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Original Research Article

# The Possible Antigenotoxic Potential of Ginger Oil on Etoposide–Treated Albino Rats

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**Abstract:** Etoposide is an anticancer drug that belonging to topoisomerase II inhibitors, it used to treat various human malignancies. Ginger (*Zingiber officinale*) is a medicinal plant belonging to the family Zingiberaceae. The present study evaluated the possible protective potential of oral treatment of ginger oil (75&150 mg/kg body weight) three times weekly for 21 days against the genotoxic effects of etoposide oral administration (1mg/kg body weight) three times weekly for 21 days on bone marrow in male albino rat (*Rattus norvegicus*). Sixty adult male albino rats were used as the following, 30 rats (5 for each group) were prepared for DNA, evaluated the oxidative status in liver tissue (they were received the treatments for 21 days). The other 30 rats were used for chromosomal aberrations in bone marrow in addition to mitotic index in bone marrow (they were received the treatments for 72 hours only). Animals treated with etoposide showed DNA fragmentations on agarose gel electrophoresis and a significant increase in the percentage of bone marrow total chromosomal aberrations (TCA: 183.3±2.7) with significant decrease ($P$<0.01) in mitotic index in bone marrow (22.3± 2.25 ). Malondialdehyde and nitric oxide as indicators for oxidative stress showed an increase (2.42 ±0.05, 24.25 ±0.41) respectively, in contrast superoxide dismutase, catalase and glutathione showed decrease ($P$<0.01) with values (15.2 ±0.20, 5.42±0.31, 1.43±0.09) respectively, after etoposide treatment. While treatments with ginger oil (either 75 or 150 mg/kg b. wt.) normalize the oxidative status in liver tissues. In conclusion, the results of the present study indicated that ginger oil exerted a protective effect against genotoxicity and cytotoxicity induced by etoposide that may be due to its antioxidant effects. Consequently, we recommended that ginger oil can be suggested to be administrated as co-medicine in chemotherapeutic treatments of cancer.

**Keywords:** Etoposide, *Zingiber officinale*, DNA fragmentation, Chromosomal aberrations, Biochemical assay

## INTRODUCTION

Etoposide is a cytotoxic anticancer drug which is generally used to treat human malignancy as leukemia, lung cancer, testicular cancer, lymphocytic and non lymphocytic cancer[1]. Etoposide is belonging to topoisomerase inhibitor drug class that interferes with the action of topoisomerase enzymes (topoisomerase I and II). Topoisomerase enzymes control the manipulation of the structure of DNA necessary for replication, but etoposide caused cross-linking in DNA double helix strands this lead to the strands unable to uncoil and separate which is necessary in DNA replication and inhibit DNA synthesis by forming etoposide topoisomerase II DNA complex[1,2]. Also treatment with etoposide was joined by numerous symptoms such as bone marrow suppression, anemia, leukopenia, neutropenia, low blood pressure, low platelets counts, nausea, vomiting, fever, rash, hair loss, acute myeloid leukemia and bleeding [3]. Steato hepatitis is the more serious event, especially if accompanied by an increase in bilirubin levels related to administration of etoposide [4]. Treatment with etoposide induced fragmentation of centromeric DNA in rats [5,6]. Mitotic index suppression and elevated frequencies of centromeric hybridization signals and chromosome fragmentation at centromeres were recorded due to etoposide treatment [7]. Etoposide has cytogenetic effect and increase the frequency chromosomal abnormalities in peripheral blood lymphocytes [8]. SCEs and chromosomal aberrations in mouse bone marrow due to etoposide treatment [9,10]. Etoposide induced oxidative stress in hepatic tissue of rats, were Glutathione, catalase and superoxide dismutase levels were significantly decreased in contrast nitric oxide and lipid peroxidation showed a significant increase after treatment[11]. Etoposide induced changes in Protein Content, decrease in liver and kidney GSH levels of rat whereas, lipid peroxidation showed significant increase compared to control[12]. Etoposide caused hepatotoxicity in albino rats after direct treatment by elevation of MDA and NO in liver tissues as well as a decrease in GSH and the activities of antioxidant enzymes, including SOD, CAT in liver tissues [13]. Oxidative stress in non-small cell

lung cancer patients after chemotherapy showed LPO and NO were low, while GSH and SOD levels were high compared to control [14,15]. Administration of etoposide caused significantly higher kidney MDA and NO levels and significantly lower kidney GSH, CAT levels [16,17]. Etoposide induced mitochondrial oxidative stress and increase in (ALT) and (AST). So it caused hepatotoxicity [18].

Large numbers of plants are now used in medicine and treatment of various diseases due to the biological effects of these substances which have antioxidant properties. Ginger (*Zingibe officinale*) is an example of botanicals which play an important role in pharmacology and treatment of various diseases [19]. Ginger improved the liver function and prevents hepatotoxicity against lamotrigine [20]. It also has a protective effect against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats [21]. Ginger has anti- genotoxic effect against Benzo(a)pyrene induced DNA damage [22]. and ameliorated the genotoxicity that induced by carbimazole which increased the chromosomal aberrations, DNA damage in albino rats [23]. Ginger extract has protective effect against cyclophosphamide which causes induction of chromosomal abnormalitis in somatic cells of mice [24]. and it has anti-mutagenic action against the anti-cancer drug Taxol genotoxicicty [25].

Ginger has the antioxidant potential to ameliorate lead-induced hepatic injury by increase of superoxide dismutase (SOD), GSH activity [26,27]. Ginger has antioxidant effect against Carbon Tetrachloride induced- liver fibrosis in rats, it caused decreased malondialdhyde (MDA) content but increased glutathione (GSH), superoxide dismutase (SOD) and hydroxyproline in liver tissues [28,29]. Ginger extract improved ALT and AST activity, reduced the level of malondialdehyde and increased the activity of superoxide dismutase against adriamycin-induced hepatotoxicity in albino rats [30]. Choline deficient diet (CDE) induced the formation of liver nodules in rats and ethionine induced rat hepatocarcinogenesis in contrast treatment with ginger showed reduced levels of SOD and MDA, increased catalase activity [31,32]. DMBA induced genotoxicity in albino rats and caused increase MDA, NO but treatment with ginger lead to increase in MDA, NO and increase in CAT, SOD, and GSH in liver [33]. Ginger treatment exerts a therapeutic protective effect in diabetes by decreasing oxidative stress, and hepatic and renal damage [34]. Ginger has effect on hepatic antioxidant enzymes system in rats treated with ethanol which induced hepatotoxicity by decrease malondialdehyde (MDA) levels in the hepatic tissue [35,36]. The Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats is cleared by decrease in the activities of MAD, NO [37].

## MATERIAL AND METHODS

### Materials

1- Etoposide (etopophos), $C_{29}H_{32}O_{13}$, (4'-Demethylepipodophyllotoxin9-[4,6-O-(R)-ethylidene-β-D glucopyranosi- de], 4'-(dihydrogen phosphate) was purchased from a local pharmacy.

2- Ginger oil (*Zingiber officinale*) belongs to family Zingiberaceae. Ginger oil was obtained from Andalos Import And Export Company.

### Experimental animals:

### Housing of the animal:

Healthy adult male albino rats (*Rattus norvegicus*) weighting 130±10 g were obtained from the Serum and Antigen Laboratories, Helwan, Egypt. Animals were kept under constant condition of temperature (25±2ºC) for at least two weeks before and throughout the experimental work. They were maintained on a stander rodent diet composed of 20% casein, corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch obtained from Egyptian Company for Oils and Soup Kafr- Elzayat, Egypt. Water was available ad libitum. The experimental protocol and animal care ethics was approved by Zoology Department, Faculty of Science, Menoufia University.

### Groups of animals under investigation:

In the present study 60 adult male albino rats were equally divided in to 6 groups used as the following: Group(1) animals were served as negative control(untreated).Group(2) animals were treated orally with etoposide (1mg/kg b. wt.) by stomach tube three times weekly. Group(3) animals were orally given (75mg/kg b. wt.) ginger oil three times weekly as low dose. Group(4) animals were orally given (150mg/kg b. wt.) ginger oil three times weekly as high dose. Group(5), Group(6) simultaneously, animals were orally given etoposide (1mg/kg b. wt.) and (75 and 150 mg/kg b. wt.) ginger oil respectively, three times weekly.

Thirty rats (5 for each group) that were prepared for DNA and oxidative status, received the treatments for 21 days. While the other 30 rats (5 for each group) that were specified for chromosomal aberrations and mitotic index in bone marrow, received the treatments for only 72 hours.

### Methods

### Molecular investigations

### Total genomic DNA extraction and apoptosis detection.

Nucleic acid extraction was done according to extraction method of [38] with some modifications had

been introduced by [39]. in which the direct staining of DNA sample was done. Apoptotic bands of DNA fragmentation appeared and located at 180 bp and its multiples 360, 540 and 720 bp against therteen bands of DNA marker (100–3000 bp, Fermentas) [40]. The intensity of released DNA fragments was measured by image J software, as a mean of optical density values.

**Cytogenetic investigation:**

**Chromosomal preparation in bone marrow:**

The method was described by [41] Control and treated animals were used for the evaluation of chromosomal aberrations and mitotic index in bone marrow[42].

**Chromosomal aberrations in bone marrow:-**

For each animal 100 metaphase spreads were scored for chromosomal aberrations. Only cells with well spread chromosomes were selected for scoring. Slides were examined at (×1000) magnification by light microscope (Olympus BX41, Japan) and the representative photos were captured using digital camera.

**Mitotic index in bone marrow**

The same slides of chromosomal aberrations were used to evaluate the mitotic index in bone marrow. For mitotic examination, 500 cells of each animal were examined at (×200) magnification by light microscope (Olympus BX41, Japan). And the representative photos were captured using digital camera. Cells were classified according to their division to resting cells (non-dividing cells), prophase and metaphase. The percentage of metaphase was calculated according to this equation.

Mitotic index (%) = (metaphase) ×100 / (resting cells + prophase+ metaphase)

**Biochemical investigations**

**Preparation of liver homogenate**

Liver tissues from each group were homogenized in ice-cold 0.1 M phosphate buffered saline (PBS) at pH 7.4 to prepare 10% w/v homogenate then centrifuged at 4,000 rpm for 15 min at 4°C.

**Oxidative stress biomarkers**

The degree of lipid peroxidation in liver tissue was estimated by measuring the concentration of malondialdehyde (MDA) using thiobarbituric acid as described by**.** [43] Absorbance was measured at 532 nm and the results were expressed as nmol/mg protein. Accumulation of the stable product of nitric oxide (NO), nitrite, was measured colorimetrically in the supernatant of liver homogenate using Griess reagent**.**[44] The absorbance of the pink color developed was measured at 540 nm. Nitrite level in test samples was calculated from standard curve established using sodium nitrite and the results were expressed as nmol/mg protein.

**Liver tissue antioxidant status**

The activities of the antioxidant enzymes and GSH level were assessed in the supernatant of liver homogenate. Catalase activity was estimated by measuring the change in absorbance at 240 nm using $H_2O_2$ as a substrate and expressed as nmol/ min/ mg protein**.** [45] Superoxide dismutase (SOD) activity was estimated according to the method of [46] by evaluating its capacity to inhibit the reduction of nitroblue tetrazolium (NBT) dye mediated by the anion generating system (phenazine metholsulfate-NADH). The change in absorbance at 560 nm was recorded and the results were expressed as U/mg protein. Glutathione level was determined by the method of [47] which is based on the reduction of Ellman's reagent [5,5′ dithiobis (2-nitrobenzoic acid) DTNB] with GSH. The absorbance of the resulting yellow colored complex was measured at 412 nm. The GSH level was calculated from GSH standard curve and expressed as nmol/mg protein.

**Statistical Analysis:**

In the present work, the result were represented as Mean ± Stander Deviation. Comparisons were made between the untreated and treated groups. All numerical data were statistically analyzed using Statistical Program of Social Science (SPSS) software for windows, version 10. *(P*< 0.005) were considered statistically significant with mitotic index of bone marrow (*P*<0.00001) were considered statistically significant with total released DNA fragmentation, *(P*<0.01) total chromosomal aberrations and *(P*< 0.01) were considered statistically significant with biochemical assay.

**RESULTS**

**Molecular investigations**

**Analysis of total genomic DNA damage**

Etoposide induced a severe damage of DNA in liver tissue as appeared in the form of DNA fragmentation shape (apoptosis) after 21 days of treatment (Fig. 1, 2), Optical density value of fragmented DNA extracted from liver of etoposide-treated rats was (97.1±0.36) that showed significant increase (*P*< 0.00001) when compared with control (6.3±0.53). While animals treated with ginger oil low and high doses (75, 150 mg/kg b. wt.) showed normal appearance of intact DNA (optical denisity12.2±0.42, 13.8±0.62) respectively, that was non-significant when compared with control (6.3±0.53) and significant (*P*< 0.001)when compared with etoposide released DNA fragmentation (97.1±0.36). On the other hand, combination of etoposide with ginger oil doses (75 or 150mg/kg b. wt.) showed an improvement in DNA fragmentations (optical density 34.2±0.62, 46.8±1.16)

respectively, when compared with etoposide alone.

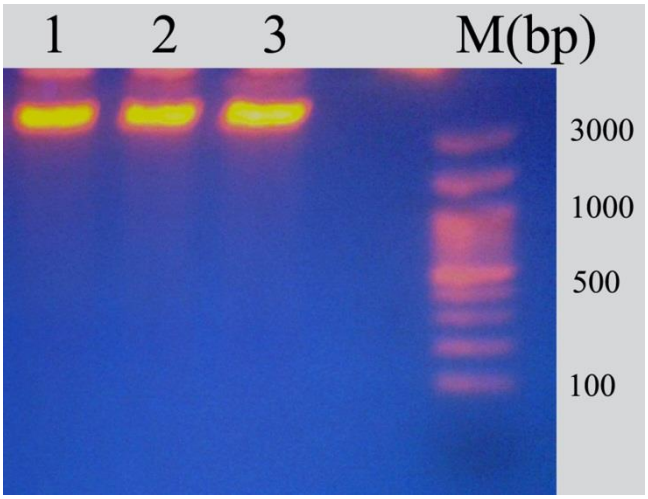


**Fig-1: Digital photograph of DNA electrophoresis showing the effect of ginger oil on rat's liver tissues. Where L1: control; L2 :G.O (75mg/kg); L3: G.O (150mg/kg) and M: DNA marker**

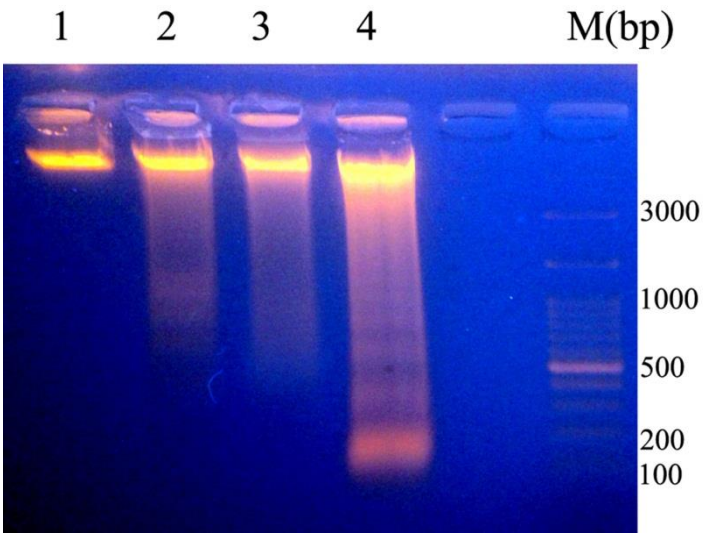


**Fig-2: Digital photograph of DNA electrophoresis of rat's liver tissues showing the protective effect of ginger oil against etoposide. Where L1: control; L2: combination of etoposide with ginger oil low dose; L3: combination of etoposide with ginger oil high dose; L4: etoposide (1g/kg b.wt.); and M: DNA marker.**

**Cytogenetic investigations results:**

**Chromosomal aberrations in bone marrow.**

Chromosomal aberrations are changes in chromosome structure or number. In this study only structural chromosomal aberrations were scored. These aberrations involve alternation of the genetic material and detected by light microscope during cell division at metaphase.

The structural aberrations include end to end association, breaks, deletions, centromeric attenuation, ring chromosome, centric fusion and chromatid gaps.

Data in Table (1) showed that etoposide significantly ($P< 0.00005$) increased total chromosomal aberrations (TCA: 183.3±2.7) when compared with control (TCA: 47.3±1.36). Moreover, etoposide statistically ($P< 0.00005$) decreased the percentage of structurally normal metaphases to (42±1.78) when compared with control (59±2.73). Data showed that administration of low dose of ginger oil (75mg/kg b. wt.) was non-significant (TCA: 57.6±2.25) when compared with control group (47.3±1.36), and significant ($P< 0.00005$) when compared with etoposide group (183.3±2.7). In addition, ginger oil low dose showed statistically non-significant increase ($P< 0.001$) in the percentage of structurally normal metaphases (55±1.78) when compared with control (59±2.73). While the high dose of ginger oil (150 mg/kg b. wt.) increased the mean values of total chromosomal

aberrations to (87.0±2.68) that increase was significant ($P$< 0.001) when compared with control (47.3±1.36) or with etoposide (183.3±2.7). The percentage of structurally normal metaphases in animals treated with high dose of ginger oil showed statistically significant ($P$< 0.001) decrease (40±1.36) when compared with control (59±2.73). Animals treated with ginger oil low dose (75 mg/kg b. wt.) and etoposide at the same time showed improvement in total chromosomal aberration (TCA: 54.6±2.73) when compared with etoposide group (183.3±2.7). This group showed good protective effect of ginger oil (low dose) on chromosomes against etoposide. Moreover, the percentage of structurally normal metaphases was increased (60±1.78) when compared with etoposide alone (42±1.78). Whereas, the combination of etoposide with high dose of ginger oil (150 mg/kg b. wt.) showed a decrease in total chromosomal aberrations (114.3±2.2) when compared with etoposide alone (183.3±2.7). In addition, the percentage of structurally normal metaphases were increased (65±2.80) when compared with etoposide alone (42±1.78).

**Table-1: The chromosomal aberrations in bone marrow of rat treated with etoposide and the protective effect of ginger oil treatment.**

| Groups | % Structurally normal metaphase | Chromosomal aberrations% | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Deletion | Break | Ring | Gap | Fragment | Centric attenuation | Centric fusion | End to end | TCA |
| Control | 59±2.73 | 2.6±1.36 | 2.0±0.89 | 0 | 1.6±1.36 | 3.0±0.89 | 2.0±0.89 | 1.3±1.36 | 2.3±1.36 | 47.3±1.36# |
| ET (1mg.kg) | 42±1.78* | 9.0±1.78 | 10.0±0.89. | 6.0±1.78 | 5.3±1.36 | 9.0±0.89 | 10.3±1.36 | 12.0±1.78 | 10.0±0.89 | 183.3±2.7* |
| G.O (75mg/kg) | 55±1.78# | 2.6±2.25 | 4.6±1.33 | 0 | 3.6±1.86 | 2.6±1.86 | 3.0±1.78 | 4.6±1.36 | 2.6±1.36 | 57.6±2.25# |
| G.O (150mg/kg) | 40±1.36*# | 3.6±1.36 | 6.0±1.78 | 5.3±1.36 | 2.3±2.25 | 3.6±1.86 | 4.6±1.36 | 3.6±1.86 | 3.0±0.89 | 87.0±2.68*# |
| ET+G.O (75mg/kg) | 60 ±1.78*# | 2.0±1.78 | 3.6±1.36 | 1.0±0.89 | 3.6±1.36 | 2.6± 2.25 | 4.0±1.76 | 4.0±0.89 | 2.6±2.25 | 54.6±2.73*# |
| ET+G.O (150mg/kg) | 65±2.80*# | 4.3±1.86 | 9.3±1.86 | 3.3±1.36 | 9.3±1.86 | 7.0±1.78 | 8.6±1.86 | 1.6±1.36 | 1.3±1.36 | 114.3±2.2*# |

Data were represented as (Mean ±S.D). *Statistically significant ($P$< 0.001) with respect to control. # Statistically significant ($P$< 0.00005) with respect to drug. ETO: Etoposide ; GO :Ginger oil; TCA: Total chromosomal aberration, n=5.

**Mitotic index in bone marrow**

Table (2) showed the mean values of mitotic index and stages of cell cycle in bone marrow cells of treated male rats (Figure 3). The results showed that etoposide significantly ($P$< 0.005) decreased the mean values of mitotic index (22.3± 2.25) when compared with control group(48.3 ± 1.36). While ginger oil low and high doses (75 and 150 mg/kg b. wt.) were non-significant ($P$< 0.005) when compared with control (48.3 ± 1.36) and significant ($P$ < 0.01) when compared with etoposide group (22.3± 2.25). The mean values of mitotic index of ginger oil low and high doses were (41.6 ±1.36 and 35.0±1.78) respectively. Animals received low dose of ginger oil (75 mg/kg b. wt.) in combination with etoposide showed an increase in mitotic index values (39.0 ±3.22) when compared with etoposide group (22.3±2.25). While animals treated with fennel oil high dose (150 mg/kg b. wt.) in combination with etoposide showed an increase in mitotic index values (32.0±0.89) which was non-significant ($P$< 0.005) when compared with control (48.3±1.36) and significant when compared with etoposide group.

**Table-2: The mitotic index in bone marrow of rat treated with etoposide and the protective effect of ginger oil treatment.**

| Groups | Mitotic index (%) |
|---|---|
| Control | 48.3 ± 1.36 |
| ET | 22.3± 2.25* |
| G.O. (75mg/kg) | 41.6 ± 1.36# |
| G.O. (150mg/kg) | 35.0± 1.78# |
| G.O. (75mg/kg) +ET | 39.0 ± 3.22# |
| G.O.(150mg/kg)+ET | 32.0± 0.89# |

Data were represented as Mean ±S.D. *Statistically significant ($P$< 0.005) with respect to control. # Statistically significant ($P$< 0.01) with respect to drug. ETO: Etoposide ; GO :Ginger oil, n=5.

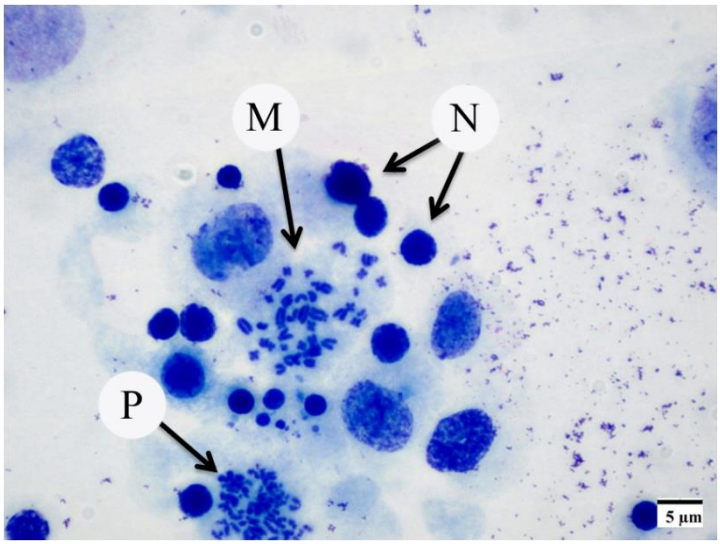


**Fig-3: Photomicrograph for rat's bone marrow chromosomal preparations (Giemsa stain) showing mitotic index and illustrating stages of cell cycle in bone marrow where, metaphase (M), prophase (P) and resting cells (N).**

**Biochemical assay**

Table (3) showed that treatment with ginger oil at 75 and 150 mg/kg had negligible effect on MDA and NO as indicators for oxidative stress, however administration of etoposide resulted in a significant increase in oxidative stress biomarkers namely MDA and NO (2.42 ±0.05 and 24.25 ±0.41 nmole/mg), respectively, compared to untreated control (1.05±0.07 and 14.42±0.46 nmole/mg). However, combining GO at a dose of 75 or 150 mg/kg with etoposide significantly reduced MDA levels by 72% and 52% and markedly reduced NO level by 90% 60%, respectively.

Analysis of the antioxidant metabolizing enzymes revealed a significant reduction in SOD, catalase, GSH levels by 26%, 55% and 32% in etoposide treated animals, respectively, as compared to control and GO treated rats. Pre-treatment of Etoposide intoxicated rats with GO (75 and 150 mg/kg) significantly restored the catalase enzyme activity to those of GO treated or control rats. However, the effect of low and high dose GO pretreatment was less prominent on SOD activity and reduced the levels by 53% and 19.2%, respectively. Interestingly, combining low dose GO with etoposide markedly increased GSH levels by 68%, while high dose GO failed to improve the GSH status in etoposide treated animals.

**Table-3: Changes in biochemical assay in liver of rats treated with etoposide and the effect of combined ginger oil treatment.**

| Groups | Malondialdehyde (nmol/mg protein) | Nitric oxide (nmol/mg protein) | Superoxide dismutase (nmol/mg protein) | Catalase (nmol/min/mg ptotein) | Glutathione (nmol/mg protein) |
|---|---|---|---|---|---|
| **Control** | 1.05 ±0.07 | 14.42 ± 0.46 | 48.45±0.20 | 12.27 ± 0.16 | 2.12 ± 0.08 |
| **ET** | 2.42 ±0.05* | 24.25 ± 0.41* | 15.02 ± 0.20* | 5.42 ± 0.31* | 1.43 ± 0.09* |
| **G.O. (75mg/kg)** | 1.18 ±0.15 | 15.38 ± 0.71 | 19.93 ±0.20 | 10.44 ± 0.21* | 2.18 ± 0.24 |
| **G.O.(150mg/kg)** | 1.07 ±0.13 | 14.68 ± 0.91* | 22.18 ± 0.31 | 7.96 ± 0.21* | 1.96 ± 0.7 |
| **G.O. (75mg/kg) +ET** | 1.42 ±0.08# | 15.15 ±0.14# | 20.35 ±0.16# | 9.11 ± 0.24# | 1.90 ± 0.07# |
| **G.O. (150mg/kg) +ET** | 1.70 ±0.11# | 18.27 ± 0.40# | 19.67 ±0.71# | 6.74 ± 0.18# | 1.52 ± 0.20 |

Data were represented as Mean ±S.D. *Statistically significant *(P*< 0.01) with respect to control. # Statistically significant (*P*< 0.01) with respect to drug. ETO: Etoposide; GO :Ginger oil, n=5.

**DISCUSSION**

The current study indicated that animals treated with etoposide for three 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 etoposide. The treatment with etoposide may caused apoptosis through DNA cleavage.[48] Etoposide caused chromosomal structural aberrations during chromosome condensation by

disruption of the cleavable complex formed by the binding of topo II to the DNA and prevention of the DNA-strand rejoining activity of topo II**.** [49] Similarly to our findings, etoposide induced apoptosis by production of reactive oxygen species, lipid peroxidation, DNA intercalation and cross-linking, cell membrane damage and p53 induction in various tissues.[50] Etoposide induced tumor cell death by induction of apoptosis and DNA damging via singling through plasma membrane lipids rafts involving the death receptor pathway and this caused damage DNA of normal cell also**.**[50] Etoposide -induced DNA damage is recognized by DNA-PK, which in turn activates p53 by phosphorylation. [51] Activated p53 increases the transcription of the gene encoding the pro-apoptotic protein Bax which accumulates in the cytosol and undergoes a conformational change that facilitates its translocation to the mitochondria**.** Topoisomerase II prevent re-ligation of DNA strand break, these breaks lead to chromosomal aberrations and non homologous recombination which caused cell death. [52,53]

The chemotherapy induced hepatic sinusoidal damage is by deposition of collagen in the perisinusoidal space, fibrosis.[54] Etoposide treatment revealed slightly enhanced changes in the liver these include increased heterochromatin and presence of a small nucleolus in the nucleus which refer to nuclear condensation and dense cytoplasm[55]**,** it also induces liver cells apoptosis by cytochrome-c release and caspase-3 release activation and causes hepatotoxicity. Administration of etoposide decreased lipid-standardized antioxidants, this caused an enhanced breakdown of these antioxidants and significantly increased lipid hydroperoxide concentrations in serum and free radical formation that may lead to oxidative stress our results. The oxidative activation of etoposide can be brought by the array of enzymes, which include CYP450 monoxygenases, prostaglandin synthetase and tyrosinase, which ultimately manifest into cytotoxicity**.**[56] Lipid peroxidation, lead to peroxidative damage of cellular lipid content it caused cellular damage by reaction of free radicals with lipids, this caused release of products as malondialdehyde, hydrogen peroxide and hydroxyl radicals.[57] Accumulation of these highly reactive free radicals and eventually generate reactive oxygen species lead to detrimental effects in different tissues. Etoposide can generate reactive oxygen species (ROS) i.e., H2O2 from O2 semiqunione or phenoxyl radicals at lower levels that can induce significant levels of antioxidant enzymes, it can serve “purposeful” roles as "regulators” of cell function**.** [58] The reduced levels in the measured biochemical parameters after treatment with etoposide might reflect the damage caused to the antioxidant defense system and consequently its capacity to neutralize the reactive oxygen species generated during the metabolic process, however the pre-treatment with a low and high dose of ginger oil promoted recovery of the antioxidant enzymes, GSH and reduced oxidative stress. Thus, suggesting that GO can enhance the antioxidant detoxification mechanism providing a favorable protective effect against etoposide liver damage.[59] Etoposide-induced formation of lipid peroxyl (LPx) radicals and accumulation of LPx products in the liver, etoposide caused the attack by free radicals and (ROS) and reducing the utilization of GSH and sulfhydryl groups of proteins might in further cascade of reaction lead to reactive thiol radicals.[60] The decreased activities of SOD and CAT may be a response to increased production of H2O2 and O2 by the auto-oxidation of the excess of glucose and non-enzymatic glycation of proteins.[61]

Ginger (*Zingiber officinal*) is used in traditional oriental medicine this is due to the major pungent constituent, [6]- Gingerol and 6-shogaol, so it have biochemical activities.[62] The phenolic contents of gingerols and shogaol in ginger have antioxidative characteristic, since it can scavenge superoxide anion and hydroxyl radicals.[63,64,65,66] Our study revealed that ginger treatment decreased the frequency of chromosomal aberration, increased mitotic index and improved DNA fragmentation. This is in parallel with the finding of [24] who illustrated the protective effect of ginger against cyclophosphamide - induced cytotoxicity, and paracetamol-induced genotoxicity.[67] Ginger can exerts antigenotoxic effect against Taxol drug especially in the total number of the chromosomal aberrations and the number of micronuclei .[68] The phytochemistry of ginger includes components that can scavenge free radicals produced in biological systems. For the purpose of energy production, some free radicals which generated during the process of oxidation are essential but increased production of free radicals results in oxidative stress that can lead to DNA damage.[69] The essential oil and oleoresin of *Zingiber officinale* exhibited significant antioxidant and antimicrobial activities.[70] This study showed that administration of ginger to rats for three times weekly improved the enzymes in rat's liver by enhanced ROS production or attenuated ROS-scavenging capacity. This may be due to the presence of many antioxidant compounds, such as gingerols, shogaols, phenolic, ketone derivatives, volatile oils and flavonoids of ginger. These antioxidant compounds may modulate the antioxidant enzymes in rats and protected the liver tissue from oxidative damage**.**[65] Ginger prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant and reacts with the basic cellular constituents - proteins, lipids, RNA and DNA by direct radical scavenging capacity.[71,72] Ginger extract prevented lipid peroxidation by enhancing SOD, CAT.[73] and that support our finding of enhanced antioxidants in liver tissues treated with ginger oil**.** All these mechanisms support our finding that illustrated

the protective effect of ginger oil against etoposide genotoxicity. On the contrary, our results showed that high dose of ginger increased chromosomal aberrations, decreased cell division and increased DNA damage and that may be due to the presence of gingerol (found in ginger) which inhibited ascorbate/ferrous complex induced lipid peroxidation in rat liver microsomes.[74] On the contrast of these results, ginger is generally considered as a safe herbal medicine of insignificant adverse side effects.[75,76] and can be classified as antimutagenic agents.[77]

## CONCLUSION

The present study concluded that ginger oil has a protective potential against etoposide-induced genocytotoxicity in male albino rats.

## REFERENCES


1. Hande, K.R. (1998). Etoposide: Our decades of development of a topoisomerase II inhibitor. Eur. J. Cancer, 34 (10), 1514–21.
2. Gordaliza, M. (2004). Derivatives cytotoxic distribution, sources, applications and new Podophyllotoxin Toxicon. Castro, 44 (4), 441–59.
3. Longe, J.L. (2014). A guide to Cancer and Its treatments. Detroit: Thomson Gale, Gale Encyclopedia Of Cancer, 1, 397–399.
4. Joshi, M., Sodhi, K.S., Pandey, R., Singh, J., Goyal, S., Prasad, S., Kaur, H., Bhaskar, N., & Mahajan, S. (2014). Cancer chemotherapy and hepatotoxicity. An update Original Article, 4(6), 2976-2984.
5. Walles, S.A., Zhou, R., & Liliemark, E. (1996). DNA damage induced by etoposide; a comparison of two different methods for determination of strand breaks in DNA**.** Cancer Lett, 105(2), 153-9.
6. Marchetti, F., Pearson, S.F., & Jack, B.B. (2005). Etoposide induces chromosomal abnormalities in mouse spermatocytes and stem cell spermatogonia. Oxford Journals, Medicine & Health, 21(4): 888-895.
7. Lähdetie, J., Keiski, A., & Suutari, A. (1994). Etoposide (VP-16) is a potent inducer of micronuclei in male rat meiosis: Spermatid micronucleus test and DNA flow cytometry after etoposide treatment. Enviromental and Molecular Mutagenesis, 24(3), 192-202.
8. Choudhury, R.C., Palo, A.K., & Sahu, P. (2004). Cytogenetic risk assessment of etoposide from mouse bone marrow. US National Library of Medicine National Institutes of Health, 24(2), 115-22.
9. Krishna, G, Nath, J, Petersen, M, & Ong,T. (1987). Cyclophosphamide-induced cytogenetic effects in mouse bone marrow and spleen cells in in vivo and in vivo/in vitro assays. Teratog Carcinog Mutagen, 7(2), 183-95.
10. Larripa, I.B., Carballo, M.A., Mudry, M.M., & Labal de Vinuesa, M.L. (1992). Etoposide and Teniposide: In invivo and in vitro genotoxic sudies. Original Research Article, 4(5), 365–375.
11. Pratibha, R., Sameer, R., Rataboli, P.V., Bhiwgade, D.A., & Dhume, C.Y. (2006). Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. European Journal of Pharmacology, 532(3), 290-3.
12. Kamblen, P.R., Kulkarni, S., & Bhiwgade., D.A. (2012). Comparitive studies of glutathione-s – transferase kinetics in control , cisplatin and etoposide treated hepatic and renal tissue of male rat. International Journal of Pharmacy and Pharmaceutical Sciences, 4(1), 121-123.
13. Mansour, H.H., Hafez, H.F., & Fahmy, N.M. (2006). Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. Journal of Biochemistry and Molecular Biology, 39(6), 656-661.
14. Gupta, A., Mittal, B., Natu, S.M., Prasad, R., & Srivastava, S. (2010). Oxidative stress in non-small cell lung cancer patients after chemotherapy: association with treatment response. Respirology, 2, 349-56.
15. Srivastava, A.N., Gupta, A., Srivastava, S., Natu, S.M., Mittal, B., Negi, M.P., & Prasad, R.. (2010). Cisplatin combination chemotherapy induces oxidative stress in advance non small cell lung cancer. Asian Pac J Cancer Prev, 11(2), 465-71.
16. Okuyan, B., Izzettin, F.V,Ozakpinar, O.B., Turan, P., Ozdemir, Z.N., Sancar, M., Cirakli, Z., Clark, M., & Ercan, F. (2012). The effects of Ginkgo biloba on nephrotoxicity induced by cisplatin-based chemotherapy protocols in rats. IUFS J Biol, 71(2), 103-111.
17. Kamble, P., Kulkarni, S., & Dayanand, A. (2013**).** Ultrastructural and antioxidant studies of etoposide treated kidney of rat, Journal of Cancer Science &Therapy, 5:137-141.
18. Martins, N.M., Santos, N.A., Curti, C., Bianchi, M.L., & Santos, A.C. (2008). Cisplatin induces mitochondrial oxidative stress with resultant energetic metabolism impairment, membrane rigidification and apoptosis in rat liver. J Appl Toxicol, 28(3), 337-44.
19. Zhou, H.L., Deng, Y.M., & Xie, Q.M. (2006). The modulatory effects of the volatile oil of ginger on the cellular immune response in vitro and in vivo in mice. Journal of Ethnopharmacology**,** 105(1-2), 301-305.
20. Poorrostami, A., Farokhi, F., & Handari, R. (2014). Effect of hydroalcoholic extract of ginger on the liver of epileptic female rats treated with lamotrigine.Avicenna J Phytomed, 4(4), 276–286.
21. Yemitan, O.K., & Izegbu, M.C. (2006). Protective effects of *Zingiber officinale (Zingiberaceae)* against carbon tetrachloride and acetaminophen-

indatotoxicity in rats).Phytother Res, 20(11), 997-1002.
22. Nirmala**,** K.**,** Krishna**,** T.P., & Polasa, K. (2007). Protective effect of ginger against Benzo(a)pyrene induced DNA damage. International Journal of Cancer Research, 3, 13-24.
23. Sakr, S.A., Hassab El Nabi, S.E., Okdah, Y.A, El-Garawani, I.M, & El-Shabka, A .M. (2016). Cytoprotective effects of aqueous ginger (*Zingiber officinale)* extract against carbimazole-induced toxicity in albino rats). European Journal of Pharmaceutical and Medical Research, 3(7), 489-497.
24. Yadamma, K., & Devi, K. R. (2014). In vivo protective effects of ginger extract on cyclophosphamide induced chromosomal aberrations in bone marrow cells of swiss mice. International Journal of Pharmacological and Pharmaceutical Sciences , 1(12), 302-307.
25. AL-Sharif, M.M.Z. (2011). Effect of therapeutic ginger on genotoxic of taxol drug (anti-cancer) in bone marrow cell of male mice. Journal of Life Sciences, 5, 897-905.
26. Attia, A.M.M, Ibrahim, F.A.A., Nabil, G.M., & Aziz, S.W. (2013). Antioxidant effects of ginger (Zingiber officinale Roscoe) against lead acetate-induced hepatotoxicity in rats. African Journal of pharmacy and pharmacology, 7(20), 1213-1219.
27. Reddy, Y.A., Chalamaiah, M., Ramesh, B., & Indira, P. (2014). Ameliorating activity of ginger (*Zingiber officinale*) extract against lead induced renal toxicity in male rats. J Food Sci Technol, 51(5), 908–914.
28. Ezz, M.K., Hamdy, G. M., Abd El Atti, R.M. (2011). The synergistic hepatoprotective effect of curcumin and ginger against Carbon Tetrachloride induced- liver fibrosis in rats. Australian Journal of Basic and Applied Sciences, 5(9), 1962-1971.
29. Abd-Allah, G.A., EL-Bakry, K.A., Bahnasawy, M.H., & El-Khodary, E.R. (2016). Protective effects of curcumin and ginger on liver cirrhosis induced by Carbon Tetrachloride in Rats. International Journal OF Pharmacology, 12, 361-369.
30. Sakr, S.A, Mahran, H.A, & Lamfon, H.A. (2011). Protective effect of ginger (*Zingiber officinale)* on adriamycin - induced hepatotoxicity in albino rats. Journal of Medicinal Plants Research, 5(1), 133-140.
31. Yusof, Y., Ahmad, N., Das, S., Sulaiman, S., & Murad, N.(2009). Chemopreventive efficacy of ginger *(Zingiber officinale)* in ethionine induced rat hepatocarcinogenesis. African Journal of Traditional, Complementary and Alternative Medicines, 6(1), 87-93.
32. Ahmad, N., Sulaiman, S., Mukti, N.A., Murad, N.A., Abd Hamid, N.A., & Yusof Y.A.M. (2006). Effects of ginger extract (*Zingiber officinale Roscoe*) on antioxidant status of hepatocarcinoma induced rats. 8(2), 85-90.
33. Rout, S., Kumar, A. ,& Rath, B. (2015). In vivo Study of Aqueous Extract of *Zingiber officinale* in modulating DMBA induced genotoxicity in albino Rats. American Journal of Phytomedicine and Clinical TherapeuticsAJPC, 3(4), 330-338.
34. Shanmugam, K.R., Mallikarjuna, K., Nishanth, K., Kuo, C.H., & Reddy, K.S. (2011). Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues. Food Chemistry, 124(4), 1436-1442.
35. Mallikarjuna, K., Sahitya, C.P., Sathyavelu, R.K., & Rajendra W. (2008). Ethanol toxicity: rehabilitation of hepatic antioxidant defense system with dietary ginger. Fitoterapia, 79, 174-178.
36. Nwozo, S.O., Osunmadewa, D.A., & Oyinloye, B.E. (2014). Anti-fatty liver effects of oils from *Zingiber officinale* and *Curcuma longa* on ethanol-induced fatty liver in rats. Journal of Integrative Medicine, 12(1), 59–65.
37. El-sharaky, A., Newairy, A.S., Kamel, M.A, Eweda, S. (2009). Protective effect of ginger extract against bromobenzene-induced hepatotoxicity in male rats. Food and Chemical Toxicology, 47(7), 1584–1590.
38. Aljanabi, S.M., & Martinez, I. (1997). Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res J, 25, 4692-4693.
39. El-Garawani, I.M., & Hassab El-Nabi, S.E. (2016). Increased sensitivity of apoptosis detection using direct staining method and integration of acridine orange as an alternative safer fluorescent dye in agarose gel electrophoresis and micronucleus test. CJPAS, 10(2), 3865-3871.
40. Hassab El-Nabi, S.E., & Elhassaneen, Y.A. (2008). Detection of DNA damage, molecular apoptosis and production of home-made ladder by using simple techniques. Biotechnol. J, 7(3), 514-522.
41. Evans, H.J. (1976). Cytological methods for detecting chemical mutagens. Chemical mutagens, principles and methods for their detection, Hollaender, A. (ed) Plenum Press, New York and London, 4, 1-29.
42. Evans, E.P., Breckon, G. & Ford, C.E. (1964). An air-drying method for meiotic preparations from mammalian testes. Cytogenet Cell Genet J, 3, 289–294.
43. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95, 351-358.
44. Granger, D.L., Taintor, R.R., Boockvar, K.S., & Hibbs, J.B. (1996) (Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction). Methods Enzymol, 268, 142-151.

45. Lubinsky, S.G.C.(1979). Genetics of catalase in Drosophila melangaster rates of synthesis and degradation of the enzyme in flies aneuploid and cuploid for the structural gene. Genetics, 19, 723-749.
46. Nishikimi, M., Appaji, N., & Yagi, K. (1972). The occurrence of superoxide anion in thereaction of reduced phenazine methosulfate and molecular oxygen). Biochem Biophys Res Commun, 46, 849-854.
47. Moron, M.S, Despierre, J.W., & Minnervik, B. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochimica Biophysica Acta-Biomembranes, 582, 67-71.
48. Buttike, T., & Sandstrom, P.A. (1994).Oxidative stress as mediator of apoptosis. Immunol.Today, 15, 7-10.
49. Kallio M., & Lähdetie, J. (1997). Effects of the DNA topoisomerase II inhibitor merbarone in male mouse meiotic division invivo: cell cycle arrest and induction of aneuploidy. Environ Mol Mutagen, 9, 16-27.
50. Dimanch-Boitrel, M.T, Meurette, O., Rebillard, A., & Lacour S. (2005). Role of early plasma membrane events in chemotherapy-induced cell death. Drug Resist update, 8(1-2), 5-14.
51. Karpinich, N.O., Tafani, M., Rothman, R.J., Russo, M.A., & Farber, J.L. (2002). (The course of etoposide-induced apoptosis from damage to DNA and p53 activation to mitochondrial release of cytochrome c). J Biol Chem, 277, 16547–16552.
52. Pommier, Y., Kerrigan, D., & Civey, J.M. (1988). Sister chromatide exchanges and chromosomal aberration and cytotoxicity produced by antitumour topoisomerase II inhibitor in sensitive(DC3F) and resistant (DC3F/(-OHE) Chinese hamster cell. Cancer Res, 48, 512-516.
53. Khun, F., Sherwani, A.F., & Afzal, M. (2009). Chromosomal aberration and micronucleus studies of two topoisomerase (II) targeting anthracycline. J Environ.Biol, 39(3), 173-174.
54. Jardim, D.L., Rodrigues, C.A., Novis, A.S., Rocha, V., & Hoff, M. (2012). Oxaliplatin-related thrombocytopenia. Annals of oncology, 23(8), 1937-1942.
55. Remmer, H., & Merker, H.J. (1965). Effect of drugs on the formation of smooth endoplasmic reticulum and drug-metabolizing enzymes. Ann. NY.Acad Sci, 123, 79-91.
56. Kagan, V.E., Kuzmenko, A.I., Tyurina, Y.Y., Shvedova, A.A., & Matsura, T., Yalowich, J.C. (2001). Pro-oxidant and antioxidant mechanisms: of etoposide in HL-60 cells: Roleof myeloperoxidase). Cancer Res, 61, 7777-778.
57. Halliwell B., Chiricos S.(1993). Lipid peroxidation :it is mechanism,measurement and significance.Am J Clin Nutr, 57, 715-724.
58. Thannickal, V.J., & Fanburg, B.L. (2000). Reactive oxygen species in cell signaling). Am.J.Physiol. Lung. Cell Mol Physiol, 279, 1005-1028.
59. Katki, A.G., Kalyanaraman, B., & Sinha, B.K. (1987). Interaction of the antitumor drug, etoposide, with reduced thiols in vitro and in vivo.Chem. Biol Interact, 62, 237-47.
60. Tyurina, Y.Y., Tyurin, V.A., Yalowich, J.C., Quinn, P.J., Claycamp, H.G., Schor, N.F., Pitt, B.R., & Kagan, V.E.(1995). (Phenoxyl radicals of etoposide (VP-16) can directly oxidize intracellular thiols: Protective versus damaging effects of phenolic antioxidants. Toxicol Appl Pharmacol, 131, 227-288.
61. Argano, M., Brignardello, E., Tamagno, E., Gatto, V., Danni, O., Boccuzzi G. (1997). Dehydroeppiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. J Endocrinol, 155, 233-240.
62. Lee, E., & Surh, Y.J. (1998). Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. Cancer Lett, 134, 163-168.
63. Chakraborty, D., Mukherjee, A., Sikdar, S., Paul, A., Ghosh, S., & Khuda-Bukhsh, A.R. (2012). [6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice.Toxicology. Lett, 210(1), 34 –43.
64. Young, H.Y., Luo, Y.L., Cheng, H.Y., Hsieh, W.C., Liao, J.C., & Peng, W.H. (2005). Analgesic and anti-inflammatory activities of [6]-gingerol. Journal of Ethnopharmacology, 96(1–2), 207–210.
65. Cao, Z.F., Chen, Z.G., Guo, P., Zhang, S.M., Lian, L.X., Luo, L., & Hu, W.M. (1993).Scavenging effects of ginger on superoxide anion and hydroxyl radical). Chung-Kuo Chung Yao Tsa Chih, 8, 750–764.
66. Krishnakantha, T.P, & Lokesh, B.R. (1993). Scavenging of superoxide anions by spice principles. Indian J Biochem. Biophys, 30, 133-134.
67. Salah, S.H., Abdouh, S. & Abdel Rahim, E.A. (2012). Effect of *Zingiber Officinale* on paracetamol-induced genotoxicity in male rats. Journal of Medicinal Plants Research, 6(41), 5425-5434.
68. AL-Sharif, M.M.Z. (2011). Effect of therapeutic ginger on genotoxic of taxol drug (Anti-Cancer) in bone marrow cell of male mice. Journal of Life Sciences, 5, 897-905.
69. Ramaa, C.S., Shirode, A.R., Mundada, A.S. & Kadam, V.J. (2006). Nutraceuticals an emerging era in the treatment and prevention of cardiovascular diseases. Curr Pharm Biotechnol, 7, 15–23.
70. Bellik, Y. (2014). Total antioxidant activity and antimicrobial potency of the essential oil and

oleoresin of *Zingiber officinale Roscoe*. Asian Pac J Trop Dis, 4, 40–44.

71. Sabina, E.P., Pragasam, S.J., Kumar, S., & Rasool, M. (2011). 6-gingerol, an active ingredient of ginger, protects acetaminophen-induced hepatotoxicity in mice. Zhong Xi Yi Jie He Xue Bao, 9, 1264–9.
72. Ajith, T.A., Hema, U., Aswathy, M.S. (2007). *Zingiber officinale Roscoe* prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status. Food Chem Toxicol, 45, 2267-2272.
73. Ajith, T.A., Jose, N., & Janardhanan, K. (2006). Amelioration of cisplatin induced nephrotoxicity in mice by ethyl acetate extract of polypore fungus, phellinus rimosus). Journal of Experimental and Clinical Cancer Research, 21, 487-491.
74. Reddy, A.C.P, & Lokesh, B.R. (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol. Cell. Biochem, 111(1-2), 117–124.
75. Weidner, M.S., & Sigwart, K. (2000). The safety of a ginger extract in the rat. J. Ethnopharmacol, 73, 513-520.
76. Langner, E., Greifenberg, S., Gruenwald, J. (1998). Ginger: history and use) Advances in Therapy, 15(1), 25–44.
77. Kuroda, Y., Jain, A.K., Tezuka, H., Kada, T. (1992). Antimutagenicity in cultured mammalian cells. Mutat. Res, 267, 201-209.