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Turmeric extract decreased frequency of polychromatic erythrocytes micronuclei induced by iodine-131

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► ***Original article***

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ABSTRACT

Background: Ionizing radiation irradiated from iodine-131 can induce DNA damage and cell death. The cellular DNA damage is the cause of mutation and cancer. The micronucleus assay in polychromatic erythrocytes was applied to assess the radio-protective effect of *Turmeric* extract on genotoxic potential of iodine therapy. ***Materials and Methods***: Thirty six male albino rats were randomly divided in six groups. A single dose (200 or 500 mg/kg) of *Turmeric* extract was injected to the rats 30 min before iodine therapy. Iodine-131 (5.55 MBq) was administrated intra peritoneal to the experimental animals. The percentage of micronuclei in PCE, NCE and ratio of PCE / (PCE + NCE) was determined 48 h after iodine injection for each experimental group to assess iodine-131 radiation effects with or without *Turmeric* extract. ***Results***: Iodine therapy showed a significant increase in the number of micronucleus formation. The animals treated with different doses of *Turmeric* extract + iodine showed a significant reduction in the frequency of micronucleus compared to the animals treated with iodine-131 alone. Both doses of *Turmeric* extract had the same effect when injected 30 min prior to iodine therapy. ***Conclusion:*** Our results indicate protective effect of *Turmeric* extract against genetic damages induced by iodine-131 administration.

Keywords: *Turmeric, iodine-131, micronucleus.*

INTRODUCTION

Iodine-131 (^{131}I) was successfully used in the treatment of hyperthyroidism and differentiated thyroid diseases [1]. ^{131}I also cause side effects in many organs, glands and increased risk of second malignancy [2]. ^{131}I emits Beta and Gamma ionizing radiations thus cause biologic effects [3,4]. Radiation of ^{131}I generates reactive oxygen species (ROS) in the cells. These free radicals can damage macromolecules such as DNA. The cellular DNA damage is the cause of mutation and cancer [5].

Development of a suitable radio-protector with minimum toxicity that could be used in clinical conditions, is an obligation [6], consequently recent studies focused on natural radio-protectors, regarding to less toxicity and side effects. There are many plant derived natural antioxidants that interfere with free radicals before they can damage the body. Antioxidants work in several ways, either by reducing the energy of the free radicals, stopping the free radicals from forming in the first place, or interrupting an oxidizing chain reaction and, thus, minimizing the damage this causes [7].

Curcuma longa (family Zingiberaceae), known as Haldi in India and *Turmeric* in English, has demonstrated a wide spectrum of therapeutic effects such as anti inflammatory, antioxidant, anti mutagenic and antitumor [8].

Curcumin, a yellow colored phenolic pigment, is the most important fraction which is responsible for the biological activities of

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turmeric [9]. No acute toxicity in mice was observed on administration of *Turmeric* powder with dose as high as 10g/kgbw [10]. Protective action for normal cells and sensitizing effect on malignant cells would make it an ideal adjuvant agent for patients undergoing radiotherapy for cancer treatment [11]. Therefore, *in-vivo* radio-protective activity of *Turmeric* extract was investigated by using ^{131}I as a damaging agent. The aims of the present study, carried out in rats receiving therapeutic dose of ^{131}I, were (1) to evaluate the micronuclei appearance in bone marrow cells after ^{131}I therapy. (2) To ascertain the possibility for appearance of micronuclei in the non-irradiated rats were treated with *Turmeric* extract, and (3) to find out any reduction in the frequency of micronuclei in irradiated rats were pre-treated with a single dose of *Turmeric* extract.

MATERIALS AND METHODS

Animals

Thirty six male albino rats (≥ 250 gr) were obtained from the breeding colony of Semnan University of Medical Sciences, Semnan, Iran. They were housed six per cage randomly, maintained at constant temperature on a standard 12:12 h light/dark cycle, standard mouse pellets and water ad libitum. Rats were injected intra peritoneal for all experiments. All procedures were conducted in agreement with the National Institutes of Health Guide for care and use of laboratory animals. Six rats were used for each treatment group.

Chemicals and treatment

The dried rhizome of *Turmeric* was purchased from local market and were identified, classified and extracted, in the Applied Scientific educational center of Jihad-e Agriculture of Semnan. The voucher specimen (No. 93-11) was preserved and deposited in herbal library of research center of medicinal plants (Semnan University of medical sciences). For extraction, 200 gr of dried rhizome were grinded and then extracted with 350 mL 80% methanol and 650 ml water for 24 hrs in a continuous extraction by soxhlet apparatus. Extracted *Turmeric* used in a single dose (200 and 500 mg/kg) [12] intraperitoneal injection by 26-gauge syringe 30 min before iodine therapy tests. The control animals received the same volume of normal saline. Six rats were used for each treatment group.

Dosage of iodine-131 was extrapolated from human dose (40 mCi) that could be used for iodine therapy in clinical trial. According to rats weight, nearby 5.5 MBq (150 μCi) ^{131}I was injected with an insulin syringe intra peritoneal to the experimental animals.

Micronucleus assay

The animals were euthanized by cervical dislocation 48 h after iodine injection [13]. The bone marrow from both femurs was flushed in the form of a fine suspension into a centrifuge tube containing fetal Bovine serum (FBS) (Gibco). The cells were dispersed by gentle pipetting and collected by centrifuge at 1000 rpm for 8 min at 4°C. The cell pellet was resuspended in a drop of FBS and bone marrow smears were prepared. The slides were coded to avoid observer bias. After 24 h air-drying, the smears were stained with May-Grunwald/ Giemsa (Sigma). With this method polychromatic erythrocytes (PCEs) stain reddish-blue and normochromatic erythrocytes (NCEs) stain orange, while nuclear material is dark purple. For each animal, a total of 1000 PCEs were scored from prepared slides to determine the percentage of micronucleated polychromatic erythrocytes (MnPCEs), micronucleated normochromatic erythrocytes (MnNCEs) and ratio of PCE to (PCE + NCE). The ratio of PCE to (PCE + NCE) was determined for each experimental group to assess iodine radiation effects with or without *Turmeric* extract on chromosome aberrations [14]. To diminish the bias of observer, some of slides were randomly scored by another witness.

Statistical analysis

Micronucleus data were analyzed using Student's *t-test*. Statistical analysis was performed using SPSS statistical software (16.0, USA); P values of <0.05 were considered to indicate statistical significance. All results were expressed as mean ± SD for six animals in each group.

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RESULTS

Microscopic examination of PCEs showed that there were several MN formations in the control and *Turmeric* extract (TE) treated groups. The MN frequency of PCEs belonging to TE treated groups was fairly similar to control group. Iodine therapy showed a significant increase ($P<0.0001$) in the number of MN formation (figure. 1). The animals treated with different doses of TE + iodine showed a significant reduction ($P<0.05$) in the frequency of MN compared to the animals treated with ^{131}I alone. Both doses of *Turmeric* extract (250 and 500 mg/kg) had same effect ($P<0.05$), when injected 30 min prior to iodine therapy but, The total MnPCEs values were 9 and 36 fold less in the 250 and 500 mg/kg TE groups after being exposed to ^{131}I, respectively, than those in the respective ^{131}I alone. Pre-treatment of irradiated groups with TE caused significantly less damage and resulted in a much lower frequency of MnNCEs which reached about 3 and 7 fold less in the 250 and 500 mg/kg TE + iodine groups, respectively, than those in ^{131}I alone (figure. 2). With a further increase in the *Turmeric* dose to 500 mg/kg, there was no increase effect of TE on the frequency of MnNCEs induced by iodine-irradiation ($P>0.05$).

As shown in figure. 3, the ratio of PCE/PCE+NCE reduced significantly (p0.<001) after iodine therapy. Determination of ratio of PCE/PCE+NCE in the irradiated rats showed a cytotoxic effect of radiation on bone marrow proliferation. Treatment of rats with *Turmeric* extract blocked the radiation-induced decline in the PCE/PCE+NCE ratio and this increase in the PCE/PCE+NCE ratio in the TE + iodine groups was higher than that of the irradiated-alone group (p0.<05). Both doses were effective in significantly reducing (p0.<001) the frequency of PCE/PCE+NCE induced by ^{131}I irradiation.

DISCUSSION

Micronuclei (MN) are the result of chromosome acentric fragments (clastogenic effect) or whole chromosomes that, through incomplete migration, have been excluded from the main core (aneugenic effect). It has been shown that the micronuclei frequency assay can be used as a valuable endpoint and is a sensitive method for assessment of genetic damage by toxic agents [15, 16]. ^{131}I acts as a genotoxic agent on living cells and can increase the DNA damages. We observed that ^{131}I has genotoxic effects in rat polychromatic erythrocytes at a dose 5.55 MBq (150 μCi) and the number of micronuclei increases significantly after iodine therapy.

In this study, we showed, *Turmeric* extract (Curcuma longa) protects rat's bone marrow polychromatic erythrocytes against genotoxicity induced by iodine internal irradiation. Natural compounds, for instance flavonoids, may play a role in scavenging free radicals, such as hydroxyl, that is the result of gamma rays in cells. Ionizing radiation produce free radical and this radicals damage DNA and induces genotoxic effects and death in the cells [5]. When an organism is exposed to ionizing radiation, many systems are affected with maximum effects over rapidly dividing cells like hematopoietic stem cells and mucosal epithelium and morbidity or mortality depends upon initial damage at cellular level that depends upon presence of oxygen, sulfhydril group and cellular protective enzymes like superoxide dismutase, catalase etc. [17].

However preservation at cellular level would depend primarily on prevention of genotoxicity at cellular level and this would imply that presence of cellular protective agents or a change in cellular milieu interior in favor of cell against radiation assault must be achieved before the exposure; so pretreatment design was preferred [11]. In our study, Pretreatment with *Turmeric* extract had protection effect on irradiation hazardous. *Turmeric* extract have strong antioxidant activity, even at low dosage, and in different forms, like spice, traditional preparation or herbal medicine, can provide a significant protection against oxidative damage [18]. Lethal dose of *Turmeric* extract is more than 1000 mg/kg [19], and in our experiment, both doses of *Turmeric* extract (250 and 500 mg/kg) had same effect ($P<0.05$). The percentage of

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PCE/PCE+NCE ratio declined in iodine treatment rat, since this ratio gives an index of cell division, *Turmeric* extract protected rats against radiation induced decline in cell proliferation, as evidenced by the increased of PCE/PCE+NCE ratio; and it is possible that *Turmeric* extract protects bone marrow cells with its antioxidant activity. Protective action for normal cells and sensitizing effect on malignant cells would make it an ideal adjuvant agent for patients undergoing radiotherapy for cancer treatment [11]. So far, the potential of *Turmeric* extract in radio-iodine therapy has not been demonstrated in patients. Subsequently, to find more effective and ideal dose we need more research.

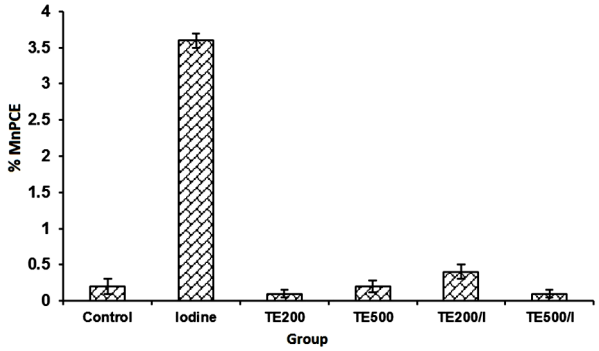


Figure 1. Effect of different dose of Turmeric extract on the frequency of MnPCE in the bone marrow of rat. (TE 200, Turmeric extract 200 mg/Kg; TE 500, Turmeric extract 500 mg/Kg; TE200/I, Turmeric extract 200 mg/Kg + Iodine 5.55 MBq; TE500/I, Turmeric extract 500 mg/Kg + Iodine 5.55 MBq). (* P<0.05).

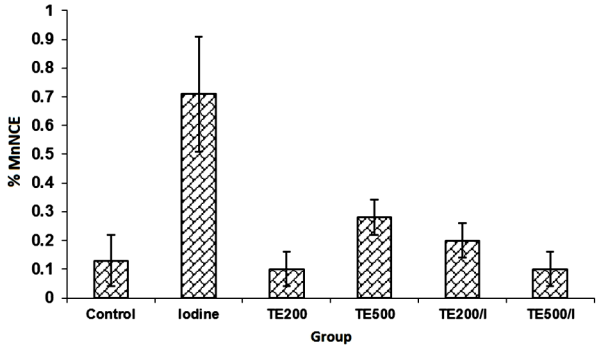


Figure 2. Effect of different dose of Turmeric extract on the frequency of MnNCE in the bone marrow of rat. (TE 200, Turmeric extract 200 mg/Kg; TE 500, Turmeric extract 500 mg/Kg; TE200/I, Turmeric extract 200 mg/Kg + Iodine 5.55 MBq; TE500/I, Turmeric extract 500 mg/Kg + Iodine 5.55 MBq). (* P<0.05).

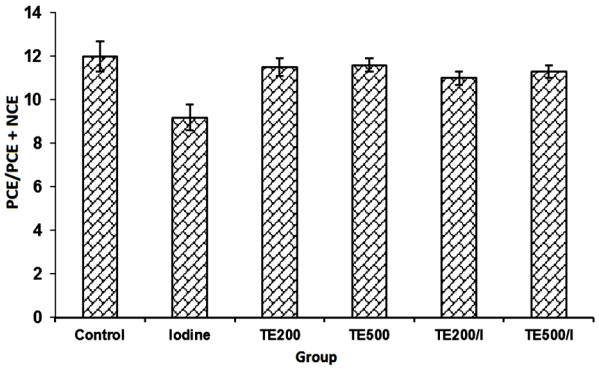


Figure 3. Effects of two doses of Turmeric extract on the radiation induced PCE/PCE+NCE ratio in the bone marrow of rat. (TE 200, Turmeric extract 200 mg/Kg; TE 500, Turmeric extract 500 mg/Kg; TE200/I, Turmeric extract 200 mg/Kg + Iodine 5.55 MBq; TE500/I, Turmeric extract 500 mg/Kg + Iodine 5.55 MBq). (* P<0.05).

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CONCLUSION

Our results demonstrate that iodine therapy had a significant increase in the frequency of MN formation in PCEs and NCEs, while *Turmeric* extract gives pronounce protection to rat bone marrow against the clastogenic effects of iodine therapy. The possibility of reducing the genotoxic activity of radionuclide therapy by *Turmeric* extracts to be explored.

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Conflicts of interest: *none to declare.*

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