal transit (GIT).

INTRODUCTION

In recent decades, we have evidenced a tremendous emergence of ultra-processed food products in the food industries, to fulfil the demands of the human population worldwide because ultra-processed food products are more desired with their longer shelf-life and are ruling the global market. Preservatives such as Tert-Butylhydroquinone (TBHQ) plays an important role in the processing of such ultra-processed food stuffs. TBHQ is a synthetic phenolic compound, is widely used additive in food industry as well as cosmetics and pharmaceutical industries as a preservative to increase the shelf-life of the products[1]. In food industry, the anti-oxidant property of TBHQ is used to prevent fats, oils, fish and meat products from undergoing oxidative deterioration, often used in ultra-processed foods like processed breakfast items, cereal based food products, snacks, instant foods, noodles, fried stuff, baked products, frozen meals, edible oils, edible animal fats, seasoning and condiments, etc., without changing the organoleptic properties of the products[2]. TBHQ is also preferred because of its low cost, high performance and high chemical stability as compared to other synthetic phenolic antioxidants (SPAs)[3]. TBHQ preserves food products by producing active hydrogen that combines with free radicals released by oxidation process, providing long shelf-life to the products[1]. As per regulatory agencies, the maximum permitted level of TBHQ allowed in the food products is 0.02% or 200mg/kg of fat or oil content. The acceptable daily intake (ADI) of TBHQ is 0-0.7mg/kg body weight allocated by Joint FAO/WHO Expert Committee on Food Additives (JECFA), an international expert scientific committee of Food and Agriculture Organization (FAO), of the United Nations and the World Health Organization (WHO)[4-5]. It has also been reported that using maximum permitted levels, exposure estimates for TBHQ have exceeded the ADI value for toddlers and children[6]. The consumption of excessive amounts of TBHQ may be detrimental for human health, as cytotoxic effects including genotoxic, mutagenic, carcinogenic, teratogenic, endocrine disrupting as well as immune-modulatory effects of TBHQ on various biological models had been reported earlier[5,7-10]. Since, TBHQ is an extensively used additive in the food industry, humans are subjected to it majorly through dietary exposure, where it comes in direct contact of gastrointestinal tract. However, effects of TBHQ on the small intestine where digestion and absorption of TBHQ containing ultra-processed food products takes place, is not reported till date. The small intestinal visceral smooth muscles (SiVSM) located in the wall structure of small intestine are responsible for the contractile function of the small intestine which helps in digestion and absorption by inducing propulsion of luminal content in aboral direction as well mixing of luminal content with digestive enzymes. So, this study is hereby designed to understand the effect(s)

of TBHQ on the contractile function of SiVSM, through a set of *ex vivo* and *in vivo* experiments.

MATERIALS AND METHODS

Chemicals

All the chemicals including reagents used during experimental study were of analytical grade and were purchased from Sigma Aldrich, USA; Merk Life Science Private Limited, Mumbai, India; Sisco Research Laboratories Private Limited (SRL), Mumbai, India. TBHQ (CAS-No: 1948-33-0) was purchased from Sigma Aldrich, USA.

Animal model for experiments

Adult male albino rats, weighing 120-150 grams, were selected as experimental models. The animals are maintained as per the recommendations of animal ethics committee of the University of Kalyani in accordance with National guidelines. The animals were housed under standard environmental conditions with an approximate 12hours light-dark cycle, room temperature of 25-27°C. Standard laboratory food and water are provided *ad libitum.*

Study of the effect(s) of TBHQ on the contractile activity of small intestinal visceral smooth muscle (SiVSM) *ex vivo*

To examine the effect(s) of TBHQ on the movement of duodenum, the animals were divided into four groups. Amongst them, TBHQ exposed groups namely treated 1 (t1), treated 2 (t2), treated 3 (t3) and treated 4 (t4) were exposed to 25µM, 50µM, 100µM and 200µM of TBHQ respectively. All the animals were fasted overnight prior to experiment with free access to water. To record small intestinal (duodenal) motility, Dale's apparatus along with isotonic transducer coupled with RMS Polyrite-D was adjusted as per requirements prior to experiment. The organ bath of the Dale's apparatus was filled with Tyrode's solution with continuous supply of 95% O_2 and 5% CO_2 and the temperature was maintained within a range of 37±0.5°C. After overnight fasting the animals were sacrificed by cervical dislocation and instantly abdomen was opened and a 3cm long duodenal segment was removed and placed in Tyrode's solution. The luminal content of the duodenal segment was gently flushed out and the segment was placed longitudinally in the organ bath of Dale's apparatus. The continuous recording of the duodenal motility was acquired with the help of isotonic transducer (IT-2245) coupled with RMS Polyrite-D (RMS, Chandigarh, India). The duodenal segment is then allowed to adapt for 45 minutes within the given experimental setup, while repeatedly changing the Tyrode's solution at an interval of 10 minutes to avoid the accumulation of metabolites in the organ bath[11]. Afterwards, graded doses of TBHQ (25µM, 50µM, 100µM and 200µM) were applied and the movement pattern of the duodenal segment is recorded for further analysis.

Study of the effect(s) of TBHQ on the contractile activity of small intestinal visceral smooth muscle (SiVSM) *in vivo*

Charcoal meal test was used to assess the effect of single-dose exposure of TBHQ on intestinal transit *in vivo*. After acclimatization period, experimental animals (albino rats) were divided into five groups namely control (C), treated 1 (TI), treated 2 (TII), treated 3 (TIII) and treated 4 (TIV). The animals of all the aforementioned groups were fasted overnight prior to experiment with free access to water. Amongst these five groups of animals, control group was not exposed to TBHQ and the remaining TI, TII, TIII and TIV groups were exposed to 25µM, 50µM, 100µM and 200µM of TBHQ respectively administered intraperitoneally. Thereafter, charcoal meal (0.5 ml of aqueous suspension of 10% charcoal and 5% gum acacia) was administered orally. The animals were sacrificed 20 minute later. The abdomen was opened and the small intestine (from pylorus to ileo-caecal junction) was removed and stretched on a plain surface. The total length of the small intestine and the distance travelled by charcoal was measured with the help of a scale and recorded[12-14]. The percentage of gastro-intestinal transit (GIT) is calculated with the help of the equation mention below:

$$\text{GIT } \% = \frac{\text{Total length of small intestine (in cm)} - \text{distance travelled by charcoal (in cm)}}{\text{Total length of small intestine (in cm)}} \times 100$$

Statistical analysis

All the data were expressed as mean ± SEM. Statistical analysis between the experimental groups were carried out using one way ANOVA (analysis of variance) followed by Tukey's Test. GraphPad Prism version 8.4.3 (686), GraphPad Software Inc. software was used for statistical analysis of the experimental data obtained.

RESULTS AND DISCUSSION

Effect(s) of TBHQ on the contractile activity of small intestinal visceral smooth muscle (SiVSM) *ex vivo*

Exposure of graded doses of TBHQ on the isolated segments of small intestine (duodenum) in the organ bath significantly decreases the amplitude and frequency of duodenal movement in a dose dependent manner.

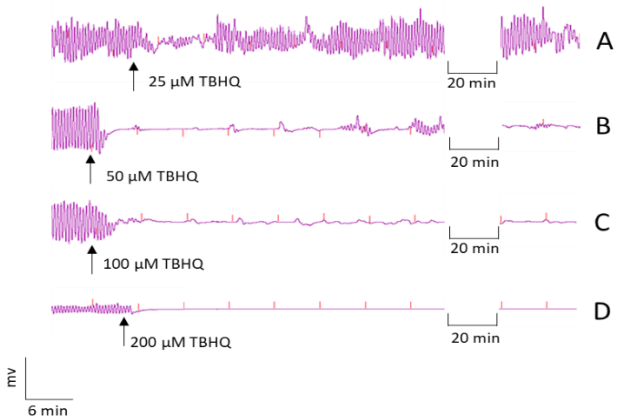

Figure 1: Representative records showing the effects of the exposure of graded doses of TBHQ on small intestinal

(duodenal) motility, recorded *ex vivo* through isotonic transducer coupled to RMS-Polyrite-D. ↑ Indicates the point of application of TBHQ. A represents the effects of the exposure of 25µM of TBHQ on small intestinal (duodenal) motility, B represents the effects of the exposure of 50µM of TBHQ on small intestinal (duodenal) motility, C represents the effects of the exposure of 100µM of TBHQ on small intestinal (duodenal) motility, D represents the effects of the exposure of 200µM of TBHQ on small intestinal (duodenal) motility.

The decrease in the amplitude and frequency of the duodenal movement can be observed from the tracings recorded *ex vivo* through an isotonic transducer coupled to RMS Polyrite-D, as shown in Figure 1. The degree of inhibition of duodenal motility increases in a dose dependent manner as represented in Figure 2.

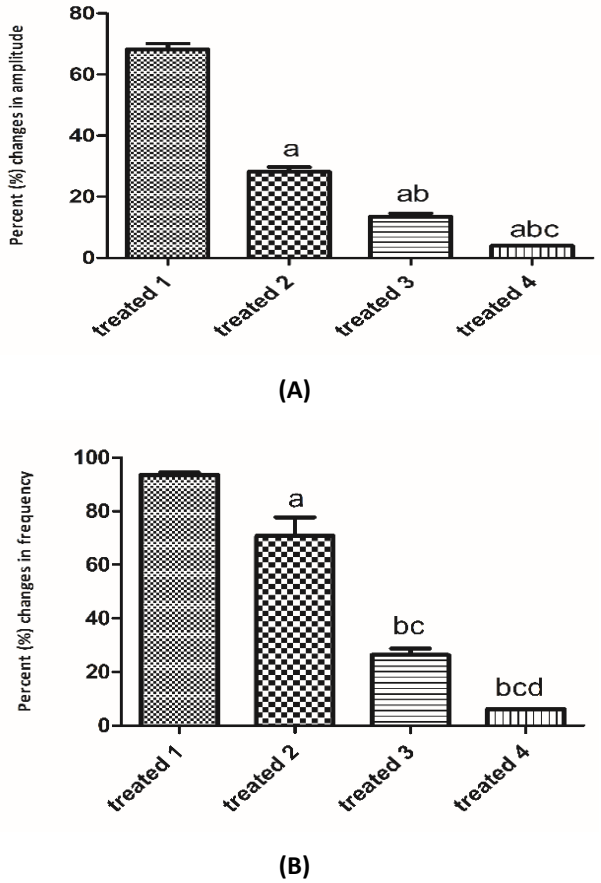

Figure 2: Showing percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in TBHQ exposed groups of rats *ex vivo*. The values are represented as mean ± SEM, (A) [a]*p<0.001* vs treated 1, [b]*p<0.001* vs treated 2, [c]*p<0.05* vs treated 3, (B) [b, a]*p<0.001, 0.01* vs. treated 1, [c]*p<0.001* vs treated 2 and [d]*p<0.05* vs treated 3.

Effect(s) of TBHQ on the contractile activity of small intestinal visceral smooth muscle (SiVSM) *in vivo*

To access the effects of exposure of graded doses of TBHQ on gastro-intestinal transit (GIT) *in vivo,* charcoal meal test was performed. In this study we have found that, exposure of TBHQ decreases the GIT which is expressed as GIT%, in a dose dependent manner as represented in Figure 3. The decreased percent in GIT is due to TBHQ induced inhibitions of the contractions of the visceral smooth muscle located in the wall structure of the small intestine, that provides motility to it.

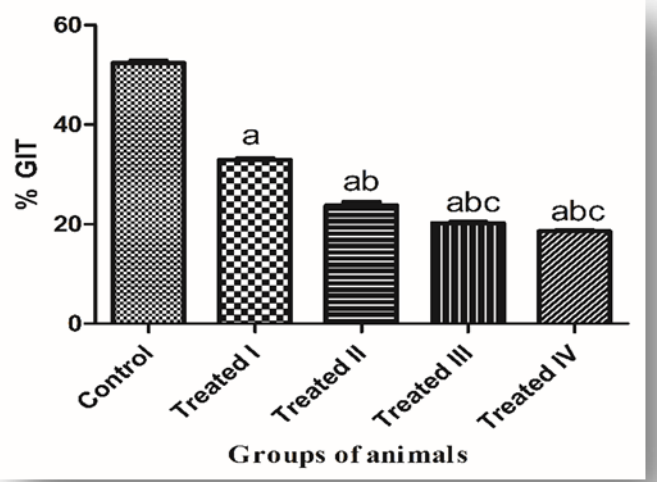

Figure 3: Graphical representation showing percent (%) changes in GIT (gastro-intestinal transit) *in vivo* in Treated I, Treated II, Treated III and Treated IV groups of animals in comparison to control group of animals, where Control group of animals were not exposed to TBHQ, Treated I group of animals were exposed to 25µM TBHQ, Treated II group of animals were exposed to 50µM TBHQ, Treated III group of animals were exposed to 100µM TBHQ and Treated IV group of animals were exposed to 200µM TBHQ. The values are represented as mean ± SEM, [a]*p<0.001* vs Control, [b]*p<0.001* vs Treated I and [c]*p<0.001* vs Treated II.

The small intestine has digestive, absorptive, secretory, and immunological functions[15]. These functions of small intestine are entrained by the contractions of visceral smooth muscles (VSM) located in the muscularis externa layer within the wall structure of the small intestine that provides motility to the small intestine, which leads to the mixing of ingested food stuffs with pancreaticobiliary secretions and their propulsion in aboral direction facilitating the digestion and absorption process[16]. These functions of small intestine are regulated by interstitial cells of Cajal (ICC) present in the musculature of small intestine. ICC generates electrical slow waves (ESWs) as well as propagates them throughout the intestinal tract which regulates the excitability and contraction of smooth muscle cells and also participates in the transduction of neural inputs from the enteric nervous system (ENS), in order to control the contractile activity of SiVSM[17-18]. In our results, we have observed inhibitory effect of TBHQ on the contractile function of SiVSM in both *ex vivo* and *in vivo* experiments in a dose dependent manner. So, from the results of the studies, we can hypothesize that TBHQ inhibits the frequency of contractions of SiVSM probably by inhibiting the generation of ESWs from ICC, inhibiting their propagation throughout intestinal tract and/or by inhibiting the transduction of neural inputs from the enteric nervous system through ICC by altering the neurotransmission

pathways or membrane potential gradient of smooth muscle cells which results in inhibition in the rhythmicity of the ESWs or basal electrical rhythms (BERs). It can also be assumed that the TBHQ induced inhibition of the contraction of the SiVSM might be due to the inhibition of the activation of excitatory intrinsic cholinergic myenteric efferents and/or potentiation of the activity of inhibitory adrenergic and/or nitrergic (NANC, non-adrenergic non-cholinergic) intrinsic myenteric efferents innervating the SiVSM. Further, the TBHQ induced decrease in GIT% might be due to TBHQ induced inhibition of contraction of VSM located at the muscularis externa of the small intestine, that decreases the motility which results in delayed GIT.

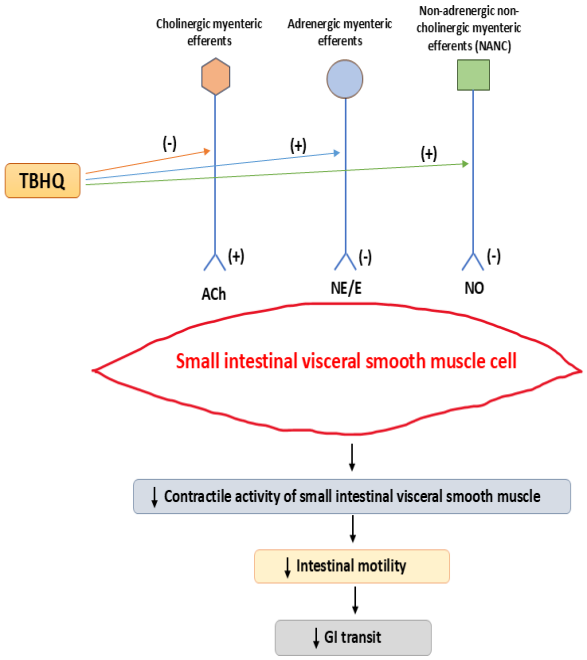

Figure 4: Schematic representation showing the probable neurocrine mechanisms involved in TBHQ induced inhibition of contractile activity of small intestinal visceral smooth muscle resulting in inhibition of intestinal motility and gastro-intestinal (GI) transit. (-) indicates inhibition, (+) indicates stimulation or potentiation and ↓ indicates decrease in activity.

CONCLUSION

In conclusion, TBHQ suppresses the contractile activity of SiVSM located in the muscularis externa layer of the small intestine in rat. TBHQ induced inhibitions of the contraction of the SiVSM might be due to inhibition of the activity of excitatory cholinergic myenteric efferents and/or stimulation of activity of inhibitory noradrenergic and/or non-adrenergic non-cholinergic (NANC) myenteric efferents innervating the SiVSM. Further, TBHQ was found to decrease the GI transit as a result of TBHQ induced inhibition of contractions of the SiVSM that provides motility. Thus, it could be suggested that TBHQ exposure through TBHQ tainted foods to small intestine might impairs digestive, absorptive and other functions of small intestine in humans.

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