**Effects of Ginger Extract (Zingiber officinale Roscoe) on Antioxidant Status of Hepatocarcinoma Induced Rats**

Norliza Ahmad¹, Suhaniza Sulaiman¹, Nor Ashikeen Mukti¹, Nor Azian Murad², Noor Aini Abd Hamid¹ and Yasmin Anum Mohd Yusof¹

¹Department of Biochemistry, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, ²Centre of Lipids, Engineering and Applied Research, Universiti Teknologi Malaysia, Kuala Lumpur, Malaysia

**Abstract**

Ginger or *Zingiber officinale* which is used in traditional medicine has been found to possess antioxidant effect that can control the generation of free radicals. Free radical level has been reported to be high in cancer cells. The aim of this study was to observe the effect of ginger extract on antioxidant status in rats induced with liver cancer. Twenty-six male Wistar rats were divided into 5 groups: 2 control groups were fed with normal rat chow and olive oil respectively. Treatment groups consisted of rats fed with ginger extract at 100 mg/kg body weight (ginger group), rats induced with liver cancer by choline deficient diet plus ethionine in drinking water (CDE group) and rats with CDE diet plus ginger extract (CDE + ginger group). Blood samples were taken from the orbital sinus at 0 and 8 weeks of experiment for the determination of antioxidant enzyme activities and MDA level. Comparison between the control group and the CDE group showed significant increase (*p*<0.05) in SOD activity at 8 week of experiment, whereas no significant differences were observed in the activities of GPx, catalase and the MDA level. Antioxidant effect of ginger extract was observed by the significant decrease (*p*<0.05) in SOD activity and the level of MDA in CDE group after treatment with ginger when compared to CDE group alone and with the control group. Catalase activity increased significantly (*p*<0.05) in CDE group after supplementation with ginger at week 8 of experiment when compared to CDE group alone, while there were no significant changes observed for GPx. From this study, it can be concluded that free radicals are increased in liver carcinogenesis (as evidenced by an increase in SOD activity) and ginger extract exhibits anticancer effect by scavenging the free radicals (decrease in SOD activity).

**Keywords:** *Zingiber officinale*, antioxidant, hepatocarcinoma induced rats.

**Introduction**

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world with an annual incidence of 250,000 to 1,000,000 cases worldwide [1]. In Malaysia, HCC is ranked 11th in malignant diseases reported [2]. Hepatitis B (HBV) and Hepatitis C virus (HCV) are the risk factors attributed to 80% of HCC cases globally [3]. Cirrhosis, radiation, free radicals, genetic changes, metabolic disorder and exposure to certain chemical carcinogens such as aflatoxin, ethionine, diethylnitosamine (DEN) and 2-acetylaminofluorene (AAF) have also been implicated in the pathogenesis of hepatocarcinoma [4-5].

Continuous production of oxygen radicals leads to the formation of covalent bond adduct with DNA nucleic acid, which will subsequently result in mutagenicity. Endogenous antioxidant enzymes such as SOD, GPx and catalase have been reported to reduce the free radical formation and prevent oxidative damage [6-7].

*Zingiber officinale* is the botanical name for ginger, a tropical herbal plant found in abundance in Asia. It belongs to the family of *Zingiberaceae* [8]. Generally it is widely used as a spice in traditional and modern cookings [8], but the use of ginger is also common in Chinese, Ayurvedic and Tibb Unani medicine [9]. Biochemically, the main active components in ginger from its phenolic substance are gingerol and shogaol, while zingiberene is obtained from ginger oil [8].

Ginger extract has been extensively studied for its pharmacological and biological activities such as antibacterial, anticonvulsion, analgesic, antiulcer, antitumour, antifungal and antiallergen [10]. Previous studies have shown that ginger could act as an antioxidant. Aeschbach et al. [11] reported that gingerol inhibited lipid peroxidation induced by FeCl₃-ascorbate system. A study by Chang et al. [12] showed that gingerol inhibited the oxidation activity of xanthine which generated reactive oxygen species (ROS), for example superoxide anions. Based on the data from an in vivo study by Jeyakumar et al. [13] on rats given high fat diet, ginger supplementation managed to increase the concentration of SOD and catalase in the tissues while the level of oxidized glutathione was decreased. A recent in vivo study by Manju and Nalini (2005) [14] reported that ginger supplementation...
suppressed colon carcinogenesis induced by a procarcinogen, dimethylhydrazine (DMH). There were not many studies that have explored the effect of ginger extract on the status of antioxidant in liver carcinogenesis. This study focused on the effect of ginger extract on antioxidant status in rats induced with hepatocellular carcinoma.

Materials and Methods

Animal and diet

A total of 26 male Wistar albino rats were supplied by Animal Care Unit, Universiti Kebangsaan Malaysia (Kuala Lumpur, Malaysia). The study was approved by the Animal Ethics of Faculty of Medicine, Universiti Kebangsaan Malaysia. At the time of study, the rats were 3-4 months of age and weighed in the range of 200-250 g. They were kept in polycarbonate cages in a room at optimum temperature, humidity and light-dark-cycle. They were divided into 5 groups according to different diets. The first group which served as the control group consisted of 4 rats and was given normal rat chow. The second group consisted of 4 rats given olive oil. The third group, consisted of 6 rats and given ginger extract at 100 mg/kg body weight dosage. The fourth group of 6 rats was fed with a diet deficient in choline (ICN Biochemicals, USA) plus 0.1% ethionine (Sigma Chemical Co., USA) in drinking water in order to induce liver cancer. The last group of 6 rats were also induced with liver cancer, but treated with ginger extract at 100 mg/kg body weight. Blood sample was taken from the rats via the orbital sinus at 0- and 8-week for the analysis of endogenous antioxidant enzymes (SOD, GPx and catalase), and for the measurement of lipid peroxidation active metabolite (MDA).

Zingiber officinale

Ethanolic crude extract of ginger was kindly supplied by Dr. Nor Azian Murad of Centre of Lipids, Engineering and Applied Research, Universiti Teknologi Malaysia, Kuala Lumpur, Malaysia. The ginger oleoresin extract must first be solubilised with olive oil to facilitate force-feeding of the respective rat groups at 100 mg/kg body weight dosage.

Preparation of blood sample for enzyme assays

Blood obtained from the orbital sinus was collected in test tubes with heparin to prevent blood coagulation, and the plasma was separated. The blood samples were then rinsed with the same volume of 0.9% NaCl (normal saline) and centrifuged at 3000 rpm for 10 minutes at 4 °C. The upper layer was removed and the above procedure was repeated with 0.9% NaCl until it became clear. The lower layer, termed hemolysate was then used for the antioxidant enzyme assays.

Superoxide dismutase (SOD) activity measurement

Superoxide dismutase activity was measured according to the method of Beyer and Fridovich [15]. Reaction mixture consisting of 50 mM potassium phosphate buffer pH 7.8, 0.1 mM EDTA, 9.9 mM L-methionine, 5.7 x 10⁻⁵ nitro blue tetrazolium (NBT) and 2.5 x 10⁻² % (w/v) Triton X-100 (all purchased from Sigma Chemical Co., USA) and riboflavin (0.01 ml of 4.4%) were freshly prepared on the day of assay. 1.0 ml of reaction mixture was added to 20 µl of hemolysate in a cuvette to initiate the reaction which was then placed in a box illuminated with 20 W neon lamp for 7 min. The reduction of NBT was measured at 560 nm in a Shimadzu-UV 160A spectrophotometer. Results were calculated by subtracting the changes in absorbance of the blank from the sample and dividing ∆ A by 0.012 to obtain the McCord-Fridovich units of activity.

Glutathione peroxidase (GPx) activity measurement

Glutathione peroxidase measurement was determined using the method of Paglia and Valentine [16] at 340 nm spectrophotometrically. Both the blank and the system cuvettes contained 0.1 M KPO₄ buffer (pH 7.0), 2 µM EDTA, 10 units/ml glutathione reductase, 4 mM sodium azide, (Sigma Chemical Co., USA), 200 mM NADPH (Calbiochem, USA) and hemolysate. In addition, the system cuvette contained 1.0 mM glutathione, GSH (Sigma Chemical Co., USA). After 10 minutes of pre incubation at 37° C, the reaction was started by adding 1.0 mM H₂O₂ (Sigma Chemical Co., USA) to the blank and system cuvettes. An additional blank assay in which the buffer was substituted for the hemolysate was performed in order to correct the non enzymatic oxidation of GSH and NADPH by H₂O₂.

Catalase (CAT) activity measurement

Catalase activity was measured following the method of Aebi [17]. 50 mM phosphate buffer (pH 7.0) and 30% H₂O₂ were freshly prepared on the day of assay. 2 ml of sample solution was mixed with 1 ml of H₂O₂, and the decomposition of hydrogen peroxide was measured spectrophotometrically at 240 nm against a blank containing 2 ml of sample solution and 1 ml of phosphate buffer. To avoid the intervention by bubbling, the reaction time was controlled by not exceeding 30 s.

Determination of lipid peroxidation (MDA)

Plasma lipid peroxidation was determined by the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Ledwozyw et al. [18]. Malondialdehyde is formed as an end product of lipid peroxidation which reacts with TBA reagent under acidic condition to generate a pink coloured product. Plasma (0.1 ml) was added to 0.4 ml of distilled water, followed by the addition of 2.5 ml of trichloroacetic acid
(TCA) and later left at room temperature for 15 minutes. TBA (1.5 ml) was then added and heated in a water bath at 100°C for 30 minutes until a faint pink colour was obtained. After cooling, the colour was extracted in 1 ml of butanol and the intensity was measured using the spectrophotometer at EX 515 nm and EM 553 nm. 1,1,1,3 - tetraethoxypropane (Sigma, USA) was used as the standard.

**Statistical analysis**

Data were analysed using the SPSS package. Results are expressed as mean ± SEM with the experiment repeated at least three times. Statistical evaluations were done using the analysis of variance (Anova). A p value of < 0.05 was considered significant.

**Results**

Table 1 summarizes the incidence of liver neoplasms in rats on a low lipotrope diet with ethionine in drinking water and the effect of ginger on cancer incidence. There was no tumor in both controls (normal rat chow and olive oil), and in ginger treated rats. However, in CDE rats the tumor incidence was 100% and the average size of the tumor was 0.5-1 cm. When CDE rats were treated with ginger extract, the tumor incidence reduced to 17%.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats examined</th>
<th>Numbers of rats with tumor</th>
<th>Incidence of tumor (%)**</th>
<th>Tumor size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CDE</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>Olive oil</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ginger*</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ginger + CDE</td>
<td>6</td>
<td>1</td>
<td>17</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* 100mg/kg body weight
**The percentage was calculated by dividing the number of rats with preneoplastic nodules over the total number of rats per group.

The activity of SOD enzyme decreased significantly at week 8 compared with week 0 in all groups except in liver cancer group (CDE), as shown in figure 1. When compared with the control group, SOD activity was found to increase significantly in CDE group at week 8 but no significant changes were observed in ginger extract group and CDE treated with ginger extract. On the other hand, there was a significant decrease of SOD activity in CDE group treated with ginger extract when compared with CDE group alone at week 8 of experiment. The activity of GPx enzyme also showed similar pattern to that of SOD. At week 8, the GPx level was significantly lowered in all groups when compared with week 0 (figure 2). However, no significant changes were observed in CDE group, ginger extract group as well as CDE group treated with ginger extract when compared with the control group, respectively, or when comparing between CDE group treated with ginger extract and the CDE group alone.

Figure 1: Effect of ginger extract on SOD activity in rats treated with choline deficient diet and 0.1% ethionine in drinking water (CDE) to induce liver cancer. Data are expressed as mean ± SEM of 6 animals per group.

Figure 2: Effect of ginger extract on glutathione peroxidase enzyme activity in CDE rats. Data expressed as mean ± SEM of 6 animals per group. * p<0.05 relative to week 0. a p<0.05 relative to control group (normal rat chow or olive oil); a,c p<0.05 relative to CDE group.
Figure 3 shows that there were no significant changes observed in the level of catalase enzyme in the control group, olive oil group and CDE group supplemented with ginger extract at week 8 when compared with week 0. However, the level increased significantly in ginger extract group, but a significant decrease was observed in CDE group between week 8 and 0, respectively. When compared to the control group, there was a significant increase in ginger group whereas no significant changes in catalase activity were observed in CDE group alone and CDE group treated with ginger. On the other hand, a significant increase was observed in CDE group treated with ginger extract when compared with CDE group alone at week 8.

The level of MDA was found to increase significantly in the control group, olive oil group as well as in CDE group at week 8 when compared with week 0, whereas no significant change was observed in ginger group (figure 4). On the contrary, the level of MDA was found to decrease significantly in CDE + ginger group at week 8 of experiment. Comparison with the control group showed significant decrease in MDA level in ginger group and CDE group supplemented with ginger. Significant decline ($p<0.05$) was also observed when the CDE group treated with ginger extract compared to the CDE group alone.

Discussion

Free radicals and reactive oxygen species (ROS) are continuously produced in the human body. These oxygen species are the cause of cell damage and the progression of tumour cells to cancer cells. Therefore, tissues must be protected from oxidative injury through intracellular (SOD, GPx and catalase) as well as extracellular (vitamins, micronutrients, antioxidants originated from herbs) antioxidants [19].

Results from this study showed significant decrease in all endogenous antioxidant levels at week 8 compared to week 0 in all groups except in the liver cancer induced group. This could be due to the increasing age of the rats. The capacity for free radical detoxification was found to decrease with age as stated by Hazelton & Lang [20].

Significant increase in SOD level in the liver cancer induced rats compared to control rats could probably be a response to the build-up of superoxide radicals which took place during cancer cell progression. This is in agreement with Cerutti [21] who stated that the production of free radicals increased in cancerous condition. Additionally, Capel et al. [22] showed that antioxidant enzyme activity increases accordingly to control the high production of free radicals in cancer cells. SOD activity in liver cancer induced group which was fed with ginger extract was found to decrease at week 8. This indicates that ginger extract may have antioxidant effect by replacing SOD activities and reducing the level of superoxide radicals in liver cancer induced rats. This is similar to the findings of Park et al. [23], in which the bioactive component in ginger reduced the production of ROS such as superoxide anions. However, SOD activity in the control group at week 8 was also found to decrease significantly in comparison to week 0 and this could be attributed to low antioxidant status in older rats (at 8 week of experiment) compared to younger rats (at 0 week), because during ageing, low enzymic antioxidant status has been reported [24].

The effect of ginger on catalase activity was observed to be different from that of SOD and GPx. The catalase activity was found to increase significantly in CDE group treated with ginger extract at week 8 in comparison to CDE group alone. The ginger extract however did not
Effects of ginger extract on antioxidant status of hepatocarcinoma induced rats

substitute the function of catalase in eliminating \(H_2O_2\), but instead it enhanced the scavenging activity of catalase. This is in agreement with a study by Chandra Mohan et al. and Manju & Nalini which reported that ginger enhanced the catalytic activity of catalase in buccal pouch carcinogenesis induced hamster and colon carcinogenesis induced rats due to its ability to scavenge free radicals and toxic carcinogenic electrophiles [14, 25]. SOD activity in both ginger and control rats was (at week 0 and 8) found to be at similar level, which perhaps explains the fact that the baseline level of enzymic antioxidant in control rats are at maximal level that does not need to be boosted up by the ginger extract.

Results obtained from this study showed that the GPx activity for all groups at week 8 decreased significantly in comparison with week 0. However, there were no significant changes in the GPx activity between the groups at week 8. The absence of GPx activity in CDE group treated with ginger could be due to the compensational relationship between the antioxidant enzymes, catalase and GPx [26]. This phenomenon could probably be due to the fact that both enzymes reacted with the same substrate, namely the \(H_2O_2\) radicals. Even though GPx was said to be more efficient in the scavenging of \(H_2O_2\) [27], the occurrence of this natural balancing of antioxidants only serves to strengthen the defense mechanism of antioxidants [28].

MDA level has been used as the marker for lipid peroxidation in cancer [29-31]. The high level of MDA in cancerous condition could have resulted from the deterioration of antioxidant defense, as studied by Szatrowski and Nathan [32].

At week 8, the level of MDA was found to decrease in ginger extract group as well as in liver cancer rats treated with ginger. This indicated that the ginger extract has successfully lowered lipid peroxidation induced by carcinogen ethionine. This is in accordance with a study by Chang et al. [12] who found the bioactive component of ginger, namely gingerol, possessed antioxidative effect by inhibiting peroxidation of phospholipids induced by xanthine oxidase activity. However, the control group showed significant increase of MDA level at week 8 when compared to week 0. This could have resulted from the normal process of ageing in the rats, therefore the capacity of free radical detoxification was reduced [20].

In conclusion, ginger extract may have bioactive components with antioxidant activity in scavenging free radicals such as superoxide anions and \(H_2O_2\) as well as decreasing the MDA level for the reduction of lipid peroxidation.

Acknowledgement

This study was financially aided by IRPA Grant (06-02-02-0023-PR0008/09-08) from the Ministry of Science, Technology and Innovation (MOSTI), Malaysia.

References

2. Kementerian Kesihatan Malaysia. 2003