The Effects of Caffeic Acid Phenethyl Ester and Ellagic Acid on the Levels of Malondialdehyde, Reduced Glutathione and Nitric Oxide in the Lung, Liver and Kidney Tissues in Acute Diazinon Toxicity in Rats

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Abstract: The aim of this study was to investigate the effects of Caffeic Acid Phenethyl Ester (CAPE) and Ellagic Acid (EA) on activities of Malondialdehyde (MDA), reduced Glutathione (GSH) and Nitric Oxide (NO) in rat lung, liver and kidney tissues in acute Diazinon (DI) toxicity. Six groups of 6 Sprague Dawley rats were used comprising control, CAPE, EA, DI control, DI+CAPE and DI+EA. Tissue samples were analyzed for GSH, MDA and NO levels in lung, liver and kidney tissues. Biochemical parameters were measured colorimetrically by spectrophotometer. Control, CAPE and EA groups showed no statistically significant difference whereas DI+medication groups revealed that CAPE and EA increased the level of GSH in liver tissue by blocking the DI effect. NO levels in lung, liver and kidney tissues were significantly increased by DI but CAPE and EA attenuated those levels. In DI+medication groups, MDA levels showed no significant change in kidney and liver tissues but in lung tissues, CAPE and EA reduced the MDA level by blocking the DI effect. It was concluded that CAPE and EA which showed similar effects to each other could be used for protection and support against oxidative stress caused by acute DI intoxication.

Key words: Caffeic acid phenethyl ester, diazinon, ellagic acid, malondialdehyde, nitric oxide, reduced glutathione

INTRODUCTION

Organophosphates (OPs) are one of the main classes of insecticides in use since the mid 1940s (Gallo and Lawryk, 1991). Organophosphorus Insecticides (OPIs), widely used in agriculture show several interesting features for environmental safety such as limited persistence and selective toxicity to insects with respect to mammals (Vittozzi et al., 2001; Sutcu et al., 2007). Therefore, the toxic material chos for this study was Diazinon (DI) as an OP often causing human and animal poisoning.

\[ \text{DI(\text{o,o-diethyl-o-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphorothioate}) has been greatly and effectively used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruits, vegetables and as a pesticide in domestic applications (International Program on Chemical Safety and WHO, 1997; Garfitt et al., 2002; Dutta and Meijer, 2003; Amirkabirian et al., 2007). Whatever the route of exposure, DI by the process of inhibiting Acetylcholinesterase (AChE), affects particularly the nervous system causing poisoning. The indications of DI poisoning are nausea, vomiting, stomach cramps, slow} \]
pulse, diarrhea, headache, dizziness, general weakness, anxiety, blurred vision, respiratory difficulties, coma and finally death. Previous studies have revealed the important role played by oxidative tissue damage in the pathogenesis of the toxic effects (hepatotoxicity, neurotoxicity, genotoxicity, embryotoxicity, immunotoxicity, etc.) of acute and chronic DI application (Bachowski et al., 1997; Dutta and Meijer, 2003). However, there are still insufficient studies on why DI cause oxidative damage and on protective and supportive medications for that damage. Therefore potentially protective materials selected for this study was Caffeic Acid Phenethyl Ester (CAPE) and Ellagic Acid (EA). CAPE is an active component of honeybee propolis and it is known to have powerful antimicrobial, anti-inflammatory, antineoplastic and anti-oxidizing effects (Koltukszu et al., 2000). According to previous studies, CAPE exerts its beneficial effects by decreasing free oxygen radicals and prevents consumption of free radical scavenging enzymes acting in parallel to these antioxidant enzymes (Koltukszu et al., 2000; Ilter et al., 2004; Ogeturk et al., 2005). It has been shown that CAPE suppresses lipid peroxidation, inhibits lipoxygenase activities and tumour promotion. At a concentration of 10 μmol, CAPE completely blocks production of Reactive Oxygen Species (ROS) in human neoprophils and Xanthine/Xanthine Oxidase (NO) system (Yilmaz et al., 2005).

EA which is the other potentially protective agent to compare with CAPE in the this research is a polyphenol antioxidant found in certain fruits and nuts such as raspberries, strawberries, walnuts, loganberries, mango kernel and pomegranate (Thomas, 2001) which has been seen to have a variety of biological activities including potent anti-oxidant (Micek et al., 1992), anti-cancer (Shumilina et al., 2008) and anti-mutagen properties (Siebenlist et al., 1994) in a number of in vitro and small animal models. The aim of this study was to investigate the effects of CAPE and EA on activities of Malondialdehyde (MDA), reduced Glutathione (GSH) and Nitric Oxide (NO) in rat lung, liver and kidney tissues in acute DI toxicity.

MATERIALS AND METHODS

Animals, care and nutrition: A total of 36 adult female Sprague-Dawley rats weighing 200-250 g were used in the study. The rats were randomly allocated to 6 groups of 6 rats each. The animals were kept in laboratory conditions of a 12 h light/dark cycle at room temperature (21±3°C). The study was approved by Selcuk University Experimental Medical Research Centre Experimental Animals Ethics Committee.

Animals and treatment: The total 36 rats in the study were divided into 6 groups including 6 each: control (Group I) CAPE (Group II) (Sigma C8221) (10 μmol kg⁻¹ dose administered intra peritoneal (ip)), EA (Group III) (E2250 Sigma, CAS Number: 476-66-4) (85 mg kg⁻¹ dose administered orally (po)), DI (Group IV) (Basudan, Syngenta, Turkey) (200 mg kg⁻¹ dose administered p.o) DI+CAPE (Group V) and DI+EA (Group VI). A total of 24 h after the medication administration, the animals were anesthetised with ketamine (50 mg kg⁻¹ dose ip) + xylazine (5 mg kg⁻¹ dose ip) then euthanised via cervical dislocation. Measurements were taken of Malondialdehyde (MDA), reduced Glutathione (GSH) and Nitric Oxide (NO) in the lung, liver and kidney tissues.

Biochemical parameters: Tissue samples from the liver, lung and kidney were collected for analysis of GSH, MDA and NO concentrations. Tissues were immediately removed and washed with 0.15 M KCl (at 4°C). The tissues were then homogenized in ice cold (A: 50 mM, H₂PO₄, ve B: 50 mM Na₂HPO₄, 2H₂O, A:B (v/v) = 1:1.5) by a homogenizer at 1600 rpm for 3 min. The homogenates were centrifuged at 5000 g at 4°C for 15 min. The supernatants were stored at -25°C until they were analyzed.

NO was measured colorimetrically using a spectrophotometer (PowerWave XS, BioTek, Instruments, Winooski, VT, USA) by the method of Miranda et al. (2001). For GSH and MDA concentrations, analyses (UV-1201, Shimadzu, Japan) were carried out by the method of Beutler et al. (1963) and Yoshioka et al. (1979), respectively.

Statistical evaluation: The data for biochemical parameters were analyzed by ANOVA then the post hoc Tukey test. All data were presented as mean±SE using SPSS Windows 10.0. A value of p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results obtained and the statistical analysis of the results are shown in the Table 1. No statistically significant difference was determined between the groups in a comparison of the effects on MDA, GSH and NO parameters in liver kidney and lung tissue in the control CAPE and EA groups. These results showed that CAPE and EA used alone created no effect on MDA, GSH and NO levels in lung, liver and kidney tissues and as both medications showed similar effects on these molecules, their use alone in respect of oxidant-antioxidant defence mechanism did not generate any problems.
Table 1: The effects on MDA, GSH and NO parameters in liver kidney and lung tissue in the control CAPE and EA groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>CAPE</th>
<th>Ellagic Acid (EA)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver MDA (µmol g⁻¹ protein)</td>
<td>16.41±1.320</td>
<td>13.59±1.490</td>
<td>11.94±1.480</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>15.53±1.050</td>
<td>16.34±0.980</td>
<td>16.80±0.820</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>275.53±41.40</td>
<td>250.94±30.75</td>
<td>266.31±30.74</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney MDA (µmol g⁻¹ protein)</td>
<td>10.78±0.880</td>
<td>10.59±1.210</td>
<td>10.65±1.050</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>9.59±0.360</td>
<td>11.61±0.880</td>
<td>9.98±0.880</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>271.96±24.00</td>
<td>266.74±53.980</td>
<td>208.47±156.18</td>
<td>NS</td>
</tr>
<tr>
<td>Lung MDA (µmol g⁻¹ protein)</td>
<td>25.56±2.800</td>
<td>24.61±1.700</td>
<td>22.64±1.460</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>15.44±1.850</td>
<td>12.32±0.8100</td>
<td>14.18±2.190</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>902.32±138.8</td>
<td>930.65±192.41</td>
<td>959.55±144.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for groups; NS: Not statistically significant

Table 2: The effects on MDA, GSH and NO levels in liver kidney and lung tissue in the DI+CAPE, DI+EA and DI groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Diazon</th>
<th>DI+CAPE</th>
<th>DI+Ellagic acid</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver MDA (µmol g⁻¹ protein)</td>
<td>16.41±1.320</td>
<td>15.60±0.65</td>
<td>15.53±1.480</td>
<td>15.42±1.750</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>15.53±1.050</td>
<td>15.07±2.240</td>
<td>15.69±0.900</td>
<td>17.13±1.360</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>275.53±41.40</td>
<td>405.99±63.15</td>
<td>365.28±39.190</td>
<td>364.38±54.20</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney MDA (µmol g⁻¹ protein)</td>
<td>10.78±0.880</td>
<td>13.27±1.190</td>
<td>13.25±1.550</td>
<td>13.15±1.470</td>
<td>NS</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>9.59±0.360</td>
<td>9.46±0.590</td>
<td>10.83±0.400</td>
<td>8.50±0.990</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>271.96±24.00</td>
<td>1134.0±183.5</td>
<td>762.34±106.89</td>
<td>929.19±120.19</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Lung MDA (µmol g⁻¹ protein)</td>
<td>25.56±2.800</td>
<td>26.01±2.060</td>
<td>19.94±2.5900</td>
<td>23.54±1.540</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>GSH (µg g⁻¹ protein)</td>
<td>15.44±1.850</td>
<td>22.87±3.170</td>
<td>17.44±1.4200</td>
<td>15.32±1.910</td>
<td>NS</td>
</tr>
<tr>
<td>NO (µmol g⁻¹ protein)</td>
<td>902.32±138.8</td>
<td>932.23±120.1</td>
<td>927.99±90.010</td>
<td>929.18±247.95</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD for groups; NS: Not statistically Significant; (a-c) Mean values with same superscripts (a-c) within the same row are different

An examination of the statistically significant values showed that in acute DI intoxication, CAPE and EA increased the level of the antioxidant agent GSH in the liver tissue by blocking the DI effect. In particular, EA was observed to increase the GSH level in the liver by a greater amount, in comparison with CAPE. When the DI, DI+CAPE and DI+EA groups were compared in respect of NO levels, DI had increased the NO levels in both liver and kidney tissues by a significant amount in comparison to the control group. The reason for DI to cause a great increase in NO levels originates from a large amount of DI having been administered over an acute 24 h period thus creating severe damage so the conclusion was reached that excessive amounts of DI increase NO expression. In a comparison of MDA levels between the DI, DI+CAPE and DI+EA groups, no significant change was determined in the kidney and liver tissues. However, in the lung tissues, it was determined that the increase in MDA level caused by DI had been reduced by CAPE and EA blocking the DI effect (Table 2).

The most important feature of DI and OPI toxicity is related to their irreversible blood AChE inhibition which at high doses could lead to animal and human death (Neishabouri et al., 2004). However, some studies indicate that oxidative stress could be an important component in the mechanism of toxicity of OPIs. OPIs may induce oxidative stress, leading to generation of free radicals and alterations in antioxidants (Bagchi et al., 1995; Ahmed et al., 2000; Gultekin et al., 2000; Sutcu et al., 2007). The cell has several mechanisms to deal with the effects of oxidative stress either by repairing the damage or by directly diminishing the occurrence of oxidative damage by means of enzymatic and nonenzymatic antioxidants. Some studies have shown that LPO has been suggested as one of the molecular mechanisms involved in OPI-induced toxicity (Yamano and Morita, 1992; Bagchi et al., 1995; Sutcu et al., 2007).

Acute exposure of rats to dimethane was found to alter the activity of several antioxidant enzymes in the liver and brain (Sharma et al., 2005). Following acute administration of chlorpyrifos in rats, levels of Thiobarbituric Acid Reactive Substances (TBARS) increased in the liver (Bagchi et al., 1995), brain (Verma and Srivastava, 2001), kidney (Onceu et al., 2002) and erythrocytes (Gultekin et al., 2001). High doses of diisopropylfluorophosphate (an OP not used as an insecticide) were found to trigger excessive nitric oxide production in the brain (Gupta et al., 2001). Repeated
administrations of malathion, endosulfan or chlorpyrifos to rats were also found to increase lipid peroxidation and alter antioxidant enzymes in blood, liver and lungs (Ahmed et al., 2000; Akhgar et al., 2003; Bebe and Panemangalore, 2003). When various antioxidant were administered, they were reported to antagonize the oxidative stress induced by OPs (Ahmed et al., 2000; Gultekin et al., 2001; Gupta et al., 2001; Karasoz et al., 2002; Onu et al., 2002; Cankayali et al., 2005; Giordano et al., 2007).

This study researched the effects of CAPE and EA on MDA, NO and GSH levels in acute DI toxicity. One of the marker agents of this study, NO, NO has been implicated in the mechanisms of cell injury and long-term physiological changes in cellular excitability. Celik et al. (2007) found that NO levels were significantly increased in the Fyelonephritis groups (FYN) caused by Escherichia coli compared with the control group \(p<0.05\). On the other hand, CAPE treatments significantly decreased the pathogen-induced elevation in NO levels similar to that seen with MDA.

Altug et al. (2008) found that CAPE treatment significantly attenuated the elevation of plasma MDA content \(p<0.05\) whereas it significantly increased the levels of plasma GSH and NO \(p<0.05\) in adult male New Zealand rabbits which was induced by microsurgical procedures producing right focal Permanent Middle Cerebral Artery Occlusion (PMCAO).

Ozyurt et al. (2007a) revealed that IR injury led to increases in MDA and NO levels in both skeletal muscle tissue and plasma. In contrast, CAPE led to a decrease in MDA and NO levels in both plasma and muscle tissue.

Ozyurt et al. (2007b) found that Malondialdehyde (MDA) and Nitric Oxide (NO) enzyme activities were found to be increased significantly in the Prefrontal Cortex (PFC) of MK-801 (diazocilpine maleate) group compared to the control group. In CAPE treated rats, prefrontal tissue MDA and NO enzyme activities were significantly decreased.

Devipriya et al. (2007) studied the antioxidant properties of EA against alcohol-induced toxicity and determined that EA resulted in a significant decrease in the levels of Thiobarbituric Acid Reactive Substances (TBARS) in both the liver and kidneys. The levels of NO were significantly increased in the alcohol-fed rats when compared to the rats in the control group. Treatment with EA restored the levels of NO to near normal.

Yildirim et al. (2003) found that NO level in the kidney tissue was increased by the cisplatin management and erdosteine prevented this increment at a statistically significant level. Increasing evidence suggested that NO has an important role in modulating oxidant stress and tissue damage. Peresleni et al. (1996) showed that oxidant stress to the epithelial cells caused an increase in immunodetectable Inducible NO Synthase (iNOS) which results in an elevation in NO release, nitrite production and decreased cell viability. Yilmaz et al. (2005) found a marked elevation in NO level in damaged liver tissue of cisplatin treated rats and CAPE significantly attenuated this increment. Song et al. (2002) demonstrated that CAPE inhibited NO production.

Similar to the findings reported by Yildirim et al. (2003) and Peresleni et al. (1996), the results of this study determined that the reason for severe damage in liver and kidney tissues was connected to the oxidative stress created by DI because of an increase in NO release and by the process of CAPE and EA reducing the greatly increased NO levels there was a balanced attenuation of NO levels.

Malondialdehyde (MDA) and TBARS levels are known to be indicators of lipid peroxidation, free radical generation and oxidative stress which result either from increased oxidant production or reduced antioxidant levels and increase at the end of lipid peroxidation. In some in vitro and in vivo studies, MDA formation increased with the administration of chlorpyrifos-ethyl, ferthion, phosalone and DI (Gultekin et al., 2001; Altuntas et al., 2002, 2003, 2004; Sutcu et al., 2007).

However, some studies have reported no change in MDA levels from the application of OP and DI. For example, Yildirim et al. (2003) found that the unchanged MDA levels in a cisplatin administrated group seemed to be contrary when compared to the NO levels. MDA is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation in the liver. The unchanged MDA level in liver tissue leads us to think that the catabolic pathway for MDA is very rapid in the liver. MDA released by lipid peroxidation in liver tissues might be metabolized immediately by a mitochondrial MDA-metabolizing enzyme (low specific aldehyde dehydrogenase) and an increase in MDA level is not seen.

In the present study, similar to the reason mentioned by Yildirim et al. (2003), no significant change was noted in the MDA levels of the kidney and liver tissues from DI and the lasting effect of DI in the DI+CAPE and the DI+EA groups. However, in contrast the results of Gultekin et al. (2001), Altuntas et al. (2002, 2003, 2004) and Sutcu et al. (2007) reported that although DI had caused increased MDA levels in the lung tissue, CAPE and EA by blocking the effect of DI had reduced the MDA level.

GSH is a major player in cellular defense against reactive oxygen species. GSTs catalyze the conjugation of
GSH with electrophilic metabolites which are involved in the detoxification of both reactive intermediates and oxygen radicals. Increased activities of GST are known to serve as protective responses to eliminate xenobiotics (Smith and Litwack, 1980). Elevated GSH activity may reflect the possibility of better protection against pesticide toxicity and it is used as a biomarker for environmental biomonitoring. When GSH activity is inhibited, accumulation of lipid peroxidation products occurs. GSH play a primary important role in cellu-detoxification of toxic aldehydes (Ozcan et al., 2006; Oruc, 2010).

Pari and Sivasankari (2008) found that EA increased levels of GSH would therefore be important in protecting cells with significant hepatoprotection against CdA-induced toxicity. In addition, it was reported that the levels of TBARS and hydroperoxides were significantly decreased and the levels of enzymatic and non-enzymatic antioxidants significantly increased on treatment with EA in the liver (Pari and Sivasankari, 2008). An examination of the statistically significant levels in the present study determined that DI had reduced the GSH level in the liver tissue but looking at the DI+CAPE and DI+EA groups CAPE and EA had increased the GSH levels against the reducing effect of DI. EA especially, in comparison to CAPE was determined to have increased the GSH level by a greater amount in the liver.

CONCLUSION

It is concluded that CAPE and EA showed positive effects similar to each other on oxidative stress in acute DI intoxication and as such could be used for protective purposes against acute DI poisoning.

REFERENCES


