Chronic administration of caffeine: Effect on the activities of hepatic antioxidant enzymes of Ehrlich ascites tumor-bearing mice

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Chronic ingestion (for 22-30 consecutive days) of caffeine (20 mg/kg/day, po) increased the activities of the hepatic enzymes- catalase (CAT) and superoxide dismutase (SOD) and decreased its lipid peroxidation (LP) in mice. Development of Ehrlich ascites carcinoma (EAC) cell decreased the activities of hepatic CAT and SOD and increased LP. But pretreatment of caffeine for 12 consecutive days and continuation of its treatment during the course of development of EAC cells restored the EAC cell-induced changes in liver CAT, SOD and LP to their corresponding control values. Thus, the present results by confirming the results of others previously published, suggest that caffeine is an antioxidant and may act as an anticarcinogen.

Caffeine (1,3,7-trimethyl xanthine) one of the methylxanthine compounds has long been known to be a natural ingredient present in coffee, tea and cocoa. At least half of the population of the world has long been consumed coffee as a hot drink. It is a highly lipophilic compound that can elevate mood, decrease fatigue and relieve tension. Caffeine can relax smooth muscle, specially bronchial muscle, stimulate central nervous system (CNS) and cardiac muscle and also acts on kidney as a diuretic. These pharmacological activities of caffeine vary depending on the dose and type of cell on which it acts. It is a well established fact that long-term caffeine intake inhibits the development of the spontaneous as well as inducible tumors.

It is well-known that induction of superoxide radical and related oxygen species cause cell damage, which have been found to be involved in the formation of malignancy. Reactive oxygen free radicals have been also known to damage tissue through lipid peroxidation (LP). Protection of cellular structures from damage by free radicals can be accomplished through enzymatic and non-enzymatic defense mechanisms. Among the free radical metabolizing enzymes, superoxide dismutase (SOD) and catalase (CAT) are of particular importance. SOD catalyses the dismutation of superoxide radical to hydrogen peroxide, thereby eliminating its toxic effect.

Abbreviations: AHH, aryl hydrocarbon hydroxylase; CAT, catalase; CNS, central nervous system; EAC, Ehrlich ascites carcinoma; ESR, electron spin resonance; GST, glutathione-s-transferase, GPx, glutathione peroxidase; LP, lipid peroxidation; SOD, superoxide dismutase.
showing its (caffeine) inhibitory effect of lipid peroxidation against the three reactive species, such as hydroxyl radical (‘OH), peroxyl radical (ROO) and singlet oxygen (‘O_2)\textsuperscript{18}.

In the present investigation, considering all those informations, authors are intend to study the effect of caffeine on the activities of antioxidant enzymes in the course of development of Ehrlich ascites carcinoma (EAC) cells.

Materials and Methods

Chemicals — Caffeine was purchased from Fluka-Chemica-Biochemica (Switzerland). Catalase, glutathione reductase, GSH, NADPH, pyrogallol, ADP were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used in the present study were of analytical grade.

Experimental animals — Adult female Swiss mice (20-25g body weight), kept in a 12 hr dark-12 hr light cycle at room temperature (28\textdegree \pm 0.5\textdegree C) with constant relative humidity (80\pm 5\%) were maintained with standard laboratory diet and water ad libitum.

Transplantation of Ehrlich ascites carcinoma (EAC) Cells — The EAC cells were transplanted from donor mice into recipient mice by intraperitoneal inoculation with 0.2 ml of ascites fluid containing 10^7 cells (approx). This cell concentration of mother ascites fluid from donor mice was made up by sterile normal saline.

Treatment of caffeine in mice with or without EAC cells — Mice were divided into 7 groups. Each group was divided individually into four subgroups a, b, c and d. Each subgroup contains 4 animals. Animals of subgroups 2a, 2b, 2c and 2d were treated with caffeine (20 mg/kg/day, po) in a volume of 0.2 ml for 22, 24, 27 and 30 consecutive days respectively. Animals of subgroups 1a, 1b, 1c and 1d were treated with an equal volume of vehicle (water) of caffeine through the same route for the same period under similar conditions as described in animals of subgroups 2a-2d. The subgroups 1a, 1b, 1c and 1d were considered as control of subgroups of experimental animals 2a, 2b, 2c and 2d respectively. Animals of subgroups 4a, 4b, 4c and 4d were treated with an equal volume of vehicle (water) of caffeine through the same route (ip) with EAC cells after 12 consecutive days of caffeine treatment and the caffeine treatment of the subgroups 6a, 6b, 6c and 6d was continued for another 10, 12, 15 and 18 consecutive days as did in experimental groups and were considered as control of corresponding experimental subgroups 6a, 6b, 6c and 6d respectively, as well as subgroups 7a, 7b, 7c and 7d respectively.

Collection of tissue and cells — Mice of both control and experimental groups were killed by cervical dislocation. Caffeine treated animals were killed 30 min after the last caffeine administration. Liver was perfused immediately with cold 0.15 M KCl, containing 2 mM EDTA (pH 7.4), minced with scissors and the tissues were collected under ice-cold condition.

Preparation of hepatic microsomes — Liver microsomes were prepared according to the method of Mukhopadhyay et al\textsuperscript{20}. Liver tissue was minced in ice-cold 0.25M sucrose solution and homogenized with a teflon-glass homogenizer in the same sucrose solution to have a 10\% (w/v) liver homogenate. The homogenate was centrifuged at 20,000g for 20 min under cold condition (0\textdegree -4\textdegree C) to remove cell debris, nuclear fraction, mitochondria and lysosomal particles as pellet. The supernatant known as post-mitochondrial fraction, was used for SOD assay. This supernatant was also centrifuged under cold condition (0\textdegree -4\textdegree C) at 105,000 g for 60 min in an ultracentrifuge (Hitachi SCP70H) to obtain the desired microosomal fraction as pellet. This microsomal pellet was used for biochemical assay of enzymes (CAT, glutathione peroxidase) and LP.

Biochemical assay — The activity of microsomal CAT (EC 1.11.1.6) was measured according to the method of Cohen et al\textsuperscript{21}, SOD (EC 1.15.1.1) was estimated according to the method of Marklund and Marklund\textsuperscript{22} and Nandi et al\textsuperscript{23} using post-mitochondrial fraction of liver. The activity of microsomal
glutathione peroxidase (GPX) (EC 1.11.1.9) was measured following the method of Pierce and Tappe21. Microsomal LP was estimated according to the method of Sinnhuber et al.25. Protein was measured by following the method of Lowry et al.26 using BSA as standard.

**Statistical analysis**—The statistical significance between the control and experimental values were assessed by analysis of variance (ANOVA).

**Results**

Table 1 demonstrates that there was a significant increase in hepatic (a) CAT activity following 22 (19.46%; F=34.0, df=1,6; P<0.05), 24 (25.49%; F=44.29, df=1,6; P<0.05), 27 (40.0%; F=164.0, df=1,6; P<0.05) and 30 (45.68%; F=2586.0, df=1,6; P<0.05) consecutive days of caffeine (20 mg/kg day, po) treatment with respect to their corresponding control, (b) SOD activity when caffeine (20 mg/kg day po) was administered to mice for 22 (21.63%; F=13.38, df=1,6; P<0.05), 24 (33.01%; F=9.48, df=1,6; P<0.05), 27 (45.28%; F=30.03, df=1,6; P<0.05) and 30 (58.05%; F=69.79, df=1,6; P<0.05) consecutive days with respect to their corresponding control and (c) LP activity of mice liver microsome, on the other hand was significantly decreased following 24 (24.18%; F=20.7, df=1,6; P<0.025), 27 (47.96%; F=175.38, df=1,6; P<0.025) and 30 (58.0%; F=399.18, df=1,6; P<0.025) consecutive days of caffeine treatment with respect to their corresponding control. No significant change in its LP activity was observed following 22 consecutive days of caffeine treatment with respect to its control. Further, it is evident from this table that the caffeine-induced increase in hepatic CAT and SOD activity and decrease in LP were not dependent on the number of days of caffeine treatment. No appreciable change in GPX activity of mice liver was observed under the present experimental condition.

It appears from Table 2 that both CAT and SOD activity of mice liver were significantly decreased during the development of EAC cells for 10 (21.69%; F=77.0, df=1,6; P<0.05 and 24.34%; F=90.88, df=1,6; P<0.05 respectively), 12 (26.65%; F=18.33, df=1,6; P<0.05 and 34.11%; F=19.18, df=1,6; P<0.05 respectively), 15 (49.03%; F=113.0, df=1,6; P<0.05 and 47.14%; F=25.31, df=1,6; P<0.05 respectively) and 18 (54.94%; F=296.67, df=1,6; P<0.05 and 51.75%; F=131.34, df=1,6; P<0.05) days with respect to their corresponding control. The hepatic microsomal LP activity in mice, on the other hand, was significantly increased during the development of EAC cells for 10 (28.24%; F=384.0, df=1,6; P<0.025), 12 (47.25%; F=45.69, df=1,6; P<0.025) and 18 (85.00%; F=478.83, df=1,6; P<0.025) days with respect to their corresponding control. No significant change in hepatic GPX activity was observed under similar condition of EAC cell development.

Table 3 appears to show that the activities of CAT, SOD, GPX and LP in mice liver were not significantly changed when mice were pretreated with the

<table>
<thead>
<tr>
<th>Period of caffeine treatment (days)</th>
<th>Conditions of treatment</th>
<th>CAT activity (mole of ( \text{H}_2\text{O}_2/\text{mg protein/min} ))</th>
<th>GPX activity (mole of ( \text{NADPH oxidized/mg protein/min} ))</th>
<th>SOD activity (unit/mg protein/min)</th>
<th>LP (mole of TBA reactive substances/mg protein/hr) ( \times 10^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>Control</td>
<td>0.154 ± 0.002</td>
<td>0.147 ± 0.006</td>
<td>14.39 ± 2.30</td>
<td>0.82 ± 0.040</td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>0.184 ± 0.010 ( ^a )</td>
<td>0.146 ± 0.009</td>
<td>17.50 ± 0.80 ( ^d )</td>
<td>0.67 ± 0.009 ( ^d )</td>
</tr>
<tr>
<td>27</td>
<td>Control</td>
<td>0.153 ± 0.001</td>
<td>0.145 ± 0.006</td>
<td>15.42 ± 1.20</td>
<td>0.80 ± 0.008</td>
</tr>
<tr>
<td>30</td>
<td>Control</td>
<td>0.192 ± 0.006 ( ^c )</td>
<td>0.151 ± 0.070</td>
<td>20.51 ± 1.82 ( ^d )</td>
<td>0.61 ± 0.040 ( ^d )</td>
</tr>
<tr>
<td>22</td>
<td>Experimental</td>
<td>0.155 ± 0.001</td>
<td>0.131 ± 0.003</td>
<td>16.03 ± 1.09</td>
<td>0.89 ± 0.020</td>
</tr>
<tr>
<td>24</td>
<td>Experimental</td>
<td>0.217 ± 0.014 ( ^d )</td>
<td>0.143 ± 0.005</td>
<td>23.29 ± 1.40 ( ^d )</td>
<td>0.46 ± 0.030 ( ^d )</td>
</tr>
<tr>
<td>27</td>
<td>Experimental</td>
<td>0.162 ± 0.002</td>
<td>0.139 ± 0.009</td>
<td>15.73 ± 0.82</td>
<td>0.88 ± 0.020</td>
</tr>
<tr>
<td>30</td>
<td>Experimental</td>
<td>0.236 ± 0.004 ( ^c )</td>
<td>0.142 ± 0.010</td>
<td>24.86 ± 1.94 ( ^d )</td>
<td>0.37 ± 0.020 ( ^d )</td>
</tr>
</tbody>
</table>

\( ^a \) Caffeine was administered (po) to female mice at a dose of 20 mg/kg/day.

\( ^b \) Control group corresponding to experimental group was treated with vehicle (water) of caffeine under similar conditions.

1 unit of SOD = 50% inhibition of auto-oxidation of pyrogallol.

Significantly different with respect to their corresponding control \( ^* P < 0.05; ^d P < 0.025 \) using Tukey test for ANOVA.
Table 2—Estimation of EAC cell-induced changes in the activities of catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and lipid peroxidation (LP) of female mice liver.

<table>
<thead>
<tr>
<th>Duration of development of EAC cells* (days)</th>
<th>Experimental conditions</th>
<th>CAT activity (mole of H₂O₂/mg protein/min)</th>
<th>GPX activity (mole of NADPH oxidized/mg protein/min)</th>
<th>SOD activity (unit/mg protein/min)</th>
<th>LP (mole of TBA reactive substances/mg protein/hr) × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Control</td>
<td>0.153 ± 0.010</td>
<td>0.147 ± 0.010</td>
<td>16.56 ± 2.10</td>
<td>0.99 ± 0.001</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.120 ± 0.008</td>
<td>0.156 ± 0.042</td>
<td>11.77 ± 0.092</td>
<td>1.27 ± 0.007</td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>0.155 ± 0.009</td>
<td>0.148 ± 0.060</td>
<td>16.36 ± 1.50</td>
<td>0.98 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.114 ± 0.010</td>
<td>0.162 ± 0.020</td>
<td>10.78 ± 0.032</td>
<td>1.37 ± 0.060</td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>0.156 ± 0.007</td>
<td>0.130 ± 0.010</td>
<td>16.14 ± 1.01</td>
<td>0.99 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.080 ± 0.004</td>
<td>0.150 ± 0.031</td>
<td>8.53 ± 1.02</td>
<td>1.66 ± 0.210</td>
</tr>
<tr>
<td>18</td>
<td>Control</td>
<td>0.164 ± 0.004</td>
<td>0.142 ± 0.008</td>
<td>15.89 ± 0.85</td>
<td>0.97 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.074 ± 0.001</td>
<td>0.148 ± 0.020</td>
<td>7.67 ± 0.265</td>
<td>1.70 ± 0.040</td>
</tr>
</tbody>
</table>

*EAC cells were transplanted intraperitoneally from EAC cell bearing mice to recipient mice and allowed to develop for different periods.

**Control group corresponding to experimental group was injected with 0.2 ml saline instead of EAC cells under similar conditions. Significantly different with respect to their corresponding control **P < 0.05; ***P < 0.025 using Tukey test for ANOVA.

All other experimental details are same as in Table 1.

Table 3—Effect of chronic consumption of vehicle of caffeine on EAC cell-induced changes in the activities of catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and lipid peroxidation (LP) of female mice liver.

<table>
<thead>
<tr>
<th>Duration of development of EAC cells* (days)</th>
<th>Experimental conditions</th>
<th>CAT activity (mole of H₂O₂/mg protein/min)</th>
<th>GPX activity (mole of NADPH oxidized/mg protein/min)</th>
<th>SOD activity (unit/mg protein/min)</th>
<th>LP (mole of TBA reactive substances/mg protein/hr) × 10⁻⁵</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 (22)**</td>
<td>Control</td>
<td>0.156 ± 0.009</td>
<td>0.143 ± 0.010</td>
<td>16.40 ± 1.10</td>
<td>0.93 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.115 ± 0.008</td>
<td>0.154 ± 0.005</td>
<td>12.40 ± 0.082</td>
<td>1.18 ± 0.120</td>
</tr>
<tr>
<td>12 (24)**</td>
<td>Control</td>
<td>0.153 ± 0.005</td>
<td>0.148 ± 0.030</td>
<td>16.36 ± 1.05</td>
<td>0.98 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.100 ± 0.010</td>
<td>0.165 ± 0.017</td>
<td>10.16 ± 0.022</td>
<td>1.34 ± 0.050</td>
</tr>
<tr>
<td>15 (27)**</td>
<td>Control</td>
<td>0.155 ± 0.001</td>
<td>0.110 ± 0.007</td>
<td>16.14 ± 1.01</td>
<td>0.99 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.079 ± 0.002</td>
<td>0.129 ± 0.024</td>
<td>8.46 ± 1.004</td>
<td>1.64 ± 0.140</td>
</tr>
<tr>
<td>18 (30)**</td>
<td>Control</td>
<td>0.162 ± 0.003</td>
<td>0.139 ± 0.009</td>
<td>15.89 ± 0.85</td>
<td>0.97 ± 0.030</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.073 ± 0.002</td>
<td>0.145 ± 0.011</td>
<td>7.55 ± 0.18</td>
<td>1.63 ± 0.030</td>
</tr>
</tbody>
</table>

*EAC cells were transplanted intraperitoneally from EAC cell bearing mice to recipient mice which were pretreated with vehicle (water) of caffeine for 12 consecutive days and continued for another 10, 12, 15 and 18 consecutive days.

**Control group corresponding to experimental group was injected with 0.2 ml saline instead of EAC cells under similar conditions.

All other experimental details are same as in Table 1.

vehicle (water) of caffeine for 12 consecutive days and continued for another 10 to 18 consecutive days following treatment with equivalent amount of saline instead of EAC cells with respect to their corresponding control of caffeine treated experimental group (Table 1) as well as EAC cell developing condition (Table 2). Further, it is noted that the activities of liver CAT and SOD were significantly reduced and LP activity was significantly enhanced when mice were pretreated with the vehicle (water) of caffeine for 12 consecutive days and continued for another 10, 12, 15 and 18 consecutive days after inoculation of mice with EAC cells with respect to their corresponding control. The GPX activity in mice liver remain unaffected under similar conditions of treatment.

Table 4 depicts that the activities of hepatic CAT, SOD and LP remain unchanged when mice were pretreated with caffeine for 12 consecutive days and
continued for another 10, 12, 15 or 18 consecutive days after inoculating EAC cells to mice with respect to their corresponding control. The hepatic CAT and SOD activities were significantly decreased when caffeine was administered for 24 (20.83%; F=339.5, df=1.6; P<0.05) and 27 (26.27%; F=204.4, df=1.6; P<0.05 respectively), 27 (26.27%; F=204.4, df=1.6; P<0.05 and 39.96%; F=99.08, df=1.6; P<0.05 respectively) and 30 (41.1%; F=626.67, df=1.6; P<0.05 and 28.88%; F=60.03, df=1.6; P<0.05 respectively) consecutive days including 12 consecutive days of caffeine treatment prior to EAC cell inoculation with respect to their corresponding caffeine treated conditions alone (Table 1). It is also observed from this Table that the activities of both CAT and SOD in mice liver were significantly enhanced when caffeine was administered for 22 (49.09%; F=93.5, df=1.6; P<0.05 and 51.87%; F=86.4, df=1.6; P<0.05 respectively), 24 (52%; F=21.0, df=1.6; P<0.05 and 51.18%; F=200.85, df=1.6; P<0.05 respectively), 27 (102.53%; F=184.29, df=1.6; P<0.05 and 65.02%; F=22.38, df=1.6; P<0.05 respectively) and 30 (90.41%; F=440.0, df=1.6; P<0.05 and 132.96%; F=575.1, df=1.6; P<0.05 respectively) consecutive days including 12 consecutive days of caffeine treatment prior to EAC cell inoculation with respect to their corresponding EAC cell developing conditions alone (Table 2). The hepatic microsomal LP was increased when caffeine was administered to mice for 22 (33.33%; F=29.68, df=1.6; P<0.025), 24 (46.38%; F=12.65, df=1.6; P<0.025), 27 (125.49%; F=111.57, df=1.6; P<0.025) and 30 (178.57%; F=209.79, df=1.6; P<0.025) consecutive days including 12 consecutive days of caffeine treatment prior to EAC cell inoculation in mice with respect to their corresponding caffeine treated conditions alone (Table 1). It is also observed that hepatic microsomal LP was significantly decreased when caffeine was administered to mice for 24 (26.3%; F=11.1, df=1.6; P<0.025), 27 (29.88%; F=10.69, df=1.6; P<0.025) and 30 (36.76%; F=137.01, df=1.6; P<0.025) consecutive days including 12 consecutive days of caffeine treatment prior to EAC cell inoculation in mice with respect to their corresponding caffeine treated conditions alone. No significant change was observed when caffeine was administered for 12 consecutive days before the inoculation of EAC cells and continued during the development of EAC cells for 10 consecutive days with respect to the corresponding EAC cell developing condition alone. No significant change in GPX activity of mice liver was observed in any of the above mentioned experimental conditions (Tables 1-4).

Discussion

The results of the present study provide evidence that chronic ingestion of caffeine (a) increases the activity of hepatic SOD and CAT (Table 1) and (b)
The cellular redox state is very much sensitive to the antioxidant enzymes and leads to the development of cancer. Caffeine is known to be an inhibitor of liver microsomal enzymes. Investigation into the possible mechanisms involved in the observed antioxidant effect of caffeine suggests that the quenching of the reactive oxygen species by caffeine may be considered as one of the possible factors responsible for oxidative damage to the biomembrane. Thus, caffeine as suggested by others may be considered as an antioxidant against oxidative damage of the biological membrane by potentiating the activities of antioxidant enzymes (such as SOD, CAT etc.). It is well-known that long-term caffeine consumption stimulates its own metabolism by inducting the activity of hepatic microsomal drug metabolizing enzyme through the activation of cyt.P450 and cyt.B5 dependent mixed function oxidase system. Therefore, it is not unlikely to assume that the present caffeine-induced change in the reactive oxygen species may be due to the metabolites of caffeine and not by caffeine itself.

It is known that under physiological conditions, oxygen radicals are part of normal regulatory circuits and cellular redox state is very much sensitive to the antioxidant. However, increase in influx of oxygen radicals and loss of cellular redox homeostasis can cause oxidative stress and lead to tumorigenesis. The antioxidant enzymes are of vital importance under this environment in an organism defense against oxidative stress. The most important one is SOD whose main function is to remove $O_2^-$ radicals. Therefore, the depression of hepatic CAT activity (Table 2) as well as activity in SOD (Table 2) in Ehrlich ascites tumor bearing mice observed in the present study which has also been supported by others observations suggests that hepatic antioxidant defense system of ascites tumor bearing mice may be impaired through the production of superoxide radicals, oxy-radicals and peroxide radicals possibly by perturbation of the hepatic cell membrane due to its enhanced LP in the carcinoma cell as well as in hepatic microsomal membrane (Table 2).

The administration of caffeine during the development of EAC cells, on the other hand, antagonizes the activity of the Ehrlich ascites tumor cell-induced decrease of hepatic antioxidant enzymes along with the induction of hepatic microsomal LP and restored to their (enzymes) respective control value, suggesting that caffeine may effectively scavenge $\cdot$OH, ROO$^-$ and $O_2^-$ and plays a potential antioxidant activity against the oxidative damage to the hepatic cellular system caused by the development of tumor cells. The formation of caffeine-derived oxygen-centered radical in the reaction of caffeine with $\cdot$OH as observed by electron spin resonance (ESR) spin trapping further strengthen the above explanation and suggest that caffeine may act as an anticarcinogen. The degree of caffeine-induced antioxidant effect on hepatic cell damage caused by tumorigenesis depends on the duration of caffeine treatment (Tables 1-4).

In addition to the above primary antioxidant defense system, which protect against reactive oxygen species, there is another enzyme GPX. There are two types of GPX. One is Se dependent and other is Se independent. It has been also reported that in hepatic tissue Se dependent GPX is dominant and it has been further known that Se content and Se dependent GPX activity both are enhanced in tumorigenesis or in tumor bearing mice. Though contradictory reports in hepatic GPX activity in cancerous condition have been cited in the literature, the results of the present study show no significant change in caffeine treated or EAC cell developing or treatment of caffeine in EAC cell developing mice liver GPX activity (Tables 1, 2 and 4) suggesting that present observation may be with the Se independent hepatic GPX activity. Further studies with/without Se and in other conditions are now in progress to clarify the present observations.

Finally, it may be concluded that induction of hepatic cell damage in Ehrlich ascites tumor bearing mice caused by generation of reactive oxygen species may be inhibited by caffeine due to its effective scavenging property against oxidative damage of the biological membrane. Therefore, from the present study, it is not unlikely to consider caffeine as an anticarcinogen.

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References


