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Research Article

Ginger Nanoparticles Modulate the Apoptotic Activity in Male Rats Exposed to Dioxin-Induced Cancer Initiation

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Abstract

Background and Objective: Ginger (*Zingiber officinale* Rosc.) has been known to exhibit various biological activities such as antioxidant and anti-carcinogenic actions. Nanoparticles of natural compounds believed to have high percentage of flavonoids which present a biological capability for treatment of several diseases including cancer. This study investigated the apoptotic activity as protective action of ginger nanoparticles (GNPs) against the 2,3,7,8-Tetracholorodibenzo-p-dioxin (TCDD) induced initiation of colon cancer in male rats. **Materials and Methods:** Animals were allocated in nine groups treated with single oral injection/week with TCDD (0.2, 1, 5 and 20 μg kg^{-1} 5 mL^{-1} corn oil) for one month. GNPs (50 mg kg^{-1} b.wt.,/everyday) were given to the rats after termination of TCDD injection (at the initiation stage of carcinogenesis) for 2 months. The antioxidant status of treated rats was determined by measuring of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) using standard kits. The expression of several apoptotic related genes Bcl-2, Bax and p53 were analyzed by qRT-PCR. Apoptotic alterations in colon cells were determined morphologically by fluorescent microscope. All obtained data were analyzed using the General Linear Models (GLM) technique. **Results:** Levels of the antioxidant activity of SOD, CAT, GPx and GST were decreased in TCDD-rats. However, GNPs supplementation significantly enhanced $p<0.05$ the levels of these antioxidants in TCDD-rats. Expression of Bcl-2, Bax and p53 genes in TCDD-rats was significantly up-regulated. However, GNPs decreased the expression of Bcl-2, Bax and p53 genes in TCDD-GNPs rats. Additionally, the necrotic/apoptotic rate was low in TCDD+GNPs groups, while, high necrotic/apoptotic rate was estimated in TCDD groups. **Conclusion:** It was concluded that GNPs supplementation inhibits the initiation of colon cancer due to enhancement of the antioxidant activity.

Key words: Zingiber officinale, 2,3,7,8-Tetracholorodibenzo-p-dioxin, antioxidant enzymes, apoptosis related genes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer initiation is the process in which several steps are involved in its activation to induce malignant cells. From these steps, alteration in the expression of several factors and proteins produce which involved in various biological actions such as regulation the cell cycle, differentiation, proliferation and metastasis[1,2].

It has been reported that some of Reactive Nitrogen Species (RNS) and Reactive Oxygen Species (ROS) such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitrosamines, peroxynitrite and nitrates are considered as not radical species[3,4]. However, chemicals of xenobiotic may exhibit their pathological impacts by ROS generation mechanism[5]. Thus, RNS and ROS stimulate the nitrative and oxidative stress in which they are major reasons for carcinogenesis initiation[6,7].

TCDD is considered as the most dangerous toxic dioxin and defined as environmental teratogen and mutagen[8]. It is promoting various toxic mechanisms in which the major hazardous effect caused by TCDD is the carcinogenic impact[9]. In this point of view, long exposure period of TCDD in rodents has been found to induce several tumor types such as thyroid, skin, liver and lung tumors[9,10].

The biological action of TCDD was reported to be in several ways. It has been reported that TCDD inhibit the immunity but it does not act directly as a complete carcinogen. Also, TCDD was not able to induce directly the DNA adducts. Moreover, no DNA damage was showed at the short time exposure of TCDD or from the reaction between its derivatives or metabolites[11]. On the other hand, it has been reported that TCDD is considered as a human carcinogenic agent by the IARC (International Agency for Research on Cancer) at the long exposure time intervals. Moreover, TCDD was reported to induce multisite tumors in animals. Its mechanism of action in cancer induction is that it affects the cells in humans and animals through a protein namely aryl hydrocarbon receptor (AhR) exists in many cells and tissues. The AhR protein is considered as transcription factor regulates the expression pathway of different set of genes[12].

On the other hand, there is a growing interest in discovering natural products having adverse effects against the environmental mutagens. It has been reported that several biological active compounds such as flavonoids which are the most important phytochemicals group have been recognized in plant materials. Therefore, feeding a regimen containing high percentage of plant foods can supply high levels of the phytochemicals and additionally anti-nutritive plant compounds that acquire health-protecting impacts. Furthermore, natural plant and products such as vegetables, herbs and fruits have a great interest from both the general public and the scientific community because their capability to suppress several diseases including cancers[13].

Ginger (*Zingiber officinale* Rosc.) is a medicinal plant belonging to sub-tropical and tropical regions. It has been found first in South-East Asia and then distributed to other many regions of the world. It is extensively used as a traditional medicine and spices either in the dried or fresh forms[14].

It has been found that ginger components exhibited anti-inflammatory activities during *in vitro* investigations[15]. Moreover, using of ginger components revealed other biological activities against several diseases and disorders such as migraine headaches, atherosclerosis, ulcers, high cholesterol, rheumatoid arthritis, impotence and depression[16]. Due to the high use of ginger in Asia, it has been found that Asian countries have a great resistance against many of cancer diseases related to breast, gastrointestinal, colon and prostate compared to other countries[17]. It is suggested that the constituents of the food in the Asian countries could play an important action in the cancer protection.

In fact, several *in vivo* and *in vitro* experiments revealed that phenolic compounds including flavonoids exist in medicinal plants, vegetables and fruits exhibited cancer chemo-preventive actions in animal models[18-20]. Interestingly, these compounds are supposed to inhibit the inflammatory, hyper-proliferative and transformative processes that they are capable not only to initiate carcinogenesis but also to enhance the later steps of tumorigenesis, specifically metastasis and angiogenesis. Therefore, ginger components are exerting high capability against mutagenicity and cancer due to several phenolic substances existing in its materials which possess strong anti-inflammatory and anti-oxidative[20].

The mechanism for arriving the natural products to the target tissues is playing an important role in the therapeutic regimen. Transforming the natural particles to nanoparticles is believed to give good therapeutic results. Moreover, nanoparticles of natural compounds provide a safe and competent carrier method for delivery and improved drug bioavailability within the tissues and cells [21]. Thus, an attempt has been conducted in the current study to investigate one of the recent methods of pharmaceutical interventions to originate nano-encapsulation of the ginger materials. Therefore, the main objective of the present study was to evaluate the preventive potential of GNPs against colon cancer induced by different doses of TCDD in male rats.

MATERIALS AND METHODS

Plant material and extract preparation: During June, 2015-March, 2016, ginger (*Zingiber officinale*) collected

from local market in Cairo, Egypt was dried by oven at 50°C. Dry plant material was grinded and methanol extract of ginger was collected according to Tasanarong *et al.*[22]. The extract was dried by freeze dry as water extract of *Zingiber officinale*. The samples have been preserved in the deep freezer (-20°C). Authentication of plant materials was identified by comparing against the specimens deposited National Research Center, Egypt, where herbarium vouchers have been kept.

Formation of Ginger Loaded Nanoparticles (GNPs): To prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of ginger extract solvent displacement technique of Samadder *et al.*[23] was deployed under optimal conditions.

Experimental animals: Ninety adult albino male rats (100-120 g, purchased from the Animal House Colony, National Research Center, Giza, Egypt) were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1% and metabolic energy, 0.012 MJ) and water *ad libitum*. After an acclimation period of 1 week, animals were divided into nine groups (10 rats/group) and housed in filter-top polycarbonate cages at temperature-controlled (23±1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of National Research Center, Egypt.

Experimental design: Animals were divided into following 9 groups. Each group consists of 10 rats. Group 1 -control: Animals treated orally with corn oil (C), Groups 2-5: Rats were injected by single oral dose/week with TCDD (0.2, 1, 5 and 20 μg kg^{-1} 5 mL^{-1} corn oil) for 4 weeks, Groups 6-9: Similar treatment to groups 2-5 plus 50 mg kg^{-1} b.wt.,/everyday of GNPs. The doses of TCDD used in this study were selected according to Takeda *et al.*[24], however, the ginger dose was selected according to Manju and Nalini[25].

Determination of antioxidant enzymes activities

Determination of SOD activity: Total SOD activity was assayed according to Li *et al.*[26] following the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT).

Determination of CAT activity: The CAT activity was assayed by the method of Netto *et al.*[27] using spectrophotometery. This method is based on the disappearance of H_2O_2 at 240 nm in a reaction medium containing 20 mM H_2O_2, 0.1% Triton X-100 and 10 mM potassium phosphate buffer pH 7.0. CAT activity is represented as absorption change in time unit (1 min)/per mg protein.

Determination of GPx and GST activities: GPx and GST activities measurements were carried out by a procedure according to Sakharov *et al.*[28]. The reaction mixture consisted of 8 mM H_2O_2, 40 mM guaiacol, 50 mM sodium acetate buffer, pH 5.5 and a suitable amount of the enzyme preparation. The change in absorbance at 470 nm due to guaiacol oxidation was followed at 30 sec intervals. One unit of glutathione peroxidase and glutathione-S-transferase activities was defined as the amount of enzyme which increases the optical density (O.D.) 1.0 min under standard assay conditions.

Apoptosis analysis

Staining of colon cells with acridine orange/ethidium bromide: Apoptotic changes in colon tissues were determined morphologically by fluorescent microscope after labelling with Acridine Orange/Ethidium Bromide (AO/EB) according to Czene *et al.*[29]. In addition, necrosis and necrotic index were measured according to Pitocco *et al.*[30]. The calculation of the indexes was performed using the following formula:

$$\text{Necrosis index (\%)} = \frac{\text{Necrotic cells in test-necrotic cells in control condition (\%)}}{\text{100 -dead cells in control condition (\%)}} \times 100$$

The apoptosis index was obtained by replacing the percentage of necrotic cells by the percentage of apoptotic cells.

Gene expression analysis

RNA Extraction: Total RNA was isolated from 100 μg of colon tissues of female rats by the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 μL molecular biology grade water. In order to remove any possible genomic DNA contamination, the total RNA samples were pre-treated using DNA-free™ DNase removal reagents kit (Ambion, Austin, TX, USA) following the manufacturer's protocol.

Reverse transcription: The complete Poly(A)+ RNA samples were reverse transcribed into cDNA in a total volume of 20 μL using 1 μL oligo (dT) primer. The composition of the reaction mixture, termed as Master Mix (MM), consisted of 50 mM $MgCl_2$, 10x Reverse Transcription (RT) buffer (50 mM KCl; 10 mM Tris-HCl; pH 8.3; Perkin-Elmer), 10 mM of each dNTP (Amersham, Brunswick, Germany) and 50 μM of oligo (dT) primer. The RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C and finished with denaturation step at 99°C for 5 min. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice

chamber until being used for DNA amplification through Polymerase Chain Reaction (PCR).

Quantitative real Time-PCR: The first strand cDNA from different samples was used as templates for RT-PCR with a pair of specific. The sequences of specific primer and product sizes are listed in Table 1. β-actin was used as a housekeeping gene for normalizing mRNA levels of the target genes.

PCR reactions were set up in 25 μL reaction mixtures containing 12.5 μL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), 0.5 μL 0.2 μM sense primers, 0.5 μL 0.2 μM antisense primer, 6.5 μL distilled water and 5 μL of cDNA template. The reaction program was allocated to 3 steps. First step was at 95.0°C for 3 min. Second step consisted of 40 cycles in which each cycle divided to 3 steps: (a) at 95.0°C for 15 sec; (b) at 55.0°C for 30 sec and (c) at 72.0°C for 30 sec. The third step consisted of 71 cycles which started at 60.0°C and then increased about 0.5°C every 10 sec up to 95.0°C. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers. Each experiment included a distilled water control.

The quantitative values of RT-PCR (qRT-PCR) of P53, Bcl2 and Bax were normalized on the bases ß-actin expression (Table 1). At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers.

Calculation of gene expression: The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method[21] as follows:

$$\Delta C_{T(test)} = C_{T(target,\ test)} - C_{T(reference,\ test)}$$

$$\Delta CT(_{calibrator}) = C_{T(target,\ calibrator)} - C_{T(reference,\ calibrator)}$$

$$\Delta\Delta CT = \Delta C_{T(Test)} - \Delta C_{T(calibrator)}$$

The relative expression was calculated by $2^{-\Delta\Delta CT}$.

Transmission electron microscopy: The particle size and shape were characterized using high resolution transmission electron microscopy (HR-TEM) (Tecnai model G2-F20, Hillsboro, Oregon, OR, USA) JEM 2100 LB_6 under operating voltage of 200 kV to investigate the micrograph of prepared PLGA encapsulation of *Zingiber officinale* extract under operating voltage of 200 kV for different samples (Fig. 1).

Statistical analysis: All data were analyzed using the General Linear Models (GLM) technique of Statistical Analysis System followed by Scheffé-test to assess significant differences between groups[33]. The values are uttered as Mean±SEM. All statements of significance were based on probability of $p<0.05$.

RESULTS

Effect of TCDD and GNPs on the antioxidant enzyme activities: The measurements of oxidative markers including SOD, CAT, GPx and GST activities are summarized in Table 2 and 3, respectively.

Table 2 and 3 showed suppression effect of GNPs on TCDD-induced alterations in the antioxidant enzymes SOD

Table 1: Primers and reaction parameters in RT-PCR

Target cDNA	Primer name	Primer sequence (5'–3')	References
β-Actin	F	GTG GGC CGC TCT AGG CAC CAA	Khalil and Booles[31]
	R	CTC TTT GAT GTC ACG CAC GAT TT	
p53	F	GCG GTA CCC CAG GTC GGC GAG AAT CC	Qin *et al.*[32]
	R	GGG CTC GAG TCT AGA CTT TTG AGA AGC	
Bcl-2	F	CTC AGT CAT CCA CAG GGC GA	Khalil and Booles[31]
	R	AGA GGG GCT ACG AGT GGG AT	
Bax	F	ACA AAG ATG GTC ACG GTC TGC C	Khalil and Booles[31]
	R	GGT TCA TCC AGG ATC GAG ACG G	

Table 2: Superoxide dismutase (SOD) and catalase (CAT) activities in male rats treated with TCDD alone or combined with GNPs

Treatments	SOD activity (U mg^{-1} protein)	CAT activity (U mg^{-1} protein min^{-1})
Control	52.6 ± 5.2^{a}	86.7 ± 6.4^{a}
TCDD-0.2	46.3 ± 4.8^{ab}	74.5 ± 4.2^{ab}
TCDD -1.0	41.4 ± 4.6^{b}	68.3 ± 5.2^{b}
TCDD-5.0	33.6 ± 3.8^{b}	56.4 ± 4.1^{bc}
TCDD-20	29.8 ± 3.6^{b}	52.8 ± 5.4^{c}
TCDD-0.2+GNPs	48.2 ± 4.2^{ab}	84.6 ± 6.3^{a}
TCDD-1.0+GNPs	45.7 ± 5.6^{ab}	82.2 ± 5.6^{a}
TCDD5.0+GNPs	41.1 ± 4.2^{b}	72.4 ± 4.6^{ab}
TCDD20+GNPs	38.2 ± 3.8^{b}	69.2 ± 4.2^{b}

Data are presented as Mean±SEM, ^{a,b,c}Mean values within tissue with unlike superscript letters were significantly different ($p<0.05$)

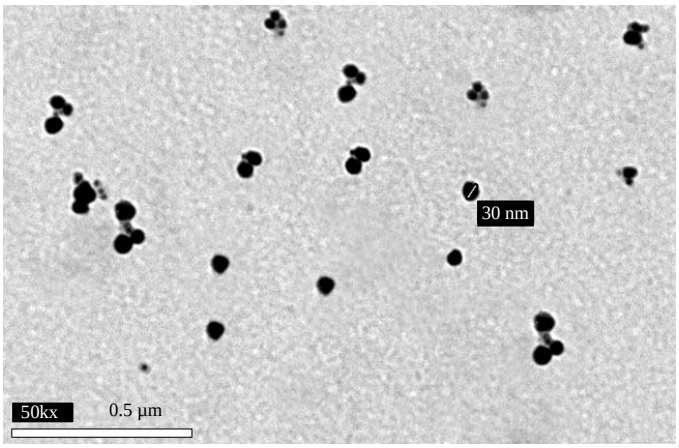

Fig. 1: Cross-sectional transmission electron microscopy image of the of poly-lactic-co-glycolic acid (PLGA) encapsulation of *Zingiber officinale* nanoparticles

Table 3: Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST) activities in male rats treated with TCDD alone or combined with GNPs

Treatments	GPx activity (U mg^{-1} tissues min^{-1})	GST activity (µmol mg^{-1} protein)
Control	6.6 ± 0.04^{a}	1.8 ± 0.01^{a}
TCDD-0.2	5.7 ± 0.03^{a}	1.3 ± 0.02^{ab}
TCDD-1.0	5.3 ± 0.06^{ab}	1.2 ± 0.02^{ab}
TCDD-5.0	4.1 ± 0.03^{b}	0.8 ± 0.03^{b}
TCDD-20	3.6 ± 0.04^{b}	0.5 ± 0.01^{b}
TCDD-0.2+GNPs	6.2 ± 0.03^{a}	1.6 ± 0.03^{a}
TCDD-1.0+GNPs	5.9 ± 0.05^{a}	1.4 ± 0.04^{ab}
TCDD-5.0+GNPs	5.6 ± 0.06^{ab}	1.2 ± 0.03^{ab}
TCDD-20+GNPs	4.8 ± 0.03^{ab}	0.9 ± 0.02^{b}

Data are presented as Mean±SEM, [a,b,c]Mean values within tissue with unlike superscript letters were significantly different ($p<0.05$)

and CAT as well as GPx and GST activities. In comparison to the control group, SOD, CAT, GPx and GST activity levels were relatively similar with the treatment of 0.2 and 1 µg kg^{-1} b.wt., of TCDD. However, the activity levels of SOD, CAT, GPx and GST decreased $p<0.05$ significantly in male rats treated with 5 and 20 µg kg b.wt., of TCDD compared with control group. On the other hand, supplementation of TCDD-treated rats with GNPs decreased $p<0.05$ significantly the alterations of SOD, CAT, GPx and GST activity levels compared those treated with TCDD alone (Table 2 and 3).

Effect of TCDD and GNPs on the induction of apoptosis: Figures 2 and 3 show the morphological changes of colon cells determined by fluorescence microscopy after double staining with AO/EB were examined to measure the type of cell death induced by GNPs in TCDD-rats. The results revealed that the AO /EB dyes used in this assay normally emit different shades of fluorescence and possess a different ability to penetrate cells. The AO penetrates into living cells, emitting green fluorescence after intercalation into DNA. However, EB emits orange to red fluorescence in the cells with an altered cell membrane. Figure 3 shows the necrotic/apoptotic rate in TCDD-rats treated with GNPs. The results revealed that the necrotic/apoptotic rate in healthy control rats and low doses of TCDD (0.2 and 1 µg kg^{-1} b.wt.) groups was relatively similar. However, the highest number of damaged cells (orange or red) showed in rats treated with 5 and 20 µg kg^{-1} b.wt., of TCDD. On the other hand, the necrotic/apoptotic rate decreased $p<0.05$ significantly in TCDD-rats treated with GNPs compared to those in rats treated with TCDD alone.

Effect of TCDD and GNPs on the expression of apoptotic related genes: The expression of p53, Bcl-2 and Bax in rats treated with different doses of TCDD and GNPs are summarized in Fig. 4-6, respectively. The results of the this study showed that expression of p53 and Bax genes in colon cancer induced by TCDD treatment was significantly up-regulated compared to those in control healthy rats (Fig. 4 and 6). The highest expression levels of p53 and Bax genes was showed in rats treated with 5 and 20 µg kg^{-1} b.wt., of TCDD compared with rats treated with 0.2 and 1 µg kg^{-1} b.wt., of TCDD. On the other hand, treatment of TCDD-rats with

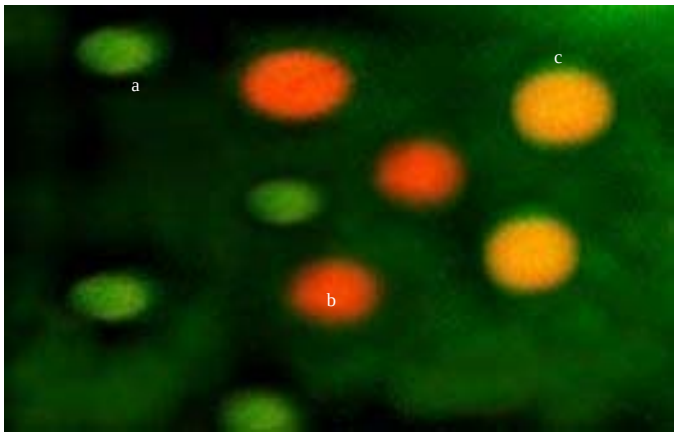

Fig. 2: Fluorescent microscope examinations demonstrating normal (a) Living cells and apoptotic cells (b) Apoptotic cells and (c) Necrotic cells as detected by Acridine Orange/Ethidium Bromide staining

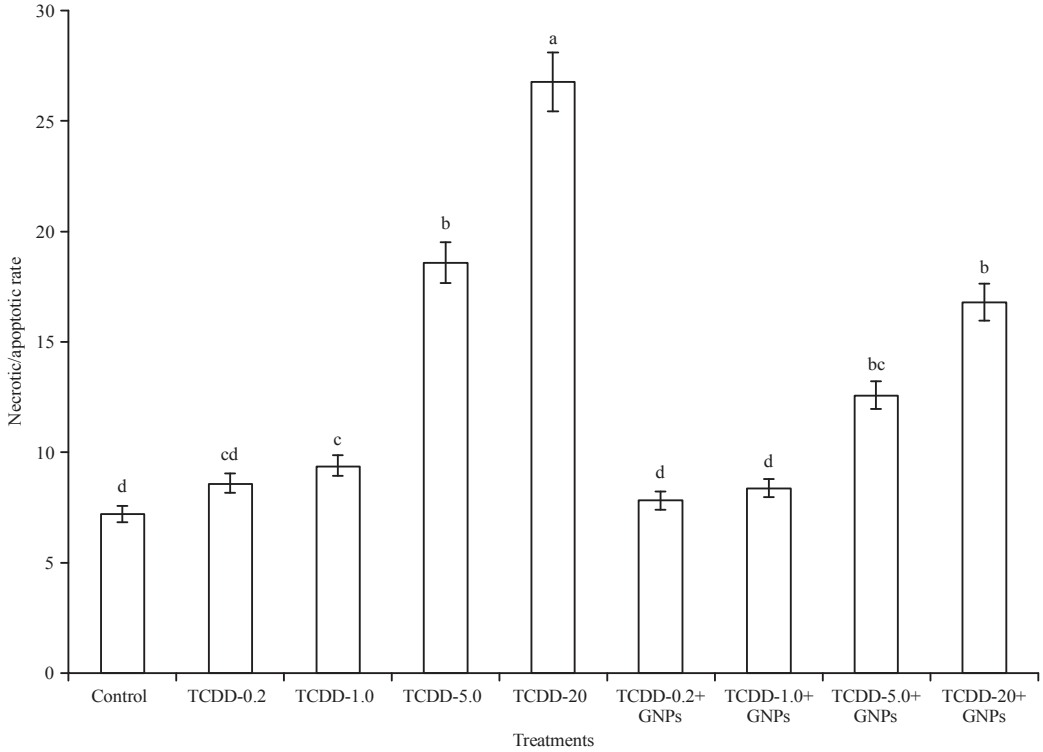

Fig. 3: Effect of TCDD and GNPs on necrotic/apoptotic rate in colon tissues of male rats, results are expressed as the Mean±SD. [a,b,c]Mean with different letters, within tissue, differ significantly ($p<0.05$)

GNPs decreased $p<0.05$ significantly the expression of p53 and Bax genes compared with rats treated with TCDD alone.

Regarding the third apoptotic related gene the results revealed that expression of Bcl2 gene in colon tissues of rats treated with TCDD was significantly $p<0.05$ down-regulated compared to those in control healthy rats (Fig. 5). The lowest expression levels of Bcl2 gene was showed in rats treated with 5 and 20 μg kg^{-1} b.wt., of TCDD compared with rats treated with 0.2 and 1 μg kg^{-1} b.wt., of TCDD. In contrast, treatment of TCDD-rats with GNPs increased $p<0.05$ significantly the expression of Bcl2 gene compared with rats treated with TCDD alone.

DISCUSSION

The present study showed that levels of the antioxidant enzymes activity of SOD and CAT as well as GPx and GST decreased $p<0.05$ significantly in male rats treated with high

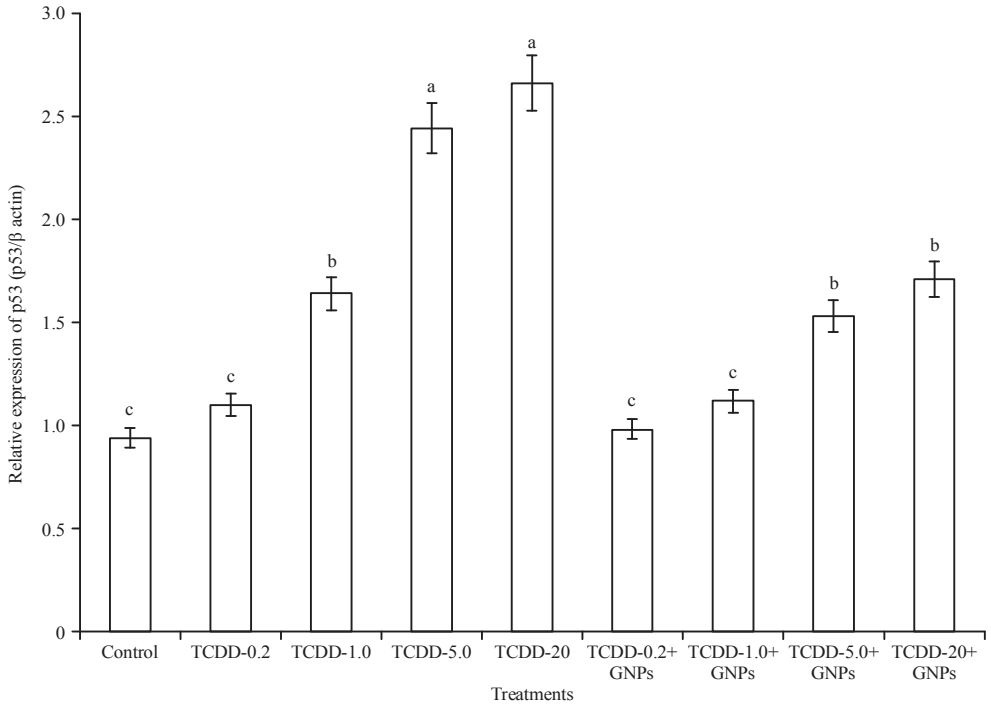

Fig. 4: Expression of p53 mRNA analyzed by quantitative Real Time-PCR in colon tissues of male rats treated with TCDD alone or combined with GNPs

[a,b,c]Mean with different letters, within tissue, differ significantly ($p<0.05$). Results are expressed as the Mean±SD

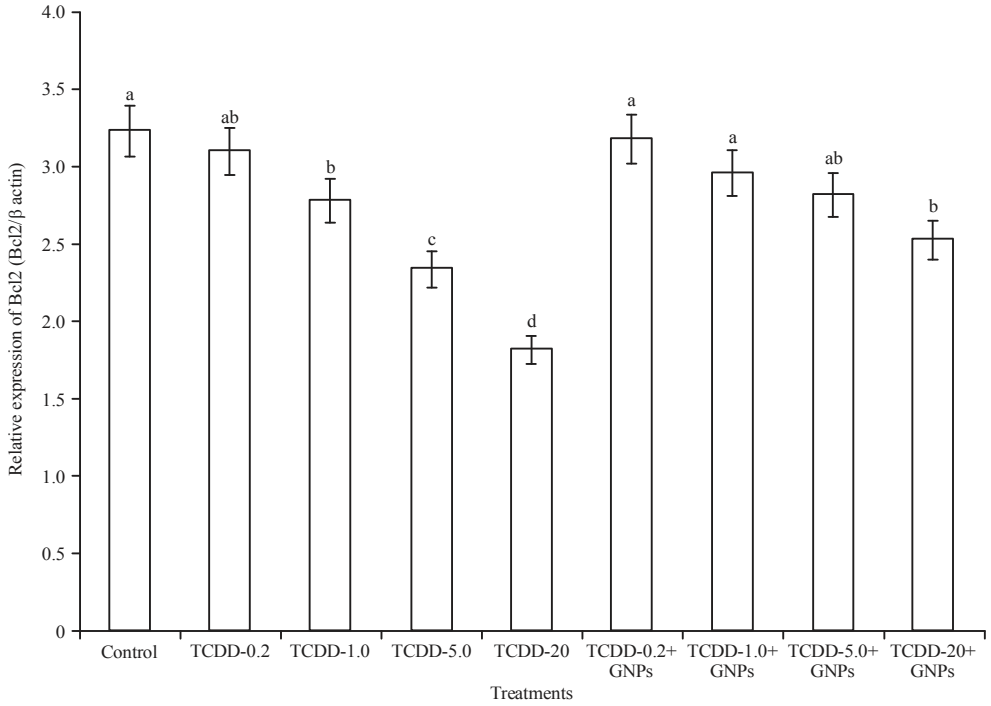

Fig. 5: Expression of Bcl2 mRNA analyzed by quantitative Real Time-PCR in colon tissues of male rats treated with TCDD alone or combined with GNPs

[a,b,c]Mean with different letters, within tissue, differ significantly ($p<0.05$). Results are expressed as the Mean±SD

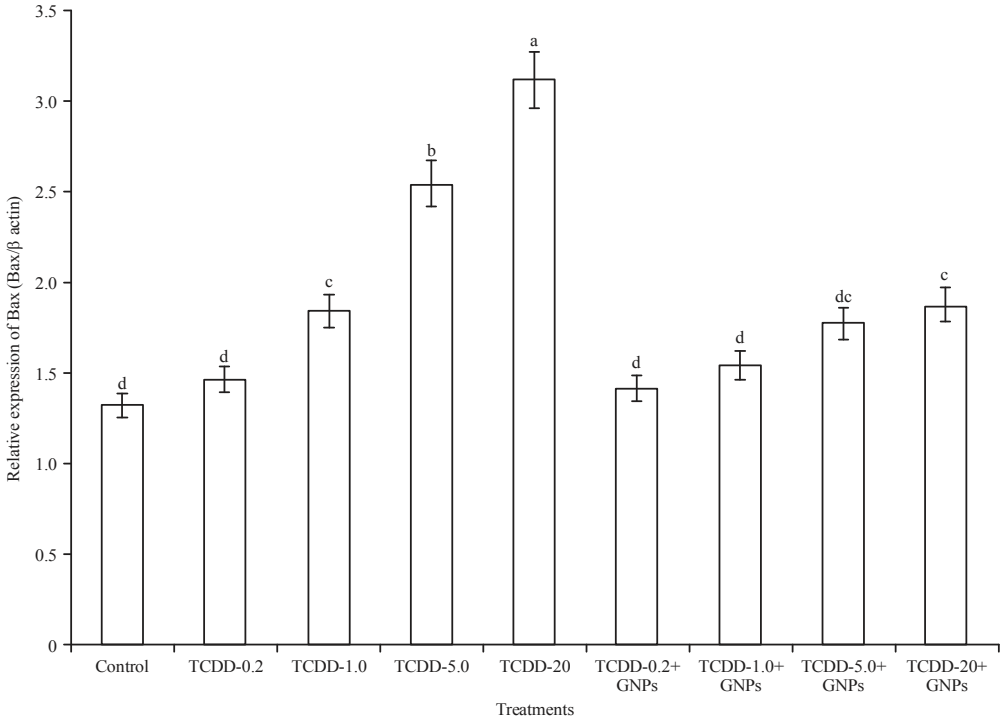

Fig. 6: Expression of Bax mRNA analyzed by quantitative Real Time-PCR in colon tissues of male rats treated with TCDD alone or combined with GNPs

[a,b,c,d]Mean with different letters, within tissue, differ significantly ($p<0.05$). Results are expressed as the Mean±SD

doses (5 and 20 μg kg^{-1} b.wt.) of TCDD compared with control group. In the same line of our results, Bulmusa *et al.*[34] found that exposure to TCDD decreased SOD, CAT and GPx activities as well as glutathione levels in liver, kidney, brain and heart tissues of male rats.

The alteration in the antioxidant enzyme activities due to exposure to TCDD was clarified by the study of MacDonald *et al.*[35]. They indicated that TCDD may induce cytochromes P450 (CYPs) enzymes to enhance ROS generation and therefore trigger lipid peroxidation, modified nitrogenous base formation and DNA strand breaks as well as decreases the levels of hepatic glutathione levels.

Moreover, Fromme *et al.*[36] indicated that TCDD enhanced blood pressure and oxidation in mice. In addition, TCDD has been found to cause impairment of the competence of the avian heart endoplasmic reticulum to appropriate $Ca^{2+}$and contractile defects[37]. Thus, change in the intracellular Ca^{2+} levels due to the oxidation effect of TCDD in male rats is the main reason in generation of high rate of ROS.

Therefore, increasing the levels of the peroxidation in rats treated with TCDD might be attributed to ROS mediating membrane damage which coincide with decrease levels of the antioxidants resulting in oxidative stress.

The present study revealed that TCDD elevated high levels of apoptosis in male rats. Expression of apoptotic related genes such as Bcl-2, Bax and p53 genes in TCDD-rats was significantly $p<0.05$ increased. Furthermore, the highest number of damaged cells (orange or red) showed in TCDD groups. In agreement with our findings, Longini *et al.*[38] and Chen *et al.*[39] suggested that the shift in the intracellular pro-oxidants stimulated by TCDD may induce cell damage/death due to direct damage in the cell membrane through lipid peroxidation[38] or due to apoptosis by transcription factors activation or by DNA damage 39. So, the observed structural changes including both forms of necrosis and apoptosis in male rats after TCDD treatment in the current study was related to oxidative stress.

The DNA damage generated by oxidation process in hepatic cells may be resulted from extremely induction of enzymes and proteins that regulated by transcriptional control of the aryl hydrocarbon receptor (AhR) activated by TCDD treatment[11].

AhR is an essential transcriptional regulator occurs during the pathophysiological and physiological processes such as

neurodegenerative diseases and cancer[40]. Besischlag *et al.*[41] reported that AhR and ARNT (AhR nuclear translocator, which defined as heterodimeric partner of AhR forming aryl hydrocarbon receptor complex) have been found to associate with the response to environmental contaminants. The AhR/ARNT complex has been found to modulate the transcription of different set of genes through the binding with Dioxin Response Elements (DRE). Moreover, it has been reported that TCDD is identified as one of the main effective exogenous AhR ligands. In addition, due to the fact that TCDD is basically not metabolized, thus, TCDD is considered as tumor promoter more than the fact it is a tumor initiator[42,43].

On the other hand, ginger products and its active ingredients including shagoals and gingerols are having the antioxidant capability and the protection against the free radicals such as ROS and H_2O_2[44,45]. We have found an increase in the antioxidant enzyme (SOD, CAT, GPx and GST) activities in male rats when ginger was supplemented with TCDD compared with TCDD alone. In this context, our results were in the same line with Reddy *et al.*[46], who recognized that ginger suppresses lipid peroxidation and inhibits ROS generation.

As ginger products increase the antoxidant capacity, it has been reported that glutathione (GSH) an effective non-protein thiol associated with glutathione peroxidase (GP_X) and glutathione transferase (GST) and they play an important role in defensive cells against teratohens and carcinogenic drugs [47]. Moreover, the antioxidant enzymes GST and GPx are found to be regulating enzymes in the transformation processes involved in inhibiting the toxicity of several carcinogenic and free radicals promoting agents. The regulation process of the GST and GPx results from their binding with the toxic agents and promoting the function of the GSH to protect the cells from the toxicity triggered by the mutagenic agents. The mechanism of action of the antioxidant enzymes in protection the cells from any carcinogenic agents is suggesting as follows: First, GSH is acting a neutralization process for the radicals species that are essential to antitumor competence. Second, GST is promoting a breakdown for the reaction between GSH and any hydrophobic components. Third, GPx is acting a catalyzation and reconversion processes for gutathione and other biological components such as NADH and GSSG[47]. Furthermore, other antioxidant enzymes such as CAT and SO are extensively distributed in all tissues and cells and existed in high amounts especially in the erythrocytes[48]. Therefore, present these enzymes in the blood red cells protect it against several free radicals such as O^{2-} and H_2O_2 which regulate the lipid peroxidation[49,50].

Natural products are considered as a main sourse of the antioxidants which having adverse effects against the environmental mutagens. It has been reported that ginger compounds enhance the activity of the antioxidant enzymes which are responsible for the protective mechanism of TCDD. In this regard, we have observed that ginger products decreased the oxidative stress inducing DNA damage and gene expression alteration by increase the levels of the antioxidant enzymes. In the same line, numerous studies have also shown that ginger products stimulated the chemopreventive actions against several mutagenic and carcinogenic agents induced several types of tumors[46,51].

The cascades of apoptosis are strongly regulated by a several factors; from these factors, Bcl2 protein family which plays central actions in the apoptotic events. The members of this family are Bcl-2 as anti-apoptotic members and Bax as pro-apoptotic members[52]. Therefore, RT-PCR tool was conducted in this study in order to determine the apoptotic action of ginger extract through assessment the expression levels of Bcl-2 and p53 genes (as apoptotic inhibitor) and Bax gene (apoptotic promoter) in male rats treated with TCDD.

The p53 gene is defined as tumor suppressor gene that performs its role as protector of the genome. The p53 protein acts as regulating downstream gene and transcription factor implicated in apoptosis. Therefore, absence the p53 role resulted in reduce cell cycle restraint, deteriorate apoptosis and genomic instability. Therefore, occurrence of p53 mutations in the genome is responsible for onset of several types of malignancy. Moreover, mutation in P53 gene sequence is the most frequent mutation cancer in human[53]. The current results revealed that GNPs decreased the expression of Bcl-2, Bax and p53 genes in GNPs-treated rats. Additionally, the necrotic/apoptotic rate was low in GNPs groups compared with TCDD treatments. In agreement with our observations, Elkady *et al.*[54] indicated that suppression of gene expression changes of apoptotic genes (Bax and Bcl-2) was coincided with inhibition of the proliferation of breast cancer cell lines by ginger treatment. These findings indicate that the ginger extract modulating Bax up-regulation and Bcl-2 down-regulation might tip the equilibrium on the way to occurrence of the apoptosis.

In the same way, it is assumed that Bcl-2 family proteins play a significant regulatory action in the apoptosis process and, therefore, it is believed to be main target molecules for anticancer therapy[55]. From the members of Bcl-2 family are the Bcl-2 and Bax proteins. The ratio of these proteins has been documented as a main indicator of the apoptotic process regulation, because Bax/Bcl-2 proteins ratio increases throughout the apoptosis[56]. The results of the current study

revealed that GNPs obviously decreased expression levels of Bcl-2 mRNA and protein and increased those for Bax. The modulation in the expression ratio of Bax to Bcl-2 attributed by ginger nanoparticles could be the main key in induction of the apoptosis in male rats treated with TCDD. While, the action mechanism of ginger nanoparticles regulated the expression of the genes encoding Bcl-2 and Bax proteins needs to be more clarified in the near future.

CONCLUSION

This study demonstrated that supplementation of *Zingiber officinale* nanoparticles (GNPs) improved the antioxidant activity, suppressed the expression of cancer related genes and decreased the necrotic/apoptotic rates in TCDD-treated rats. Thus, these results suggested that GNPs could be used as protective agents against colon cancer initiation induced by the procarcinogen TCDD in male rats.

SIGNIFICANCE STATEMENTS

This study discovers the possible protective effect of Ginger (*Zingiber officinale*) nanoparticles that can be beneficial for suppression of colon cancer induced by TCDD in male rats. This study will help the pharmaceutical researchers to uncover safer drugs which might strengthen the immune system for cancer patients that many researchers were not able to explore. Thus, a new theory on these nanoparticles of the *Zingiber officinale* as a novel compounds, and possibly other structure of the medicinal plant, may be arrived at novel structure of drug discovery.

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