Protective effect of Aqueous Extract of Ginger against Mitronidazole Induced Chromosomal aberration in bone marrow cells and sperm abnormalities in Albino Rats

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Abstract

Background: A wide variety of phenolic substances derived from spice possess potent antimutagenic and anticarcinogenic activities. Some of these phenolic substances are present in ginger, possessing strong anti-inflammatory and anti-oxidative properties as well as exert substantial anti-carcinogenic and anti-mutagenic activities.

Objective: The aim of the present study to exam the protective effect of ginger extract on Metronidazole (MT) induce bone marrow chromosomal aberrations and sperm abnormalities, cell inoculated in male albino rats.

Patients and Methods: The present experiment included four groups of male albino rats. The experimental group was administered five consecutive oral dose of metronidazole (250mg/kg/day). The subsequent experimental group was administered ginger extract (150mg/kg/day) for 9 days and on the 10th day Metronidazole (250mg/kg/day) was administered for five consecutive days. For each experimental group a parallel control group was maintained, one group administered 1ml of D.W. for 14 days the seconded group administered (150mg/kg/day) of ginger extract for 9 days . Twenty two hours after the administration of the last dose, rats were sacrificed for both chromosomal aberration and sperm abnormalities.

Results: In animals treated with only mitronidazole there was a significant increases of chromosomal aberration and sperm abnormalities. mitronidazole, beside other chromosomal aberrations such as polyploidy, anuploidy were also observed. In comparison to normal group animals were pre-treated with plant extract and subsequently treated with mitronidazole and an overall decline in the mutation rate was also discernible clearly indicating the protective effect of plant extract.

Conclusion: the results of this study indicate that metronidazole has the ability to induce chromosome aberrations including (centromeric breake, centromeric gap, chromatid break, centromeric fusion, polyploidy, aneuploidy and chromatid gap) in bone marrow cells and sperm abnormalities in male albino rats. Ability of aqueous extract of ginger to reduce both chromosomal aberrations and sperm abnormalities of mutated rats

Key words: : Mitronidazole; plant extract; Chromosome aberration; sperm abnormalities, Albino Rats.

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Introduction

Metronidazole is a synthetic antibacterial and antiprotozoal agent that belongs to the nitroimidazole class [1] .It is recently being used in man and animals in the treatment of trichomoniasis, giardiasis, amoebiasis and obligate anaerobic bacteria [2]. It is rapidly and completely absorbed from the gastrointestinal tract [3] and widely distributed in most tissues and body fluids [4]. Administration high doses of metronidazole caused harmful effects on some organs and affects males' fertility in rats [5][6]. Reported the direct hazardous effects of MT (Metronidazol) on the germ and Leydig cells after penetration into the blood-testis, MT administration (200 or 400 mg/kg), for 8 weeks, caused a harmful effect on the testes of male rats [7]. The mode of action of metronidazole has four successive steps [8]. Eentry into a susceptible organism, reductive activation, toxic effect of reduced intermediates by binding to DNA, causing loss of helical structure, strand breakage, and impairment of DNA function, and finaly release of inactive end products. The reduction of the nitro group by low redox potential electron transport proteins causes the molecule to act as a preferential electron acceptor. Similar to the microorganisms, the genotoxic potential is thought to be due to reduction of the nitro group to radicals and other reactive metabolites. They are considered as the actual mediators of metronidazole biological activity and cause biochemical lesions that which are responsible for its cytotoxic and mutagenic activity. The prerequisite for this is nitroreductases which are capable of reducing the nitro group in animal organs and tissues [9]. Metronidazole induced a reversible bone marrow depression [10][11]

Observed an increase in the mutagenicity of serum in female mice 8 hr after administration of metronidazole. It has also been found that metronidazole binds and interacts with both bacterial and mammalian DNA under anaerobic conditions. Furthermore, it has been observed that metronidazole induces DNA single-strand breaks in the lymphocyte of the patients on standard doses of the drug [12.[

Zingiber officinale commonly called ginger to a tropical and sub-tropical belongs to family Zingiberaceae, and originated in South-East Asia and was introduced to many parts of the globe, has been cultivated for thousands of years as a spice and for medicinal purposes. [13].

The ginger plant has a perennial, tuberous root or rhizome; the stems are erect, oblique, round, annual, and invested by the smooth sheaths of the leaves, 2 or 3 feet in height. Ginger rhizome is typically consumed as a fresh paste, dried powder, slices preserved in syrup, candy (crystallized ginger) or for flavoring tea in many countries, especially in India and China. The underground stem or rhizome of this plant has been used as a medicine in Asian, Indian, and Arabic herbal traditions since ancient times [14] . The important active components of the ginger rhizome are thought to be volatile oils and pungent principles like phenolic compounds such as gingerols [6-gingerol], shogaols, zingerone, and gingiberols. Ginger extracts showed different pharmacological effects such as anti-platelet, anti-oxidant, anti-tumour,anti rhinoviral,antihepatotoxicity and anti-arthritic effect [15). The antigenotoxic action of ginger has been found as one of the possible mechanism of oxygen free radical scavenging followed by decreased production of reactive oxygen species. [16,

17] . Ginger was also found to possess a protective against DNA damage induced by H2O2 and enhanced sperm healthy parameters in rats [18, 19.[

The aim of this study is to examine the protective effect of aqueous extracts of ginger using the analysis of chromosomal aberrations in bone marrow cells and sperm abnormalities in mutated rats.

Patients and Methods

Experimental design

The study was conducted on twenty adult male rats. A group of 5 rats per experiment were taken and treated with mitronidazole and aqueous extract of ginger. The doses were prepared daily with distilled water and were administered by gastric gavages method. The dose protocol was as follows: Group-I Control group (C) were treated with only distilled water for 14 day Group-II (T1) Experimental batch were administered MT 250mg/kg/ for five consecutive days). Group-III (T2) was treated with only ginger extract (150mg/kg/day) for 9 days. Group-IV. (T3) Experimental batch were pre-treated with ginger extract 150mg/kg/day for 9 days and on the 10th day five consecutive oral doses of MT 250mg kg/day was administered.

Animals

Albino rats 8 weeks old and weighing (225-250 g) were provided. The animals were housed in standard conditions of temperature (25±2 0C) and 12 hr light-dark cycle in the animal house of Department of Biology/Soran university, Rats were fed with standard diets (rodent diet), tap water *ad libitum*.

Preparation of ginger aqueous extract

Ginger rhizomes were purchased from local market. The aqueous extracts of Zingiber officinale were prepared according to the method of (20, 21). One kg of fresh Zinger rhizome was washed, chopped, dried, powdered and then soaked in 2 lit of water for 3hrs then heated at 60- 65C for 30 minutes, the extract was collected and the processes were repeated three to four times with the residual. The collected extract was pooled and passed through fine cloth. The supernatant was concentrated by rotary evaporator and the temperature adjusted to 55˚C, then dried at room temperature. The dried aqueous extract has been dissolved in distilled water with one concentration (150mg/kg) and stored in refrigerator; then the solution of aqueous extract was given to animals.

Drugs

A commercially available formulation of metronidazole (FlagylA) tablet 250 mg was used from Glaxo Company (India).

Cytogenetic Methods

Chromosomal aberrations (CA)

The method of [22] was adopted for the chromosomal aberration. Twenty-two hours after administration of last dose, all treated groups were injected intraperitoneally with colchicine solution (newly prepared). The injected rat left for (4-6 hr.) to arrest the divided cells in metaphase, quickly after sacrificing the rats, the femoral bone marrow was flushed in to tube containing 6 ml of hypotonic KCl (0.075M). The marrow suspension was incubated at 37ºC for 15-20 min and centrifuged at 1000 rpm for 10 min. The pellet was mixed with the methanol: acetic acid fixative (3:1). Suspension was allowed to stand for 30 min and then centrifuged. The pellet was mixed thoroughly with fresh fixative and 2-3 drops of the suspension was dropped on a clean glass slides. The slides were flame-dried and stained with 10% Giemsa at pH 6.8 for 15-20 min. The slides were screened for chromosome abnormalities. One hundred well-spread metaphase plates per animal were scored for structural chromosomal aberrations and recorded. Concurrently, total chromosomal abnormalities (excluding gaps), number of abnormal metaphase plates

per one hundred metaphase plates was counted per rat, were examined using research microscope with oil immersion lens.

Sperm abnormalities test

The sperm suspension was obtained from animals by cutting the caudal epididymis of a testis in few drops of mammalian saline. The sperm suspension was spread on clean glass slides. Sperm smears were dried in air. The sperms were stained with haematoxylin and eosin. 100 sperms were examined for each animal directly under microscope to detect the morphological abnormalities in head region.

Statistical analysis

The data were expressed as mean ± SEM, statistical package for the Social Science (SPSS) program was used for analyzing chromosomal aberrations and sperm abnormalities and analysis of variance One Way ANOVA followed by Duncann's- Post Hoc Results with $p < 0.05$ were considered statistically significant.

Results

In the present experiment, an effort has been made to assess the genotoxicity induced by MT as well as to monitor the protection accorded by aqueous extract of ginger against MT toxicity.

The results from the cytological examination of bone marrow cells at metaphase stage are illustrated in table 1. It was observed that significant differences between all groups. Metronidazole significantly increased chromosomal aberration in rat bone marrow as compared to the control group. The highest value of aberration was chromatid break in C, T1and T3 (4.200±0.200, 29.000±1.702 and 3.800 ±0374) respectively, while lowest value of aberrations was polyploidy in all group (0.000±0.000, 3.400±0 .748, 1.200±0 .200 and 1.200±0.200) respectively. No significant differences were observed in the T2 group in all type of aberration with compared to the control group except in centromeric gap. In animals pretreated with plant extract, and subsequently administered MT the number of chromosomal aberrations were significantly ($p<0.05$) decrease compared to the T1 group. In all type of aberration was equivalent to the control group the greatest reduction was seen in the chromatid break. No significant difference was observed between control rats and rats fed with 150mg/kg ginger extract alone with respect to chromosomal aberrations. Both structural and numerical chromosomal aberrations were recorded. Figure 1(A-E) shows more than one type of structural chromosomal aberrations, but the arrow indicates centromeric breake, chromatid break, centromeric fusion respectively. The numerical aberrations appeared as polyploidy cells and an aneuploidy.

Table (1): Protective effects of aqueous extract of ginger on mitronidazol induced genotoxicity as manifested by chromosomal abnormalities in bone marrow cells of albino male rats.

Animal groups	chromosomal Aberrations						
	hromatid gap	Chromatid break.*	Centromeric break.*	Centromeric gap.*	polyploidy*	Aneuploidy.*	Centromeric fusion*
C	1.800±0.374a	4.200±0.200a	2.200±0.200 a	1.200±0.200 a	0.000±0.000 a	1.200±0.200 a	1.200±0.200 a
T1	6.800±0 .860b	29.000±1.702b	10.400±1.720b	6.400±1.029 b	3.400±0 .748b	24.200±2.154b	16.400±1.363b
T2	2.000±0.3162a	2.600±0.400a	2.800±0.444a	5.800±0.734b	1.200±0 .200a	1.800±0.200a	1.800± 0.374a
T3	2.200±0.200a	3.800 ±037a	3.200±0.200a	2.400±0.871a	1.200±0.200a	3.400±0.600a	1.400± 0..244a

Values are expressed as mean ± standard errors. Values with different letters at the same column are significantly different at P<0.05 (anova) with Duncan's post Hoc.C = control, T1 = 250 mg / kg bwt metronidazole-treated, T 2= 150 mg / kg bwt plant extract-treated, T3=pretreated animal with plant extract for 9 days+ MTZ 250mg/Kg/animal for 5 consecutive days

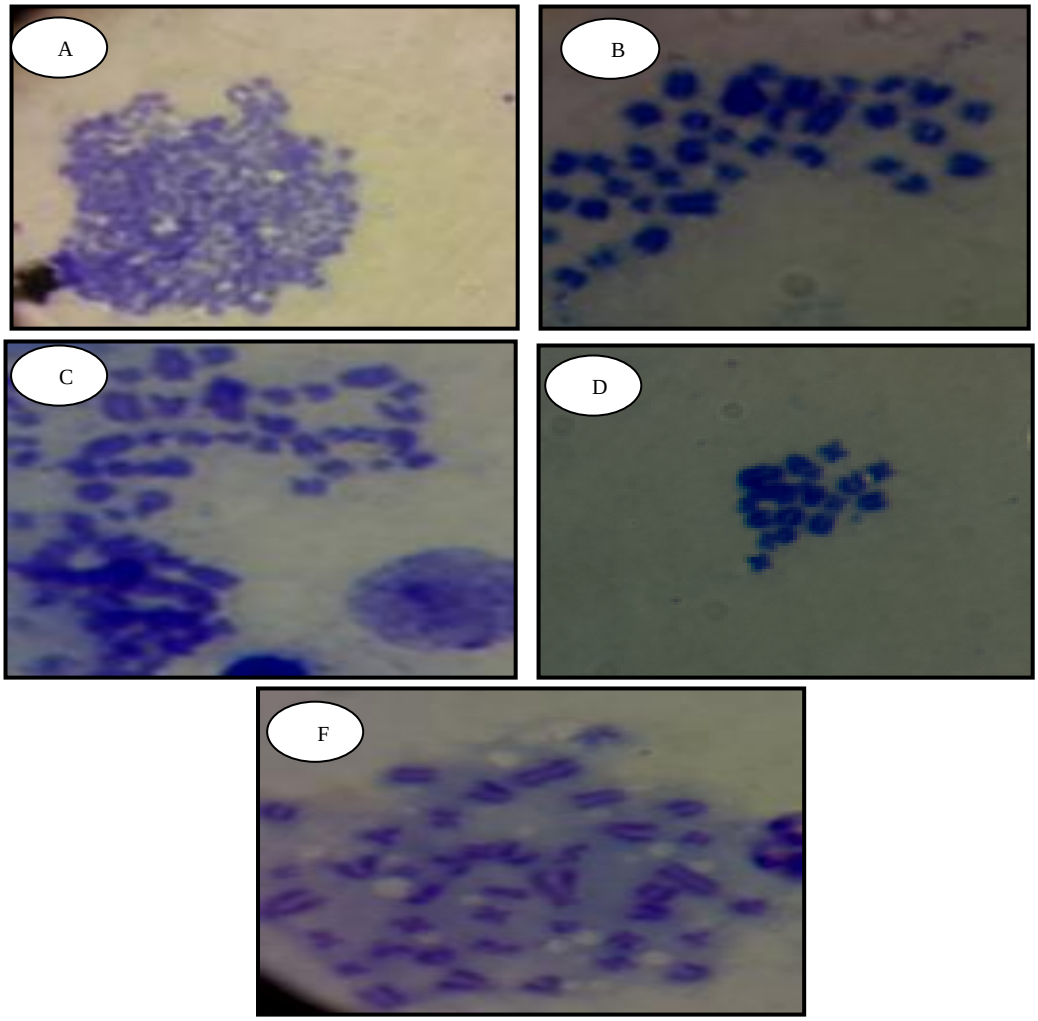

Figure (1): Chromosomal aberrations in bone marrow cells of treated rat with metronidazole. A: polyploidy B:Centromeric break C: Chromatid break. D: Aneuploidy. E.centromeric fusion F: (100X).

The sperm is formed of head and tail regions. The head region is characterized by its basophilic affinity to haematoxylin stain and it is elongated in shape. It have bent head and tail, sperm without head, blunt hook, defective hook and head ,coiled tail shape (Figure 2). Table (2) showed that there is no significant difference in mean of abnormal sperm between control and plant extract groups. Treatment with MT induced significant (P<0.05) increase in all type of sperm abnormalities after period of treatment. In animals pretreated with plant extract, and subsequently administered MT the number of sperm aberrations were significantly (p<0.05) decrease compared to the MT group except in and bent tail sperm, the greatest reduction was seen in the coiled tail.

Table (2): Protective effects of aqueous extract of ginger on mitronidazol induced sperm abnormalities of albino male rats.

Animal groups	Sperm abnormalities						
	Bent neck*	Bent tail*	Sperm without head*	Blunt hook*	Defective hook	Defective head*	Coiled tail*
C	2.200-±0.200^{b}	1.600±0 .678a	2.000±0.3162a	1.400±0.509a	0.800± 0.200a	1.200±0.583a	1.200±0.374a
T1	3.400 ±0.400^{c}	4.400± 0.748b	7.600±1.122b	5.000±0.836b	3.800±0.734b	5.600±0.600b	9.800±0.374b
T2	2.200± 0.374^{b}	1.800 ±0.583a	3.200±0.200a	0.600±0.400a	1.000±0.000a	0.800±0.200a	1.400±0.871a
T3	1.000 ±0.316^{a}	2.400± 0.678b	2.200±0.969a	0.800±0.489a	1.600±0.244a	1.800±0.374a	1.600±.0400a

Values are expressed as mean ± standard errors. Values with different letters at the same column are significantly different at P<0.05 (anova) with Duncan's post Hoc.C = control, T1 = 250 mg / kg bwt metronidazole-treated, T 2= 150 mg / kg bwt plant extract-treated, T3=pretreated animal with plant extract for 9 days+ MT 250mg/Kg/animal for 5 consecutive days.

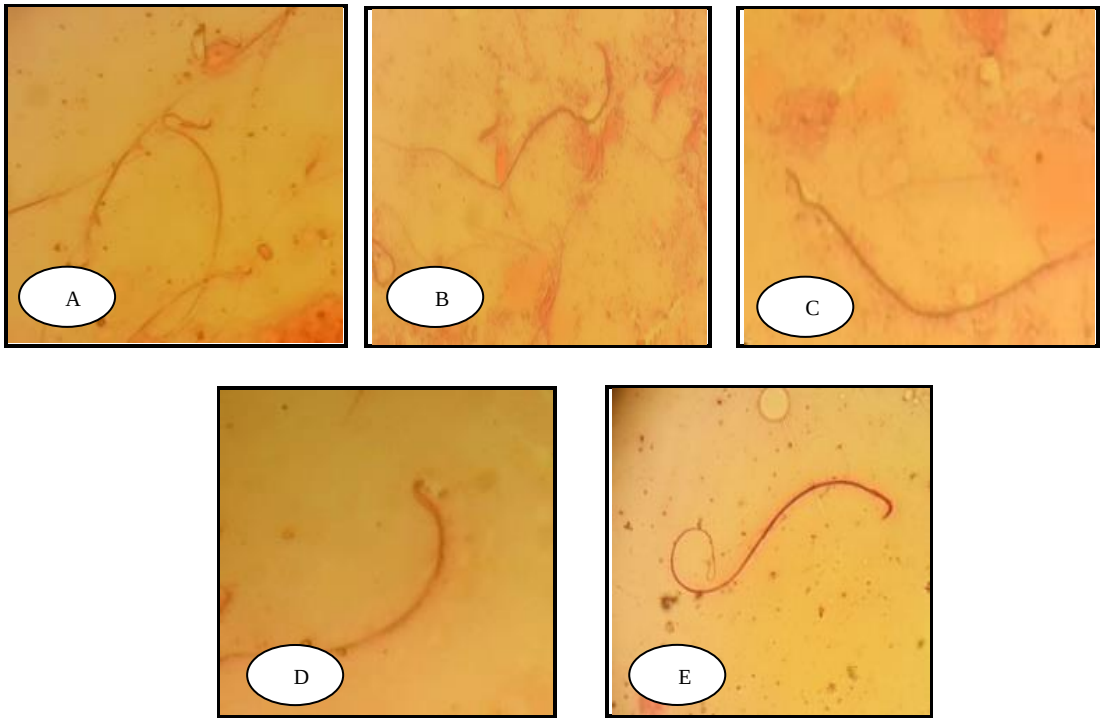

Figure (2): Sperm abnormalities in treated rat with metronidazole. A: bent neck B: bent tail C: sperm without head. D: defective hook soerm. E.coiled tail: (100X).

Discussion

During the last 20 years, the nitroimidazolic genotoxic agent metronidazole has been studied by several other authors [23]. And has been associated with differential effects due to the metabolism of this compound and with the genotype of the exposed organisms,or both. When we analyze the potential etronidazole genotoxicity, we found differences in chromosome aberrations frequencies between the control groups and the experimental groups which had received metronidazole. These finding agree with previous reports [23].

Metronidazole was found to induce chromatid breake, most probably either by alteration of the net charge of chromosomal protein (histones and/or acidic chromosomal proteins) or by induction of DNA cross linking [24, 25]. Found that metronidazole caused DNA breaks in rat hepatocytes which were directly related to the dose and the length of the exposure, which agrees with our results, that the increased percentage of the abarrant metaphases, was caused by 500 mg of metronidazole for 2 weeks [26]. An effort has been made in the current experimental design to observe whether such toxic effects induced by metronidazole, are neutralized or counter balanced by administration of plant extract. Ginger contains active phenolic compounds such as gingerol, paradol and shogoal that have antioxidant [27]. Anti-cancer [28], anti-inflammatory [29].

The present study, showed an increase in frequency of aberrant cell. Various pictures of chromosomal aberrations appeared in bone marrow cells of metronidazole treated animals. These aberrations were manifested in numerical (aneuploidy and polyploidy) and structural aberrations. Treatment with ginger extract reduced aberrant cells. The chromosomal damage was significantly repaired by plant extract, as decline in the number of chromosomal aberrations in animals pretreated with plant extract and subsequently treated with metronidazole show significant decline in chromosomal aberrations compared to the control groups. Thus, the current experiments confirm the protective role of plant extract in rat bone marrow cells .

In the present experiment metronidazole significantly increased abnormal sperm, [30] found that a single dose of 700 mg/kg b.wt. of 2 thiazolyl-5-nitroimidazole resulted in infertility in mice after 3 weeks, with a return of fertility by week 7. The reduction in percentage of motile sperm and increase in abnormal sperm might be due to metronidazole which reaches the blood testes barrier and gains access to the germ cells in the somniferous tubules. The present study shows that there was significantly decrease the number of sperm abnormalities in animals pretreated with plant extract, and subsequently administered of MT as compared to the MT group. These results were supported by previous findings who found that ginger reported in animal models that administration of Zingiber officinale, increased sperm count and normal morphology. It has protective effects against oxidative stress in rats [31]. Active Phenolic compound in Z. officinale as antioxidant specified by its ability in capturing free radical and ROS .[32, 33] .

In conclusion, the results of this study indicate that, Metronidazole 250

mg/kg by gavage has the ability to induce chromosome aberrations including (centromeric breake, centromeric gap, chromatid break, centromeric fusion, polyploidy, aneuploidy and chromatid gap) in bone marrow cells and sperm abnormalities in albino rats. Also the protective effect of aqueous extract of ginger to reduce both the chromosomal aberrations and sperm abnormalities of mutated rats may be attributed to its antioxidant properties.

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