PROTECTIVE EFFECT OF GRAPE SEEDS PROANTHOCYANIDINS AGAINST NAPHTHALENE-INDUCED HEPATOTOXICITY IN RATS

Amany A. E. Ahmed* and Amal J. Fatani

Oxidative stress with subsequent production of reactive oxygen species has been postulated as one of the mechanisms of naphthalene toxicity. In the present study, the effect of oral administration of the natural antioxidant and free radical scavenger, proanthocyanidins present in grape seeds (GSP, 10 or 50 mg kg⁻¹, p.o.), has been investigated in rats following the concomitant administration of the natural antioxidant and free radical scavenger, proanthocyanidins present in one of the mechanisms of naphthalene toxicity. In the present study, the effect of oral administration of naphthalene (1 g kg⁻¹, p.o., 15 days) and measurement of selective parameters indicative of liver function and oxidative stress. Serum aminotransferases (ALT and AST), alkaline phosphatase (AP) activities, and total bilirubin (T Bil) concentration were measured, as well as hepatic tissue lipid peroxidation (MDA), DNA fragmentation, and glutathione (GSH) contents. The effects of GSP on naphthalene-evoked changes in the above-mentioned parameters were compared with the known hepatoprotective agent, silymarin. Naphthalene hepatotoxicity was evident by the significant elevation of rat serum activities of ALT, AST, AP, and T Bil concentration. This effect was accompanied with significant increases in MDA and DNA fragmentation plus the depletion of GSH in hepatic tissues. Concurrent administration of GSP significantly attenuated the naphthalene-induced disturbances in serum liver function enzymes, and markedly antagonized the lipid peroxidation, DNA fragmentation and GSH depletion induced by naphthalene in hepatic tissues. In conclusion, GSP appears to be a potent candidate to ameliorate the oxidative stress and hepatotoxicity associated with naphthalene in rats.

Key words: Naphthalene hepatotoxicity, grape seeds proanthocyanidins, liver biomarker enzymes, oxidative stress, DNA fragmentation, bromosulphalein dye clearance.

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Introduction

The bioactivated xenobiotic naphthalene is a pervasive environmental contaminant. It is one of the volatile aromatic hydrocarbons that are widely used commercially. Humans are exposed to naphthalene from a number of different sources including workplace exposures as in the aluminum smelting industry or where naphthalene is a starting material in the production of phthalic anhydride. It is used in the synthesis of dyes, resins, plastics, plus many pharmaceuticals including veterinary medicine, insect repellents, moth balls and toilet bowel deodorants (1, 2). In addition, naphthalene is a natural constituent of coal tar and crude oil and has been identified in cigarette smoke and emission from fossil fuel combustion (3).

Naphthalene toxicity is highly species, tissue and cell selective (3). Naphthalene exposure is associated with several toxic manifestations in humans and laboratory animals, it has been found to cause cataract, hemolytic anemia, and damage of the bronchial epithelial (Clara) cells and proximal tubules of the kidney (4, 5). Moreover, naphthalene was involved in hepatocyte injury and liver dysfunction (4, 6, 7).

Toxic manifestations of naphthalene have been attributed to oxidative stress caused by generation of reactive oxygen species (ROS)(4, 8, 9). These species may be generated during phase I metabolism by cytochromes P-450 or by the redox cycling of its metabolites 1,4 & 1,2 naphthoquinone as well as its hydroxylated products 1-naphthol, 2-naphthol and 1,2-dihydroxynaphthalene (10).

Tingle et al. (6) reported that human liver is capable of metabolizing naphthalene rapidly and efficiently into stable protein-reactive and cytotoxic metabolites, but if these metabolites are not rapidly detoxified by microsomal enzymes it can bind to microsomal proteins and damage cell membrane and tissue macromolecules including DNA, proteins and lipids. Moreover, intracellular reduced glutathione was proven to provide a major detoxification process for naphthalene metabolites (11).

A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. Grapes and grape products are good sources of dietary flavonoids, which are powerful antioxidant compounds. Furthermore, the inedible component of the fruit such as seeds and skin contain some compounds that are able to scavenge superoxide radicals in living organisms’ cells (12, 13). Moreover, grape seeds polyphenols (proanthocyanidins) were found to be highly bioavailable providing significantly greater protection against free radicals, free radical-induced lipid peroxidation and DNA damage, than that observed with vitamins C, E and β-carotene, possibly due to its broad spectrum of health benefits (14). Thus, many recent studies suggest that in-vivo grape seeds proanthocyanidins exposure may protect multiple organs from a variety of toxic assaults (13, 14).

The commercially available grape seeds proanthocyanidins extract, used in the present study (GSP, Nulife international, USA) is a standardized water-ethanol extract from red grape seeds and contains monomeric, dimeric, trimeric and tetrameric proanthocyanidins, plus other oligomeric proanthocyanidin bioflavonoids. Generally, the dimer-, trimer-, and tetramer proanthocyanidins, also referred to as extractable proanthocyanidins or bioflavonoids, have been shown to be highly bioavailable and provide excellent health benefits.

Thus the objective of the present investigation was to evaluate the in-vivo effect of 15-days oral administration of the environmental xenobiotic toxicant, naphthalene, on rat liver, in an attempt to ascertain the involvement of lipid peroxidation, DNA fragmentation and glutathione depletion in naphthalene-induced hepatotoxicity. Additionally, this study examined whether the concomitant administration of the natural antioxidant, grape seeds proanthocyanidins extract would protect rats from naphthalene-evoked disturbances in the selected liver biomarkers.
Materials and methods

1. Animals:
Adult male Wistar rats (150-175 g), were provided by the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. The rats had free access to standard rat chow and water, were housed in cages, 8-10 rats per cage, and kept in a room maintained at 25 ± 2ºC with a 12 h light/dark cycle. All experiments on animals were carried out according to the Guidelines of the Animal Care and Use Committee, King Saud University, Riyadh, Saudi Arabia.

2. Drugs and chemicals:
Grape seeds proanthocyanidins extract (GSP, 95% purity) was purchased from Nulife international (USA). Bromosulphalein (BSP, 5% concentration ampoules) were obtained from Fluka AG (Germany). Diphenylamine was purchased from Koch Light Laboratories (England). All other chemicals and reagents used were of analytical grade and were obtained from Sigma- Aldrich Chemical Co. (St. Louis, USA).

3. Experimental design:
Experiments were performed on five groups (n=10 rats). The first group received naphthalene dissolved in corn oil (1 g kg⁻¹, p.o.) (4) daily for 15 days. The 2nd, 3rd, and 4th groups were given a saline solution of either GSP (10 or 50 mg kg⁻¹, p.o.) (16) or the reference hepato-protectant silymarin (100 mg kg⁻¹) (17), respectively, 2 h before administration of naphthalene (1 g kg⁻¹, p.o.) for 15 days. The fifth normal control group was administered an equal volume of the vehicle (corn oil) orally for 15 days. At the end of the experiments, two hours after administration of naphthalene, all animals were killed by decapitation, and midstream blood samples (4 ml) were collected in test tubes. After-wards, serum alanine aminotransferase (ALT) and asparatic acid aminotransferase (AST) activities were estimated according to the method of Ritman and Frankel (18), while alkaline phosphatase (AP) activity was assayed depending on the method of Bowers and Mc Comb (19). Commercial kits (Spinreact, Spain) were used and the activities of these enzymes were expressed as international units(U l⁻¹).

4. Estimation of liver injury markers:

4.1. Assay of serum enzymes and components
Serum alanine aminotransferase (ALT) and asparatic acid aminotransferase (AST) activities were estimated according to the method of Ritman and Frankel (18), while alkaline phosphatase (AP) activity was assayed depending on the method of Bowers and Mc Comb (19). Commercial kits (Spinreact, Spain) were used and the activities of these enzymes were expressed as international units(U l⁻¹). Moreover, serum total bilirubin concentration (T Bil), was assayed depending on the method of Martinek (20). Commercial kits (Spinreact, Spain) were used and concentrations were expressed as mg dl⁻¹.

4.2. Estimation of liver lipid peroxidation (MDA) and glutathione (GSH) contents:
Lipid peroxidation was assayed according to the method described by Ohkawa et al. (21), where the levels of thiobarbituric acid reactive substances (TBARS), a commonly utilized specific marker used to measure the degree of lipid peroxidation, were measured in 20% of liver homogenates, using thiobarbituric acid reaction and the results were expressed as malondialdehyde (MDA) (nmol g⁻¹ wet weight).

Glutathione (GSH) content in the liver homogenates was determined according to the method described by Ellman (22), depending on the reaction of Ellman’s reagent (5, 5-dithio-bis (2-nitrobenzoic acid), (DTNB), with the thiol group of GSH at pH 8.0 to give yellow color of 5-thiol-2-nitrobenzoate anion. This was measured spectrophotometrically at 412 nm and the results were expressed as µmol g⁻¹ wet weight.

5. Quantitative DNA fragmentation assay:
Quantitative estimation of DNA fragmentation was determined by colorimetric diphenylamine assay as described by Burton (23). Liver samples from different groups were homogenized in chilled lysis buffer (10 mmol TRIS-HCl, 20 mmol EDTA, 0.5% Triton X-100, pH 8.0). Homogenates (1 ml) were then centrifuged at 27,000 g for 20 min to separate intact DNA in the pellet from fragmented/damaged DNA in the supernatant fractions. Perchloric acid (to reach a final concentration of 0.5 M) was added separately to both the pellets and supernatant samples. Samples were heated at 90 ºC for 15 min then centrifuged at 1500 g for 10 min to remove proteins. Resulting supernatants, whether containing whole or fragmented DNA, were left to react with diphenylamine (0.088 M) for 16-20 h at room temperature, afterwards absorbance was measured at 600 nm. DNA fragmentation was expressed as a percentage.
of total to fragmented DNA. Treatment effects were reported as percentage of fragmentation observed in control group.

6. Measurement of hepatic Bromosulphalein (BSP) clearance:
   Rats were divided into five groups each of six animals. One group served as control and was given only the vehicle (corn oil). Another group was administered naphthalene dissolved in corn oil (1 g kg\(^{-1}\), p.o.) for 15 days. Three groups were given saline solutions of either GSP (10 or 50 mg kg\(^{-1}\), p.o.) or silymarin (100 mg kg\(^{-1}\), p.o.) two hours before administration of naphthalene (1 g kg\(^{-1}\), p.o.) for 15 days. After 18 h from the last dose of naphthalene, bromosulphalein (BSP) dye (100 mg kg\(^{-1}\)) was injected intravenously to all animals. Midstream blood from decapitated animals (4 ml) were collected in heparinized tubes after exactly 30 min from the dye injection. Afterwards, blood samples were centrifuged for 15 min at 3000 g and the dye concentration in plasma was estimated spectrophotometrically at 580 nm. Data were presented as both BSP retention (percentage retained of originally injected amount, 100 mg), as well as excretory capacity (%) calculated as 1- (Rt – Rn/Rc – Rn) x 100, where R= BSP retention, while t, n, c, represent treatment, naphthalene & control, respectively (24).

7. Statistical analysis:
   Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey Kramer post hoc multiple comparisons test. The results were expressed as mean ± SEM of 10 values in each group, and the level of significance was considered at p <0.05.

Results

1. Effect of treatments on serum levels of liver enzyme markers
   Administration of naphthalene for 15 days caused a significant (p<0.001) increase in the activities of serum ALT (150%), AST (143 %), AP (178%) and T Bil concentration (214%) as compared to control values (Figures 1 to 4), indicating that naphthalene induced significant liver injury with cholestasis.

   Oral administration of GSP in rats (10 or 50 mg kg\(^{-1}\), 2 hr before naphthalene for 15 days), significantly protected the liver and attenuated the naphthalene-induced changes in activities of the estimated liver enzymes ALT (76% and 73%), AST (72 % and 75 %), AP (79% and 73%) and concentration of T Bil (67% and 59%), respectively, as compared to the responses seen with naphthalene alone. In general, the effects of GSP (10 and 15 mg kg\(^{-1}\)) were comparable to that of the standard silymarin, appearing to offer more pronounced protection against naphthalene-evoked changes in AST activity and T Bil concentration (Figures 1 to 4).

2. Effect of treatments on hepatic lipid peroxidation and glutathione contents:
   The present study showed that oral administration of naphthalene for 15 days produced a significant (p<0.001) increase in hepatic lipid peroxides(5,6),(995,992) as indicated by the rise in the levels of the MDA (224%) as well as a significant (p<0.001) depletion of hepatic GSH contents (31 %) as compared with values in control group (Fig. 5 and 6).

   Concurrent administration of GSP (10 or 50 mg kg\(^{-1}\)) protected the liver and significantly attenuated the extent of naphthalene-elicited hepatic lipid peroxidation (60% and 53%, respectively) as compared to results seen in rats given naphthalene alone. Values in GSP-treated groups were not significantly different than that of control. Once again the protective effects of GSP were comparable to that of silymarin, with the higher dose appearing to be more effective (Fig. 5).

   Moreover, GSH was restored to approximately control tissue levels upon co-administration of GSP (10 or 50 mg kg\(^{-1}\)) and naphthalene, whereas values reached 300% and 357%, respectively, of those seen in group administered naphthalene alone. Silymarin administration also preserved the liver GSH, but to a lesser extent than GSP, indicating that GSP maybe more potent than silymarin in replenishing liver GSH contents. Surprisingly, in the group given the higher dose of GSP, levels of hepatic GSH were even higher than that seen in the control group (Fig. 6).
Table 1: Effect of concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (100 mg kg\(^{-1}\)) with naphthalene (1 g kg\(^{-1}\)) for 15 days on hepatic bromosulphalein dye (BSP, 100 mg kg\(^{-1}\), i.v.) clearance in adult male rats.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg kg(^{-1}))</th>
<th>BSP retention (mg%)</th>
<th>Excretory capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (corn oil)</td>
<td>_</td>
<td>0.602 ± 0.018</td>
<td>_</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1000</td>
<td>1.399 ± 0.147**##</td>
<td>_</td>
</tr>
<tr>
<td>Naphthalene + Silymarin</td>
<td>100</td>
<td>0.889 ± 0.058**#*</td>
<td>64</td>
</tr>
<tr>
<td>Naphthalene + GSP</td>
<td>10</td>
<td>0.991 ± 0.068**#*</td>
<td>51</td>
</tr>
<tr>
<td>Naphthalene + GSP</td>
<td>50</td>
<td>0.779 ± 0.079***</td>
<td>78</td>
</tr>
</tbody>
</table>

- Values represent the mean ± SEM of six rats in each group.
- BSP retention, percentage retained of originally injected amount
- Excretory capacity (%) = 1 - (Rt – Rn/Re – Rn) x 100; R: BSP retention; t, n & c: treatment, naphthalene & control, respectively.
- At #, significantly different than control (#, p<0.05; ##, p<0.001)
- At *, significantly different than naphthalene-treated group (*, p<0.05; **, p<0.01; ***, p<0.001).

Fig. 1. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on serum alanine aminotransferase (ALT) activity (U l\(^{-1}\)) in adult male rats.

Fig. 2. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on serum aspartic acid aminotransferase (AST) activity (U l\(^{-1}\)) in adult male rats.

Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.05; **, p<0.001).

Fig. 3. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on serum alkaline phosphatase (AP) activity (U l\(^{-1}\)) in adult male rats.

Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.05; **, p<0.01).
Fig. 4. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on serum total bilirubin (T Bil) concentration (mg dl\(^{-1}\)) in adult male rats. Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.01; ##, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.05; **, p<0.01). At +, values significantly different than silymarin-treated group (+, p<0.05).

Fig. 5. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on hepatic lipid peroxidation, estimated as MDA contents (nmol g\(^{-1}\) wet weight), in adult male rats. Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.01; **, p<0.001).

Fig. 6. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on hepatic reduced glutathione contents (μmol g\(^{-1}\) wet weight) in adult male rats. Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.05; **, p<0.01; ***, p<0.001).

Fig. 7. Effect of 15 days concomitant oral administration of grape seeds proanthocyanidins (GSP, 10 or 50 mg kg\(^{-1}\)) or silymarin (Sily, 100 mg kg\(^{-1}\)) with naphthalene (Naph, 1 g kg\(^{-1}\)) on hepatic DNA fragmentation (% of control) in adult male rats. Data expressed as the mean ± SEM (n=10 rats). At #, values significantly different than control (#, p<0.001). At *, values significantly different than naphthalene-treated group (*, p<0.01).
3. Effect of treatments on hepatic DNA fragmentation:

Naphthalene-induced DNA fragmentation in rat hepatic tissues is illustrated in Fig. 7. The comparative protective abilities of silymarin (100 mg kg\(^{-1}\)) and GSP (10 or 50 mg kg\(^{-1}\)) are also presented. The results showed that 15 days oral administration of naphthalene induced an increase in DNA fragmentation in the rat hepatic tissues reaching approximately 140% of control values. Concomitant administration of GSP (10 or 50 mg kg\(^{-1}\)) significantly decreased naphthalene-induced hepatic DNA fragmentation where the values reached 84% and 79% respectively, of values seen in group given naphthalene alone. The results in GSP-treated groups were not significantly different than that of control. Additionally, their effects were comparable to that of silymarin, and indicated the protective ability of GSP against naphthalene-induced DNA fragmentation in rat hepatic tissues.

4. Effect of treatments on hepatic Bromosulphalein (BSP) clearance:

As shown in table 1, BSP retention after 30 minutes from its injection into naphthalene-treated rats was significantly elevated (\(p < 0.001\)) when compared to values seen in normal rats. However, in animals treated with GSP (10 or 50 mg kg\(^{-1}\)), the naphthalene-evoked increase in BSP retention was significantly reduced to 71% (\(p<0.05\)) and 56% (\(p<0.001\)), respectively when compared to values in animals given naphthalene alone. This lead to a significant reduction in the liver excreting capacity for BSP dye reaching 51.2% and 77.8%, respectively, as compared to naphthalene-treated group. In general, the protective effects of GSP were comparable to that seen with the hepatoprotectant agent, silymarin, and the values in the group treated with the lower dose of GSP were not significantly different than that of control.

Discussion

In the present investigation, rats intoxicated with naphthalene (1 g kg\(^{-1}\), p.o.) for 15 days, showed signs of liver injury with cholestasis as observed from the significant and dramatic increase in activities of liver enzymes (ALT, AST and AP) and T Bil concentration.

Elevation in serum AST and ALT is a reflection of radical-mediated lipid peroxidation of liver cell membrane (25). It has been reported that AP is membrane bound and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites. Furthermore, AP is well documented to act as an indicator of cholestatic changes and both AP activity and T Bil concentration are indices of biliary damage (26). Several studies have reported changes in the activities of liver function enzymes which may be accompanied with cholestasis and elevation in serum T Bil concentrations during naphthalene or naphthalene derivatives intoxication. Similarly, naphthalene was reported to cause hepatocytes injury and liver dysfunction in different animal models (6, 27, 28).

Xenobiotics and hepatotoxic agents were proven to induce hepatotoxicity via various mechanisms such as activation of cytochrome P450 (29), generation of ROS (8, 30), or through the effects of the active toxic metabolites on protective actions of GSH (31). Naphthalene-induced hepatotoxicity in the present study was also accompanied by an increase in hepatic lipid peroxidation (MDA) and a decrease in GSH contents, indicating a clear association between oxidative stress plus lipid peroxidation with the development of naphthalene-elicited hepatotoxicity.

Lipid peroxidation, an important indicator of oxidative damage of biological tissues, was found to be induced in rats exposed to naphthalene (8). Similarly, oxidative stress was induced by naphthalene in different animal models, whereas its administration resulted in the elevation of the levels of serum and liver lipid peroxides in mice (28, 32), and in rats (33). In addition, naphthalene has been shown to induce oxidative stress as evidenced by hepatic and brain lipid peroxidation, glutathione depletion, DNA-single strand breaks and excretion of urinary lipid metabolites in rats (34) as well as in cultured macrophage J774A.1 cells (35).

In the present study, liver glutathione levels of rats intoxicated with naphthalene were reduced leading to liver injury which was reflected by impairment in BSP dye excretion rate. GSH is a well known protectant of liver cells against oxidative stress, through non-enzymatic and enzymatic reactions (36). The depletion of liver GSH contents is thought to result from inhibition of GSH efflux across the hepatocyte’s membrane (27). Moreover, reduced glutathione has been reported to form either nucleophil-forming conjugates with the active meta-
bolites or act as a reductant for peroxides and free radicals (37), which might explain its depletion. The resultant reduction in GSH level may thus increase susceptibility of the tissue to oxidative damage including lipid peroxidation and DNA fragmentation.

In accordance with the results of the present study, hepatic DNA fragmentation has been reported in many models of hepatotoxicity following treatment with certain drugs or chemicals (25, 38), indicating crucial associations between the generation of ROS, oxidative damage to membrane lipids plus DNA molecules, lipid peroxidation and DNA fragmentation (38, 39). Moreover, naphthalene, through its active metabolites, has been reported to cause DNA damage and lipid peroxidation in cells (35), which is also consistent with the concept of the involvement of ROS as a mechanism of its toxicity. It has also been reported that GSH may provide a major detoxification process for naphthalene metabolites (11), and its depletion may cause their accumulation.

Willems et al. (3) reported that, in rats, approximately all of the naphthalene that is absorbed is metabolized. In addition, naphthalene was found to undergo bioactivation to a reactive and cytotoxic protein metabolite, thought to be the intermediate naphthalene 1, 2-epoxide. It has also been reported to be the principal toxic metabolite in rat hepatocytes (11). Moreover, previous studies have shown that, 1-naphthol is metabolized to 1, 2-naphthoquinone and 1, 4-naphthoquinone (10, 40). These compounds are cytotoxic to hepatocytes, especially in the absence of a functional metabolizing system and in the presence of depleted intracellular glutathione (40, 41). In addition, Tingle et al. (6) reported that various human P450 enzymes have been implicated in the bioactivation of naphthalene, including CYP1A2, 3A4 and 2E1. Induction of human liver CYP2E1 resulted in preferential bioactivation of naphthalene to cytotoxic species which are probably quinones derived from further metabolism of its stable metabolite, 1-naphthol.

Other suggested mechanisms of naphthalene-induced hepatotoxicity have been put forth. Amin and Hamza (38) suggested a positive correlation between hepatotoxicity and DNA fragmentation. They implicated that calcium may play a role in drug-induced oxidative stress associated with hepatotoxicity. Similarly, Aktop et al. (32) concluded that the reactive metabolites of naphthalene could result in depletion of glutathione through binding covalently to tissue macromolecules leading to loss of thiol groups and disturbances in calcium sequestration activity of the subcellular compartment.

The present investigation demonstrated that administration of grape seed proanthocyanidins extract greatly attenuated the naphthalene-elicited changes in the levels of liver biomarkers (ALT, AST, AP and T Bil) and significantly reduced lipid peroxidation, DNA fragmentation and the depletion of glutathione contents in hepatic tissues. These effects were comparable to those seen with the reference drug silymarin.

Silymarin, a hepa-to-protectant, was shown to guard the livers of experimental animals against several hepatotoxic substances. It acts by preventing or inhibiting lipid peroxide formation that can be induced by different hepatotoxins. This effect may be mediated through membrane-stabilization or free radical-quenching activity (17).

The beneficial effects of plants polyphenols or antioxidants against some xenobiotic hydrocarbons induced-hepatotoxicity has been previously studied. Miyagawa et al. (42) reported a hepato-protective effect of green tea extract and its phenolic compounds against 1, 4-naphthoquinone-induced hepatotoxicity in rats. Moreover, the antioxidant, vitamin E succinate, was reported to protect against naphthalene-evoked changes in biomarkers of oxidative injury in rats (8). Similarly, garlic extract significantly protected the liver of mice against naphthalene toxicity (28).

Many investigators have demonstrated the efficacy of GSP as an inhibitor of lipid peroxidation and as a powerful free radical scavenger in vivo as well as in vitro (25, 35). Similarly, GSP was found to reduce the oxidation of polyunsaturated fatty acids in mouse liver microsomes (12) and provide protection against lipid peroxidation and DNA fragmentation in mice (13, 35). Moreover, in rats exposed to either a vitamin E-deficient diet or a high cholesterol diet, supplementation of polymeric grape seeds tannins to their diets normalized the diet-depleted levels of liver glutathione and reduced the elevated plasma lipid peroxides. In addition, a significant decrease in acetaminophen-induced serum aminotransferases elevation, hepatic DNA damage, and mortality rate in rats administered GSP has been reported (25, 43, 44).

The protective effects of GSP on GSH changes has been documented (14, 44) and was confirmed in
the present study, where GSP was capable of restoring naphthalene-evoked depletion of GSH levels. This restoration of hepatic GSH contents in the liver of rats treated with GSP appeared to be effective in providing protection against naphthalene-affected rat livers, since a significant reduction in lipid peroxidation and DNA fragmentation were observed in the present study. This effect was also accompanied by a significant improvement in activities of the liver function enzymes and was reflected by the increased ability of the rat liver to excrete BSP dye. BSP clearance represents a simple reflected by the increased ability of the rat liver to excrete BSP dye. BSP clearance represents a simple sensitive test for the functional integrity of the liver (24, 45). The results of the present study indicated that GSP, in the doses utilized, significantly improved the capacity of the damaged liver cells to excrete BSP dye, since the plasma levels of the dye were lower in the GSP-treated rats than that of rats treated with naphthalene alone.

Plants proanthocyanidins have been demonstrated to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules (14). In addition to the previously-mentioned mechanisms, proanthocyanidins appear capable of attenuating naphthalene bioactivation in vivo. Ray et al. (46) linked the protective abilities of GSP against acetaminophen-induced hepatotoxicity through antioxidant effects and through inhibition of CYP2E liver enzymes, responsible for metabolism of acetaminophen in mice and rats.

Therefore, it can be suggested that, concomitant presence of GSP, as a powerful antioxidant, during naphthalene metabolism facilitates the detoxification process, thus minimizing naphthalene-dependent production of toxic radical species along with drastically limiting the ongoing lipid peroxidation and DNA fragmentation. This effect would result in a significant reduction of serum levels of hepatic enzyme markers and improve BSP dye excretion capacity of hepatic cells as was seen in this study.

In conclusion, it is plausible to suggest that naphthalene metabolism triggered production of ROS, coupled with impaired oxidant/antioxidant balance, leading to a state of oxidative stress that could have been partially responsible for the resultant DNA fragmentation, hepatotoxicity, and the disturbance in the level of hepatic enzymes seen in this study. Restoration of hepatic cells antioxidant capacity appeared to provide protection against naphthalene-induced oxidative stress. The extract GSP exhibited a beneficial effect as a natural hepatoprotectant and seemed to abate the oxidative insult in liver tissue, restore the altered naphthalene-sensitive hepatic biochemical markers to an appreciable extent, improve the hepatic GSH plus lipid peroxidation contents, and attenuate DNA fragmentation of the liver cells.

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References

PROTECTIVE EFFECT OF GRAPE SEEDS


