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CYTOPROTECTIVE EFFECTS OF AQUEOUS GINGER (*ZINGIBER OFFICINALE*) EXTRACT AGAINST CARBIMAZOLE-INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT

Carbimazole is an antithyroid drug used to treat over active thyroid gland. On the other hand treatment with carbimazole associated with many side effects. In our research, rats treated with carbimazole for three and six weeks showed significant increase in percentage of apoptotic and necrotic cells by using dual staining of leucocytes by acridine orange and ethidium bromide. Moreover DNA fragmentation was observed after six weeks of treatment with carbimazole. Also comet assay revealed DNA damage in rats treated with carbimazole for six weeks. An increase in chromosomal aberrations were recorded with decreased cell proliferation was reflected by a decrease in mitotic index. Combined treatment with aqueous ginger (*Zingiber officinale*) extract and carbimazole caused a reduction in chromosomal aberrations, DNA damage and increasing cell viability by decraesing apoptotic and necrotic percentage with an increase in mitotic index. The results of this study indicated that aqueous ginger extract ameliorates the genotoxicity and cytotoxicity induced by carbimazole in albino rats and this is may be due to the potent antioxidant effects of its components.

KEYWORDS: *Zingiber officinale*, Carbimazole, DNA, chromosomal aberrations, cell viability, comet assay.

INTRODUCTION

Carbimazole is an antithyroid medication which is generally used to treat hyperthyroidism[1]. Carbimazole is a prodrug of the active structure methimazole which keeps the thyroid peroxidase enzyme from coupling and iodinating the tyrosine deposits on thyroglobulin, consequently decreasing the generation of T3 and T4[2]. Treatment with carbimazole typically proceeded for 12 to year and a half took after by a trial withdrawal[3]. Also treatment with carbimazole was joined by numerous symptoms. **Vilchez et al.**[4] observed that carbimazole treatment brought on some symptoms (e.g. pruritus, rash, urticaria, fever, arthralgias, agranulocytosis, hepatotoxicity with extreme cholestatic jaundice). Following treatment of patients with propylthiouracil and methimazole, proliferative cell nuclear antigen (PCNA) expression is markedly reduced, proposing that carbimazole have an antiproliferative effect[5]. The proposed machanism of methimazole activity is intracellular: it brings down the level of proliferating cell nuclear antigen (PCNA). PCNA advances specific apoptosis in some T lymphocyte clones[6]. **Sutiakova et al.**[7] recorded that carbimazole has cytogenetic effect and increase the frequency chromosomal abnormalities in peripheral blood lymphocytes of ewe and ram sheep. **Shin et al.**[8] showed that treatment with carbimazole cuased suppression of neurogenesis and enhancement of DNA fragmentation in the hippocampus rat pups. **Heidari et al.,**[9] reported that administration of methimazole was associated with agranulocytosis and hepatotoxicity, which are the two most significant adverse effects. **Joanta et al.**[10] concluded that carbimazole increased lipid peroxides from both thyroid gland and serum.

Large number of plants and their extracts are now used in medicine and treatment of various diseases. Due to the biological effects of these substances which have antioxidant properties, they are important in medicine. The roots of ginger (*Zingibe officinale*) is an example of botanicals which play an important role in pharmacology and treatment of various diseases. **Bordbar et al.**[11] showed that *Zingiber officinale* improved the results in animals injected intraperitoneally by 5mg/kg busulfan solution. **Sakr and Al-Amoudi**[12] demonstrated that treating animals with deltamethrin and ginger revealed an improvement in the histological changes observed in animals treated with deltamethrin. Examination of liver sections of ginger and metalaxyl treated animals revealed reduction of bax and preservation of nearly normal histological structure with slight congested blood vessels and few cellular infiltration[13]. **Yadamma and Devi**[14] revealed that ginger extract has protective effect against cyclophosphamide which cause induction of chromosomal abnormalitis in somatic cells of mice. **AL-Sharif**[15] showed that ginger has anti-mutagenic action against the anti-cancer drug Taxol genotoxicicty. Ginger

exhibit antioxidant properties formalin by the increase of SOD and CAT activities[16].

MATERIALS AND METHODS

Ginger Extract

Ginger (*Z. officinale* Roscoe) rhizome was bought from the neighborhood market at Shebin El-kom, Egypt. One kilogram of ginger rhizome was cleaned, washed under running water, cut into little pieces, air dried and powdered. 125 g of this powder were macerated in 1000 ml of dist. water for 12 h at room temperature and filtered. The convergence of the concentrate is 24 mg/ml. Each rat in the present study was orally given 1 ml of the last fluid concentrate[17].

Carbimazole

Carbimazole (Antithyroid drug produced by CID, Egypt) was purchased from a local pharmacy in the form of 5 mg/tablet.

Animals and Treatments

Sexually develop male albino rats (*Ratus norvegicus*) weighingv120 ±5 g were utilized. Rats were kept in the research facility under consistent temperature (24 ± 2 ^{o}C) all through the present work. They were kept up on a standard rat diet made out of 20% casein, 15% corn oil, 55% corn starch, 5% salt blend, and 5% vitaminized starch (Egyptian Company of Oils and Soap, Kafr-Elzayat, Egypt). Water was accessible not indispensable. Every exploratory methodology conformed to the aide for consideration and utilization of animal house affirmed by Menoufia University. They were divided into 4 groups:

Group 1. These animals (10 rats) served as controls and were given physiological saline (0.9% NaCl).

Group 2. Animals of this group (10 rats) were each orally given 1 ml by stomach tube of the aqueous extract of ginger containing (24 mg/ml) daily for three & six weeks.

Group 3. Animals of this group (10 rats) were each given orally carbimazole 1.35 mg/Kg b.w, equivalent to the therapeutic dose for human dissolved in dist. water by stomach tube[18].

Group 4. Animals of this group (10 rats) were orally given (24 mg/ml) ginger by stomach tube followed by carbimazole (1.35 mg/Kg b.w) for three and six weeks.

Comet assay (single cell electrophoresis) as preliminary test to detect the acute genotoxic effect of carbimazole after 72 hrs

Leucocytes were isolated from whole blood[19]. Erythrocytes were removed from blood by suspending cells in erythrocyte lysing solution (0.015 M NH_4CI, 1mM $NaHCO_3$, 0.1m M EDTA). Whole blood was incubated with 8 ml erythrocyte lysing solution, centrifuged for 5 min at 1000 rpm. Repeat last step twice till white pellet appeared clear. The pellet were removed carefully and washed two times with culture medium [RPMI 1640 with L-glutamine (Sigma), supplemented with 10% fetal calf serum (Sigma) and 0.1 % penicillin (5000 IU/ml), streptomycin (5000 mg/ml) solution]. The cells resuspended in 300 microlitre of low melting agarose ane dissolved in phosphate buffered saline (PBS) free of Ca^{2+} and Mg^{2+}. It was placed on precleaned microscopic slide already covered with thin layer of 0.5 normal melting agarose to promote firm attachment of second layer and kept for 2 min. at -12 ^{o}C to solidify. After that, the cover slide were gently removed by sliding it away and the slide were covered with a third layer of agarose and covered by cover slide then kept 2 min. at -12 ^{o}C. After the solidification of agarose, the cover slide gently removed. Immersing slides in a jar containing lysing solution [2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% N-lauroyl-sarcosine, pH 10, 1% Triton X100 and 10% dimethyl sulfoxide (DMSO)]. The slides were kept at 4 ^{o}C for 20 min. then slides were placed on horizontal electrophoresis unit. The unit was filled with a fresh alkaline electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH 13) to a level of 0.25 cm over the slides. The cells were exposed for 10 min to allow DNA unwinding and expression alkali-labile sites. Electric current of 25 V and 300 mA was applied for 10 min. Alkali and electrophoresis treatments were performed in ice bath and in dim light to prevent additional DNA damage. After electrophoresis, the slides were placed horizontally and tris buffer (0.4 mM Tris, pH 7.5) was added to neutralize the excess alkali. The slides were allowed to sit for 5 min. Finally, 100 microlitre ethidium bromide (20 µg/ml) was added to each slide, cover with cover slide and can be stored at 4 ^{o}C for 4 days in moist environment. Scoring was performed according to[20]. Examination was done with a fluorescent microscope (Olympus BX 41). The migration was evaluated by observing the nuclear DNA, where the rounded spot of DNA was considered as normal DNA spot, while migration towards the anode appears as comet spot and was considered as damaged DNA spot. 500 randomly selected spots of DNA were examined and the percentage of damagd DNA spots were calculated.

Cytogenetic preparation (Chromosomal aberration & Mitotic index)

The method was described by **Latt et al.**[21] Animals were injected 2 hours before sacrifice with 0.5 ml (0.0012%) colchicine/20 gm body weight (3 mg/kg body wt), to increase the number of metaphase spreads. Bone marrow cells were collected from the femurs in isotonic NaCl solution for injection (0.9%) and spinned for 10 min at 1000 rpm, discarding the supernatant. To swell the cell volume, 6 ml of hypotonic solution (0.075M KCl) was added and incubated for 15 minutes at 37°C. Fixation was done by adding 3-4 ml of absolute methanol and glacial acetic acid (3:1) drop by drop. After 10 min, the tubes were centrifuged, and the supernatant was removed. This was repeated three times to complete fixation. In the second time the tubes were kept in refrigerator for 30 min at 4°C. About three drops of fresh cell suspension were dropped on a clean slide dipped in 70% ethyl alcohol and flamed. The slides were stained with Giemsa and mounted in DPX. For each

animal fifty metaphase spreads were scored for chromosomal aberrations. For studying chromosomal aberrations and mitotic index, 5 animals were selected from the treated and control groups.

DNA extraction and fragmentation detection in testis by agarose gel electrophoresis

Nucleic acid extraction and detection of DNA fragmentation (apoptosis) was done according to extraction method of **Aljanabi and Martinez**[22]. Ten milligrams of testis in Eppendorf tubes were lysed with 600 microlitre buffer (50 mM NaCl, 1 mM Na_2EDTA, 0.5% SDS, pH 8.3) and delicately shaked. The mixure was incubated overnight at 37 ^{o}C then, 20 microlitre of saturated NaCl was added the sample, shaked and centrifuged at 12,000 rpm for 10 min. the supernatant was transferred to new Eppendorf tubes and then DNA precipitated by 600 microlitre cold isoproprnol. The mix was inverted several times till fine fibres appear, and then centrifuged for 5 min. at 12,000 rpm. The supernatant is removed and the pellets were washed with 500 microlitre 70% ethyl alcohol centrifuged at 12,000 rpm for 5 min. After centrifugation the alcohol was decanted or tipped out and the tubes plotted on whatman paper to be dry. The pellets were resuspended in 50 microlitre or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, pH 8). The resuspended DNA was incubated for 30 – 60 min with loading mix (Rnase + loading buffer) and then loaded into the gel wells.

Gels were prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% polyvinyl pyrolidine (PVP; Sigma). The agarose and PVP were boiled with tris borate EDTA buffer (1 × TBE buffer; 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.8). 0.5 micro-gram/ml ethidium bromide was added to gel at 40°C. then gels were poured and allowed to solidify at room temperature for 1hr. before the samples were loaded. Electrophoresis was performed for 2hrs at 50 volt using 1X TBE buffer as running buffer. DNA was visualized using a 312 nm UV light under a transilluminator. All gels of DNA were photographed with digital camera. Apoptotic bands appeared measured by software Gel analyzer program as maximum optical density value.

Cell Viability

Dual staining of leucocyte by ethidium bromide/acridine orange was carried out to detect the evidence of apoptosis, necrosis and viability of treated and control animals, 10µl of cells were incubated for 1 minutes with 5 µl (10µg/ml) of a solution of acridine orange/ethidium bromide 1:1 ratio of (100µg/ml) in PBS. Stained cells were visualized under (Olympus fluorescence microscopes, Japan) immediately and the images were digitally photographed. When observed under the fluorescent microscope at 400×, green color indicated viable cells, whereas cells with orange and red color were apoptotic and necrotic cells, respectively[23]. The percentage of apoptotic and necrotic cells were calculated.

Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by ANOVA followed by Student's t -test using Minitab 12 computer program (Minitab Inc., State Collage, PA, USA).

RESULTS

Comet assay (single cell electrophoresis)

Treatment with carbimazole induced significant damage in leucocyte DNA in compared with control group and appeared as damaged and strongly damaged spots (Fig 1) while animals of group treated with ginger and carbimazole showed an improvement in percentage of leucocyte DNA damage. Leucocyte of animals treated with ginger showed insignificant percentage in compared with control. Data in Table (1) described the results of comet assay in leucocyte of different groups after 72 hrs. of treatment.

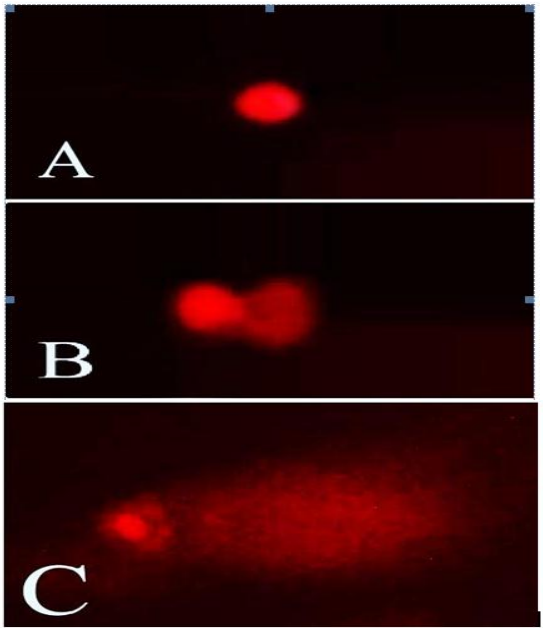

Fig. 1. Showing different treatment on DNA single strand of leucocyte in rats (A) Normal DNA spot (no migration), (B) Damaged DNA spot (migration towards the anode), (C) Strong damaged DNA spot (more migration towards the anode).

Table 1: Effect of different treatment on single cell electrophoresis or DNA damage in leucocyte.

Groups	Total damage % (Mean ± SD)
Control	5.4 ± 1.14
Ginger	6 ± 1.56
Carbimazole	26.1 ± 1.92*
Gin. + Carb.	18.2 ± 1.16*

(*) Significant at $p < 0.05$ in comparison with control

Chromosomal aberrations and mitotic index

Structural chromosomal aberrations were detected in the bone marrow cells of male rats treated with carbimazole and protected with ginger at different time intervals. The Structural aberration include chromatid deletion, centromeric attenuation, fragmentation, gap, centric fusion and break. Table (2) showed the mean value of chromosomal aberrations. There was a significant difference between control and animals treated carbimazole at P<0.05. Ginger has improvement effect at all duration against treatment with carbimazole. The animals treated with carbimazole showed a decrease in the mitotic index when compared with control group and increased in group treated with ginger and carbimazole.

Table 2: Average of structural chromosomal abnormalities observed in bone marrow cells of male rats treated with carbimazole and ginger juice (mean±SE).

Animal group	Gap	Break	Deletion	Fragment	Centromeric attenuation	Centric Fusion	Total
Control	0.8±0.83	1.4±0.54	0.6±0.54	1.2±0.83	4.4±1.14	1.2±0.83	**7**
Ginger	1.4±1.14	1.6±0.54	0.8±0.44	1±0.7	5.2±1.3	1±0.7	**7.4**
Carbimazole 3w	2±0.7*	3.2±0.83*	1.8±0.44*	2±0.7	9.4±1.81*	2.6±0.54*	**14.6**
Gin. + Car. 3w	2.6±0.63*	2.2±0.74*	1.8±0.74*	1.8±0.4	9.8±1.32*	2.8±0.74*	**13.6**
Carbimazole 6w	2.6±0.54*	4.2±0.8*	2.2±0.44*	2.2±0.83	18.6±1.6*	4±0.7*	**19.6**
Gin. + Car. 6w	2.2±0.44*	3.2±0.83*	1.8±0.83*	1.8±0.44	16.6±1.51*	3.4±1.14*	**16**

***Significant at P<0.05.**

DNA fragmentation detection in testis by agarose gel electrophoresis

a-Three weeks group results

Figure (2.a) showed the effect of carbimazole and ginger on DNA of testes. Lane A represents marker, while lanes B,C,D,E represents control, ginger, carbimazole, ginger + carbimazole respectively. The optical density of released DNA is shown in Fig. (2.b) by gel analyzer program. The results showed that there was weak effect on the intensity of released DNA in testes treated with carbimazole only or ginger and carbimazole. The optical density of DNA with values of 8.35, 8.78, 13.6, 6.76 respectively.

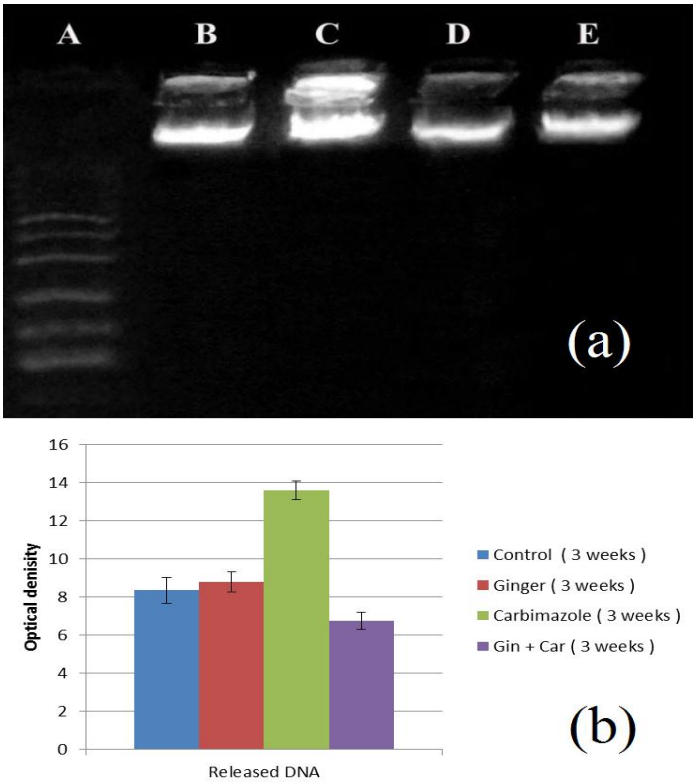

Fig. 2. DNA damage in testes of rats. (a). Marker lane (A), control lane (B), ginger lane (C). treated with carbimazole lane (D) and ginger + carbimazole lane (E). (b). Optical denisity of released DNA in testes of rats in different.

b-Six weeks group results

Fig (3.a) showed the effect of carbimazole and the effect of ginger on DNA of testes. Lane A represents marker, while lanes B,C,D,E represents control, ginger, carbimazole and ginger + carbimazole respectively. As shown in Fig (3.b) there was increase in the intensity of released DNA in testes treated with carbimazole with values of 32.22 and slightly decrease in the intensity of released DNA in testes treated with ginger and carbimazole with value of 25.7.

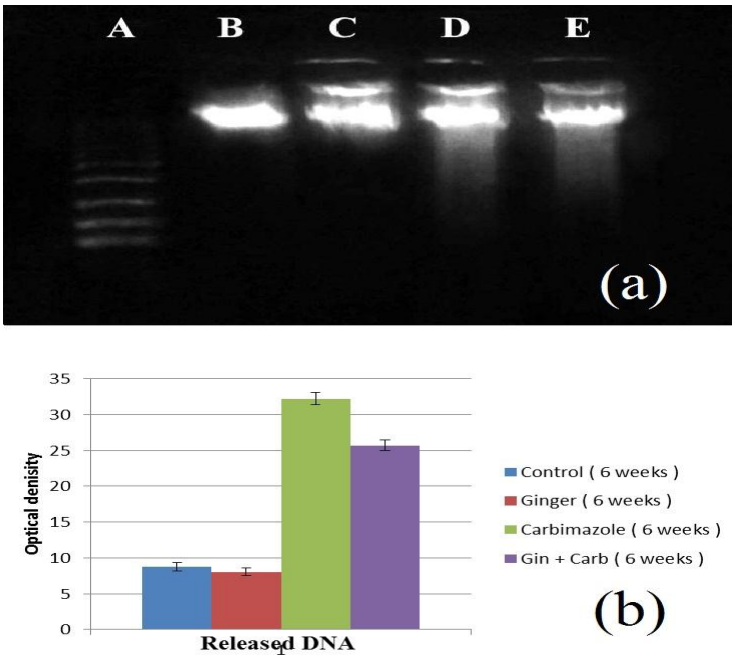

Fig. 3. DNA damage in testes of rats. (a) Marker lane (A), control lane (B), ginger lane (C). treated with carbimazole lane (D) and ginger + carbimazole lane (E). (b) Optical denisity of released DNA in testes of rats in different treated groups after six weeks.

Cell viability

Acridine orange/Ethidium bromide staining

Fig. (4.a) showed leucocytes of control animals group stained with acridine orange and ethidium bromide. Live cells have green color, apoptotic cells have orange color while necrotic cells have red color. Treatment of animals with carbimazole for three weeks and six weeks resulted in a decrease in percentage of living cells counts. The percentage of apoptotic cells gradually increased by time of treatment with carbimazole. Also necrotic cells percentage showed increase in carbimazole group in compared with control and ginger groups. While treatment of animals with ginger and carbimazole showed an improvement in percentage of apoptotic and necrotic cells in compared with carbimazole group. Figs. (4.b.c) showing the percentage of apoptotic and necrotic cell in different groups.

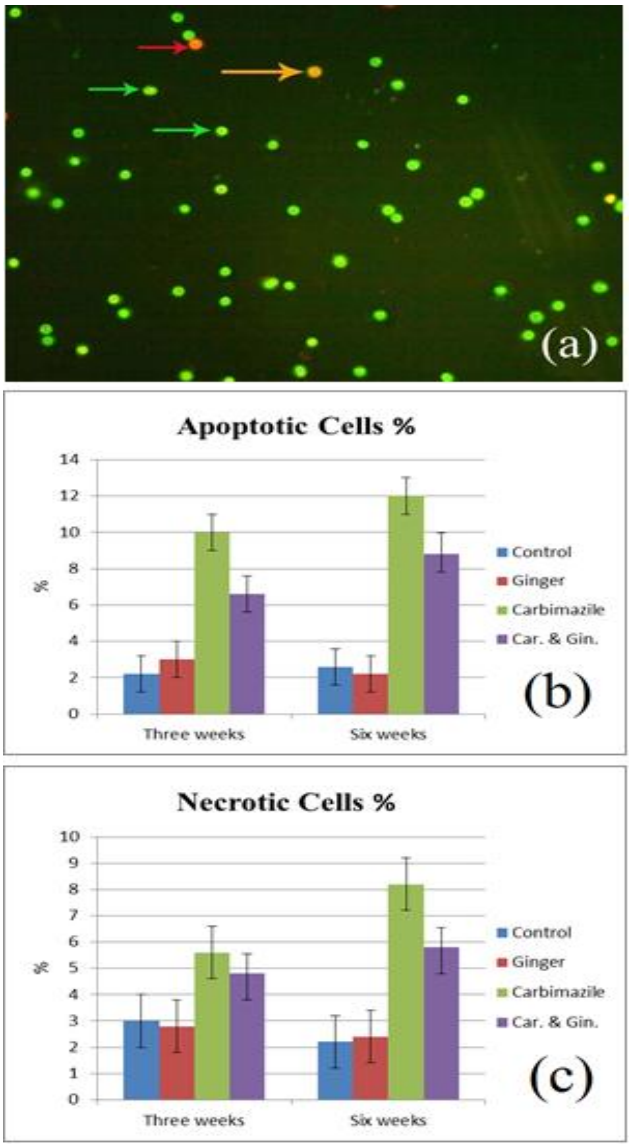

Fig. 4. (a) Control cells stained with AO/EB showing live cells (Green arrows), apoptotic cells (Orange arrow) and necrotic cells (Red arrows) (X400). (b) The effect of different treatments on the percentage of apoptotic leucocytes of rats. (c) The effect of different treatments on necrotic leucocytes of rats.

DISCUSSION

The current study indicated that animals treated with carbimazole for three and six weeks caused an increase in the frequency of chromosomal aberration include deletion, fragment, centromeric attenuation, centric fusion, break and gap. Mitotic index was also affected after treatment with carbimazole. **Kodama et al.**[24] examined the inducibility of chromosomal aberrations of cultured mammalian cells on 11 clinical medicines which are used for a long term mainly in the field of internal medicine. P-aminosalicilic acid, isonicotinic acidhydrazid, streptomycin A, hydralazine hydrochloride, methimazole and theophylline induced definite increase of chromosome aberrations. Among them P-aminosalicilic acid was a little weak in its effect. Their ratios by isonicotinic acid hydrazid, streptomycin A, methimazole, theophylline and issorbide dinitrate were very high and those by others were relatively low.

The cytogenetic effect of carbimazole was studied on the frequency of chromosome aberrations in peripheral blood lymphocytes of ewe and ram lambs. The dose of the drug was gradually increased, reaching 50 mg/animal/day at the time of blood collection. The frequency of aberrant cells in the experimental group of ram lambs was 4.21 +/- 0.82%, that in ewe lambs was 3.33 +/- 0.47% [7]. The incidence of micronucleated cells was significantly higher in the fetal blood than in the maternal bone marrow after treatment with carbimazol and methylthiouracil on mice fetal blood in the 15th and 16th day of intrauterine life. The finding demonstrates that the method of transplacental mutagenesis is a reliable test for estimating the mutagenic risk of such drugs as carbimazol and methylthiouracil[25]. Treating rats with carbimazole caused an increase in DNA fragmentation in testes after six weeks of treatment. Also, comet assay showed that there was DNA damage in leucocyte of rats treated with carbimazole for 72 hrs. This result indicated that carbimazole had cytogenetic toxicity in rats. The single-cell gel electrophoresis technique or comet assay is widely regarded as a quick and reliable method of analyzing DNA damage in individual cells[26]. DNA strand breaks allow DNA to extend from lysed and salt-extracted nuclei, or nucleoids, to form a comet-like tail on alkaline electrophoresis. The comet assay, a technique, capable of detecting DNA damage in individual cells, is a valuable approach for human biomonitoring studies[27]. Cells undergoing active cell death or apoptosis demonstrate highly fragmented DNA. Apoptosis results in the extensive formation of double-strand breaks and is readily detected using alkaline electrophoretic conditions. When viewed using the comet assay, only a small percentage of DNA of an apoptotic cell remains associated with the comet head[28],[29].

Thakkar and Jain[30] studied the cell damage in patients suffering from type 2 diabetes (T2D) and thyroid dysfunctions using comet assay. The results showed that T2D patients showed 92.24% of cell damage compared to hypothyroid (HT) or hyperthyroid (HeT) patients (51.04% or 54.64%, respectively). Further, increase in cell damage was also observed in HT + DM subjects ($P < 0.05$). Treatment of rats with PTU caused extensive DNA fragmentation throughout development in hippocampus of hypothyroid pups, but not in the euthyroid controls[31]. **Shin et al.**[8] investigated the effects of treadmill exercise on short-term memory, spatial learning ability, neurogenesis, and apoptosis in hypothyroidism rat pups. On the 14th perinatal day, the pregnant rats were divided into two groups: the maternal control group and the maternal methimazole (MMI)-treated group. For the induction of hypothyroidism in rat pups, MMI was added to the drinking water (0.02%, wt/vol), from the 14th prenatal day to the 49th postnatal day. After delivery, the male rat pups born from the maternal control group were assigned into the control group and the control and exercise group. The rat pups born from the maternal MMI-treated group were divided into the hypothyroidism-induction group and the hypothyroidism-induction and treadmill exercise group. The rat pups in the exercise groups were forced to run on a motorized treadmill for 30 min once a day, starting on the 22nd postnatal day for 4 weeks. Induction of hypothyroidism during the fetal and early postnatal period showed suppression of neurogenesis and enhancement of DNA fragmentation in the hippocampus. Short-term memory and spatial learning ability were deteriorated in the hypothyroidism rat pups. Treadmill exercise during the postnatal period increased neurogenesis and inhibited apoptosis, and resulted in the improvement of short-term memory and spatial learning ability in the hypothyroidism rat pups. A decrease in leucocyte viability was recorded after treatment with carbimazole. In this concern, **Heidari et al.**[9] reported that administration of methimazole was associated with agranulocytosis and hepatotoxicity, which are the two most significant adverse effects. They added that treating hepatocytes with methimazole resulted in cytotoxicity characterized by the reduction in cell viability.

Our studies revealed that ginger treatment decreased frequency of chromosomal aberration, increased mitotic index and improved DNA fragmentation. **Yadamma and Devi**[14] studied the protective effect of Ginger Extract against cyclophosphamide induced cytotoxicity was evaluated in vivo animal model using analysis of chromosomal aberrations in somatic cells of mice and they were observed that there was a significant decrease in the percentage of chromosomal aberrations when animals were firstly treated with different doses of Ginger Extract. Ginger decrease testes apoptosis in animals treated with gentamicin + ginger. Regarding the results, it is recommended that administration of ginger with gentamicin might be beneficial in men who receive gentamicin to treat infections[32]. **AL-Sharif**[15] determined the possible anti-mutagenic effect of ginger against the genotoxic effect of anti-cancer drug Taxol 0.6 mg/kg. The results showed that significant increase in total chromosomal aberrations and significant increase in the number of micronuclei were observed after treatment drug. Simultaneous treatment of ginger was found to be effective in reducing the genotoxic effects induced by drug Taxol especially in the total number of the chromosomal aberrations and the number of micronuclei. **Hamoudah et al.**[33] investigated the protective role of ginger and curcumin powders against some toxicological effects of thermoxidized frying cotton oil (OFO). The results indicated that OFO treated group showed significant increase in both liver enzymes (AST and ALT) and glucose levels. Significant increase in the frequencies of chromosomal aberrations in somatic and germ cells were encountered. The addition of ginger or curcumin to diet of OFO treated group produced improvement in the liver function, decrease in the glucose level, increase in the level of total antioxidants, reduction in the frequencies of chromosomal aberrations and improvement of the hepatic pathological changes. These results indicated that ginger and curcumin can

protect against toxicity of frying oil. **Salah et al.**[34] studied the effect of *Zingiber Officinale* on paracetamol-induced genotoxicity in male rats. The significant increase in chromosomal aberration, the changes in the number, position and intensity of bands, liver and renal damages induced by paracetamol may be attributed to the fact that paracetamol can induce genotoxicity through DNA damage. Paracetamol also stimulated AST and ALT activity, these stimulations indicated liver cell necrosis. The treatments with ginger presented hepatoprotective effect, also ginger can protect against oxidative kidneys tissue damage that reduced lipid peroxiation in liver and kidneys. The possible mechanism by which ginger exhibited significant protection against paracetamol- induced genotoxicity and hepatotoxicity may be due to its antioxidant effect. It may also be responsible for the hepatoprotective activity and attainment of normal frequencies of chromosomal aberration in ginger-treated rats.

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