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Grapes are one of the most consumed fruits in the world and are rich in polyphenols. Grape seed proanthocyanidins (GSP) have demonstrated chemopreventive and/or chemotherapeutic effects in various cancer cell cultures and animal models. The clinical efficacy of chemotherapy is often limited by its adverse effects. Several studies show that reactive oxygen species mediate the cardiotoxicity and neurotoxicity induced by various cancer chemotherapeutic agents. This implies that concomitant administration of antioxidants may prevent these adverse effects. The review was carried out in accordance with the PRISMA guidelines. An electronic search strategy in Medline and Embase databases was conducted. Of the 41 studies reviewed, 27 studied GSP while the remainder (14) studied grape seed or skin extracts (GSE). All the studies were published in English, except 2 in Chinese. A significant percentage (34%) of the studies we reviewed assessed the effect of GSE or GSP on cardiotoxicity induced by chemotherapy. Doxorubicin was the most common chemotherapeutic drug studied followed by cisplatin. Research studies that assessed the effect of GSE or GSP on radiation treatment accounted for 22% of the articles reviewed. GSE/GSP ameliorates some of the cytotoxic effects on normal cells/tissues induced by chemo/radiotherapy.

INTRODUCTION

Epidemiologic studies have demonstrated that the consumption of a vegetable and fruit based diet significantly reduces the overall cancer risk (1,2). The World Cancer Research Fund has estimated that up to one-third of the cancers that occur in developed countries like the United States are related to overweight or obesity, physical inactivity, and/or poor nutrition (3). Fruits and vegetables have been hypothesized to be major dietary contributors to cancer prevention (4).

Grapes are one of the most widely consumed fruits in the world and are rich in polyphenols of which about 60% to 70% is found in grape seeds as dimers, trimers, and other oligomers of flavan-3-ols, known commonly as proanthocyanidins. Grape seed proanthocyanidins (GSPs) have demonstrated chemopreventive and/or chemotherapeutic effects in various cancer cell cultures and animal models (5).

Bioactive phytochemicals, particularly those present in the diet, offer promising options for the development of more effective strategies for the prevention or treatment of cancers and they can be utilized as complementary or alternative medicine. Although phytochemicals have been used for thousands of years in various cultures/civilizations for the treatment of many diseases, wound healing, or to preserve skin beauty, their active ingredients and mechanisms of action often are not well characterized. GSPs are promising bioactive molecules that have demonstrated anticarcinogenic effects in some animal tumor models and exhibit no apparent toxicity in vivo (5–7). It is likely that at least some of the components present in the GSPs act synergistically and thus this product may be more effective than any single component. Grape seed extracts (GSE) obtained from different regions vary in their content of polyphenols. The amount of polyphenols in GSE correlated with increased antiemetic effect (8).

Advances in chemotherapy have contributed to the increased survival rates of cancer patients (9). However, the clinical efficacy of chemotherapy is often limited by its non-cancer specific effects causing damage to proliferating normal cells, especially those of the bone marrow and the lining of the gastrointestinal tract (10). Several studies show that reactive oxygen species (ROS) mediate the cardiotoxicity and neurotoxicity induced by various cancer chemotherapeutic agents. This implies that concomitant administration of antioxidants may prevent these

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adverse effects. Chemotherapeutic agents may also use ROS independent mechanisms, allowing antioxidants to reduce ROS-induced toxicities without interfering with the cytotoxic effect on cancer cells. However, as the potential role of ROS production in the mechanism of cytotoxicity toward cancer is still uncertain for some agents, the concomitant use of antioxidants with these agents is often controversial (11).

Overproduction of ROS, including superoxide anion ($O^{2\cdot -}$), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) are to a large extent responsible for radiation damage, which can overwhelm the levels of antioxidants resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur (12).

There is a need to develop nontoxic compounds that can reduce the undesirable effects of ionizing radiation used for medical purposes. Such compounds could potentially protect humans against genetic damage, mutation, changes in physiological systems, and the teratogenic effects of toxic agents (including radiation) that act through the generation of free radicals (13,14).

The aim of this review is to use the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) method to examine whether there is sufficient preclinical evidence to justify clinical trials of grape seed extract for the prevention or treatment of chemotherapy or radiotherapy induced toxicity. Both in vitro and in vivo studies were assessed.

METHODS

This review was carried out in accordance with the PRISMA guidelines (15,16). An electronic search strategy in Medline and Embase databases was conducted.

Search Strategy

With the assistance of a medical librarian, we searched Medline and Embase for original articles concerning the role of GSE in the treatment of chemo/radiotherapy induced toxicities from 1995 to May 2013. There were no articles concerning the role of GSE in the treatment of chemo/radiotherapy induced toxicities prior to 1995 in Medline and Embase. No language restriction was used. Fig. 1 summarizes the steps involved in literature selection. The following is the search strategy used for Medline:

((grape seed extract [mh] OR "grape seed extract" [tiab] OR "grape seed extract proanthocyanidins"[tiab] OR "vitis vinifera" [tiab] AND (proanthocyanidin [tiab] OR proanthocyanidins [tiab] OR proanthocyanidins [mh]))
AND
Chemopreventive[tiab] OR chemotherapeutic [tiab] OR "radiation Induced" [tiab] OR "radiotherapy induced"[tiab]

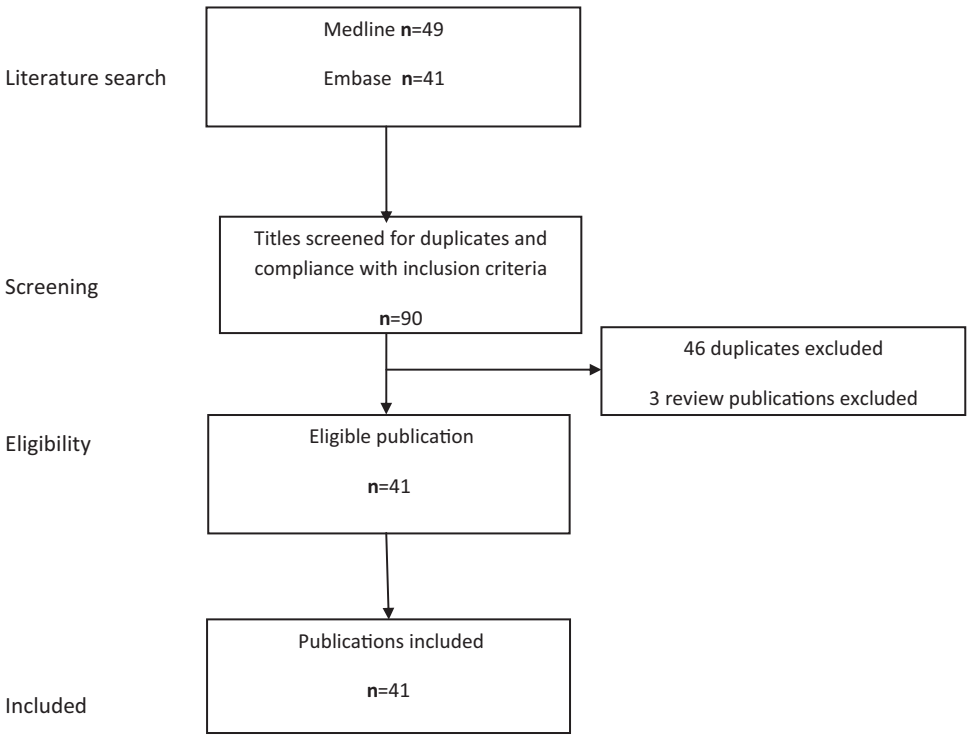

FIG. 1. Study selection summary.

OR "chemotherapy induced"[tiab] OR radiotherapy, adjuvant/ae[mh] OR chemoprotective[tiab] OR antineoplastic agents/ae[mh] OR radiotherapy/ae[mh] OR radiotherapy[tiab] OR chemotherapy, adjuvant/ae[mh] OR chemoradiotherapy/ae[mh] OR radiation/ae[mh] OR radiation effects[mh] OR radiation injuries[mh] OR radiation injuries, experimental [mh] OR irradiation[tiab] OR radiotherapeutic [tiab] OR antineoplastic agents [mh] OR antibiotics, antineoplastic [mh]

The following is the search strategy used for Embase:

'grape seed extract'/exp OR 'proanthocyanidin'/ exp OR 'grape seed extract': ab, ti OR 'grape seed proanthocyanidins': ab, ti OR 'grape seed': ab, ti OR 'grape seeds': ab, ti OR gspe: ab, ti AND ([embase]/lim OR [embase classic/lim) AND [1995–2013]/py

AND

'antineoplastic agent'/ exp/dd_ae, dd_to OR 'cancer radiotherapy'/ exp OR 'antineoplastic agent'/ exp OR 'chemoradiotherapy'/ exp OR 'radiotherapy'/ exp OR 'radiation injury'/ exp OR 'radioprotective agent'/ exp OR 'radiation response'/ exp OR 'radiotherapy induced': ab, ti OR 'chemotherapy induced' : ab, ti OR chemoprotective: ab, ti OR radiotherapy: ab, ti OR radiotherapy: ab, ti OR chemotherapy: ab, ti OR chemoradiotherapy: ab, ti OR 'radiation effects': ab, ti OR 'radiation injuries': ab, ti AND ([embase]/lim OR [embase classic]/lim) AND 1995–2013]/py

Another search using the following search strategy was performed to obtain other relevant articles:

Grape seed extract AND methotrexate.

Study Characteristics and Data Extraction

The selection of the studies was performed on the basis of the title and abstract. In case of doubt, the entire publication was reviewed. Two investigators independently screened all the abstracts for the inclusion criteria. Differences were resolved by a third investigator. Studies published in Chinese were translated by a Chinese scientist colleague. All original preclinical studies of GSE and its byproducts with chemo/radiation therapy induced toxicity were included. Clinical studies, review articles, and letters were excluded. The following data were extracted from the studies: author, year of publication, original language, intervention, toxicity, chemo/radiotherapy, type of experiments performed (i.e. cell culture vs. animal study), weight, and use of randomization.

Methodological Quality of Studies

Methodological quality of the animal studies was assessed by one reviewer. Data was extracted on study design, sample size calculation, allocation concealment, blinding, number of animals randomized, and inclusion of controls. We did not use a checklist and a score to assess study quality. According to van der Worp et al. (17) use of this is controversial and it is not clear that any particular characteristics are better than others.

RESULTS

A total of 90 articles were identified initially. Of these, 41 publications met our inclusion criteria. Eight were in vitro studies, 28 were in vivo studies, and 5 studies that were a combination of in vitro and in vivo studies (Tables 1 and 2).

Of the 41 studies reviewed, 27 studied proanthocyanidins (GSP) whereas the remainder (14) studied GSE. All the studies were published in English except 2 published in Chinese. A significant percentage (34%) of the studies we reviewed assessed the effect of GSE or GSP on cardiotoxicity induced by chemotherapy. Other toxicities assessed in the reviewed articles include gastrointestinal toxicity, hepatotoxicity, nephrotoxicity, mucositis, genotoxicity, hematological toxicity, and emesis. These accounted for 49% of the studies reviewed. In all these studies, GSE or GSP was able to ameliorate the toxic effects of chemotherapy. Doxorubicin (Dox) was the most common chemotherapeutic drug studied in the reviewed articles (45%), followed by cisplatin (12.5%). Other drugs include methothrexate, 5-fluorouracil, idarubicin, and cyclophosphamide.

Research studies that assessed the effect of GSE or GSP on radiation treatment accounted for 22% of the articles reviewed. Radiation treatment caused several toxicities. These include hepatic toxicity, chromosomal damage, white blood cell toxicity, and oxidative stress. GSE or GSP was able to minimize or prevent the toxic effects of radiotherapy in all, except 1 study. The total percentage was more than 100% because some of the studies were designed to assess more than one type of toxicity.

The dose of GSE or GSP used ranged from 10mg/kg to 500mg/kg. The route of administration was mostly oral; however, the intraperitoneal route was used in two studies (20,37). Several species of rats and mice were used for the studies including Sprague Dawley rats, Wistar albino rats, Mus musculey var albino mice, and Agouti rats. The dose used in vitro studies ranged from 25 μg/ml to 100 μg/ml.

Regarding the quality of the studies, none clearly reported a sample size calculation, allocation concealment or blinding of whether animals belonged to treatment or control group. Most of the studies mentioned randomization of the animals in the methods section (75%).

Outcome

All the studies in this review except 1 reported a positive outcome when GSE/GSP was combined with chemo/radiotherapy to prevent or treat chemo/radiotherapy induced toxicity. When GSE/GSP was combined with Dox, GSE/GSP suppressed Dox-induced EKG changes except QT interval

TABLE 1
Description of studies (chemotherapy)

Ref.	Intervention	Toxicity	Chemo-therapy	Type of study	Cell line/cell type	Animal	Randomization	Outcome
18	Proanthocyanidins 70 mg/kg	Cardiotoxicity	Doxorubicin 15 mg/kg	In vivo		Male Sprague Dawley rats	Yes	Proanthocyanidin suppressed Dox-induced EKG and biochemical changes.
19	GSP 200 mg/kg	Cardiotoxicity	Doxorubicin 20 mg/kg	In vivo		Adult male Wistar Albino rats	Yes	Proanthocyanidins suppressed Dox-induced biochemical and histological changes.
20	GSE 500 mg/kg	Cardiotoxicity	Doxorubicin 20 mg/kg	In vivo		Female Wistar rats	None	GSE counteracted Dox-induced disturbances of hemodynamic parameters, alleviated oxidative stress and increased super oxide dismutase activity.
21	Proanthocyanidin 70 mg/kg	Cardiotoxicity	Doxorubicin 15 mg/kg	In vivo		Rats	None	Proanthocyanidin prevented EKG alterations induced by Dox, except QT interval prolongation.
22	GSP 50 μg/ml	Cardiotoxicity	Doxorubicin 10 μM	In vitro	Cardiomyocytes			GSP in combination with Dox has protective effect on Dox induced toxicity in cardiomyocytes. GSP did not decrease the proliferation-inhibitory effect of Dox in MCF-7 human breast cancer cells.
23	GSE 50–150 mg/kg	Cardio/genotoxicity	Doxorubicin 2.5 mg/kg	In vivo		Mus musculus var Albino mice	Yes	GSE acts as a potent antioxidant to prevent heart damage and genotoxicity of bone marrow cells.
24**	GSP	Cardiotoxicity	Doxorubicin	In vivo/in vitro		Hann: NMRI mice	None	Proanthocyanidin decreased production of Dox metabolites and ROS.
25	GSE proantho-cyanidin 100 mg/kg	Cardiotoxicity	Doxorubicin 7.5 mg/kg & 15 mg/kg	In vivo		Wistar albino male rats	Yes	Proanthocyanidin decreased Doxo induced cardiac injury.
26	Proanthocyanidin 150 mg/dy	Cardiotoxicity	Doxorubicin 15 mg/kg	In vivo		Male Sprague Dawley rats	Yes	Proanthocyanidins protect cardiomyocytes from Dox induced cardiotoxicity. They do not attenuate the antitumor activity of Dox.
27	Proanthocyanidin B 4100 μM	Cardiotoxicity	Doxorubicin 20μM	In vitro	Rat heart cell line H9C2(2-1)			Grape seed polyphenol catechin and PcB(4) pretreatment would protect cardiomyocytes against Dox induced toxicity.
28	GSP 200 mg/kg	Cardiotoxicity and immune suppression	Doxorubicin 2 mg/kg	In vitro/In vivo		Female BALBIc mice	None	Proanthocyanidin could enhance the anti-tumor activity of Dox and ameliorate Dox induced myocardial oxidative stress and immune suppression in tumor bearing mice.
29	GSP 100 mg/kg	Cardiotoxicity	Doxorubicin 20 mg/kg	In vivo		Mice	None	Results suggest that GSP exposure is bioavailable and provides significant multiorgan protection against drug and chemical induced assaults.
30	IH 636 GSP100 mg	Cardiotoxicity	Doxorubicin 20 mg/kg	In vivo		Mice	None	GSP preexposure prior to Dox provided near complete protection in terms of serum chemistry changes and significantly reduced DNA fragmentation.
31	GSP 100 mg/kg	Cardiotoxicity	Doxorubicin 20 mg/kg	In vitro/ In vivo	Normal human oral keratinocytes	Mice		Results suggests that GSP exposure provides significant multiorgan protection against structurally diverse drug and chemical induced toxic assaults.
32	GSE and skin extract 500 mg/kg	Hematologic toxicity	Doxorubicin 20 mg/kg	In vivo		Female Wistar rats	Yes	GSE suppressed Dox induced biochemical changes. GSE exerted antioxidant properties.
33	Proanthocyanidin 1.68, 3.375, &6.75mg/ml	DNA damage	Doxorubicin 0.125 mg/ml	In vitro	Somatic cells of Drosophila			GSP suppressed the DNA damage induced by Dox in a dose dependent manner.
34	Deffated milled grape seed 2.5, 10 & 25 μg/ml	Hepatotoxicity	Doxorubicin 25 μM	In vitro	Hepatocytes from Wistar male rats			Deffated milled grape seed extract protects the cellular membrane from oxidative damage and consequently prevents protein and lipid oxidation.
35	Proanthocyanidin 100 mg/kg	Mutagenicity	Doxorubicin	In vivo		Adult male white Swiss albino mice	Yes	Proanthocyanidins can be a promising chemopreventative agent to avert secondary malignancy and abnormal reproductive outcome risks in cancer patients receiving Dox involved treatment.
36	Red GSE 37.5, 25, & 12 μg GA Eq/ml	Normal/tumor cell lines	Doxorubicin 0.2–200μM	In vitro	Normal(Hfl-1) Tumor (Hep G2 &Mis) cell lines		N/A	Red GSE treatment prior to subsequent administration of Dox afforded a differential protection against Dox negative toxic side effects.
37		Nephrotoxicity	Cisplatin 20 mg/kg	In vivo		Mice	Yes	Proanthocyanidin attenuates cisplatin induced oxidative renal toxicity.

	Proanthocyanidin 10, 20, & 40 mg/kg							
38	GSP 200 mg/kg	Nephrotoxicity	Cisplatin 7.5 mg/kg	In vivo		Male Sprague Dawley rats	Yes	GSE protected against toxic effects induced by cisplatin.
39	GSP 200 mg/kg	Cisplatin-induced toxicity	Cisplatin 7.5 mg	In vivo		Sprague Dawley rats	None	GSP extract preexposure prior to cisplatin provided near complete protection in terms of plasma and organ biochemical changes, antioxidant enzyme activity and oxidative stress.
40	GSE 100 mg/kg	Genotoxicity	Cisplatin 2.2 or 5.5 mg/kg	In vivo		Swiss albino mice	None	GSE plays a role in attenuating the genotoxicity induced by cisplatin.
41	GSE 3–30 mg/kg	Emesis	Cisplatin 3 mg/kg	In vivo		Male Wistar rats	None	Appropriate doses of GSE have therapeutic value in treating cisplatin induced emesis.
42	Proanthocyanidin 100 mg/kg	Gastrointestinal toxicity	Methotrexate 20 mg/kg	In vivo		Rats	Yes	Proanthocyanidin reduced severity of intestinal oxidant injury.
43	Proanthocyanidin 100 mg/kg	Small intestine damage	Methotrexate 20 mg/kg	In vivo		Male Wistar albino rats	Yes	Proanthocyanidin decreased jejunal damage and malon-dialdehyde level and increased superoxide dismutase and glutathione peroxidase levels.
44	GSE 50 mg/kg	Hepato/ nephrotoxicity	Methotrexate 10 mg/kg	In vivo		Male Wistar rats	Yes	Evidence supports the importance of GSE in the prevention of hepatic and nephritic damage induced by methotrexate.
45	GSE100 mg/kg	Hepatotoxicity	Methotrexate 20 mg/kg	In vivo		Male Wistar rata	None	It appears that GSE protects the rat liver and inhibits methotrexate induced oxidative stress.
46	GSE400 mg/kg	Mucositis and inflammatory bowel disease	5-fluoro-uracil 150 mg/kg	In vivo		Female dark Agouti rats	None	GSE partially ameliorated several indicators of disease severity in models of intestine mucositis and ulcerative colitis.
47	GSE400 mg/kg	Mucositis	5-fluoro-uracil 150 mg/kg	In vivo	IEC6 cells	Female dark Agouti rats	None	GSE partially protected IEC-6 cells from 5-FU induced cytotoxicity and ameliorated intestinal damage induced by 5-FU in rats.
48	Red grape seed proanthocyanidin. 25–100 mg/kg	Hepatotoxicity	Idarubicin 30 nM and 4HC 1 μg/ml	In vitro/in vivo	Normal human chang liver cells.	Swiss Webstar mice	None	GSP diminishes the cytotoxicity induced by chemotherapeutic drugs such as Ida and 4HC on normal Chang liver epithelial cells.
49	GSP25 μg/ml	Toxic effects on cultured Chang epithelial cells	Idarubicin 30 nM nd 4HC 1 μg/ml	In vitro	Chang epithelial cells			GSP can be a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents.

GSE = grape seed extract; GSP = grape seed proanthocyanidin; 4HC = 4-hydroxy-peroxycyclophosphamide.
**Poster presentation. Dose not included.

TABLE 2
Description of studies (radiation therapy)

Ref.	Language	Intervention	Toxicity	Radio-therapy	Type of study	Cell line/cell type	Animal	Randomization	Outcome
50	English	GSE proanthocyanidin 20 μl	Chromosome damage	Gamma radiation 2Gy $\pm$ 3%	In vitro	Human lymphocytes			GSE provided a cytoprotective effect (magnitude of protection = 14.8%) when added to cultured blood cells prior to irradiation.
51	Chinese	Proanthocyanidin 100 mg/l	Radiation induced injury of human derived cells	Gamma radiation 4 & 8Gy	In vitro	AHH-1& HIEC cells			Proanthocyanidin have protective effect on radiation induced cellular injury.
52	English	GSE 100 mg/kg	Radiation induced oxidative stress in heart and pancreas	Gamma radiation 5Gy	In vivo		Male albino rats	Yes	GSE protects the heart and pancreas tissues from oxidative damage induced by ionizing radiation.
53	English	GSP 10 g/l	Radiation injury	Gamma radiation 7Gy	In vivo		Wistar rats	Yes	GSP and combination of GSP and casein peptide can effectively attenuate the damage of intestinal mucosal barrier from radiation in rats.
54	English	GSP 500 mg	Spleen and WBC toxicity	Radiotherapy 7Gy	In vivo		Male and female wistar rats	None	Peroxidative impairment could be obviously attenuated and reduction in WBC could also be improved by a single GSP.
55	English	GSE 100 mg/kg	Hepato- toxicity	Gamma radiation 8Gy	In vivo		Male wistar rats	None	The level of antioxidant parameters on radiation induced liver toxicity were restored to control values with grape seed extract therapy.
56	English	GSE 50 mg/dy	Effect on blood lympho-cytes	X-radiation 6Gy	In vivo		Albino wistar rats	Yes	Results indicate that GSE enhanced the antioxidant status and decreased the incidence of free radical induced lipid peroxidation in blood samples of rats exposed to X-radiation.
57	Chinese	Grape procyanidin 150 mg/kg	Cell apoptosis	Gamma radiation 5Gy	In vitro/In vivo		Mice	None	Grape procyanidin has certain protective effect against mice pancreatic cell apoptosis and the abnormal expression of bcl2 bax protein induced by (60) Co gamma.
58	English	GSP 100 g/l	Chromosomal damage	X-radiation 48cGy	In vivo		Adult male swiss mice	None	GSE is radio-protective.

WBC = white blood count; GSE = grape seed extract; GSP = grape seed proanthocyanidin.

prolongation (18,21). GSE/GSP also suppressed Dox induced biochemical and myocardial histological changes (18,19). In other studies, GSE/GSP suppressed Dox-induced DNA damage in somatic cells of drosophila melanogaster in a dose-dependent manner, ameliorated immunosuppression in tumor bearing mice and prevented genotoxicity in bone marrow cells (23,28,33). The presence of GSE/GSP did not attenuate antitumor effect of Dox (22,26,28).

The combination of GSP with methotrexate reduced jejunal damage and the level of malondialdehyde (MDA), a marker of lipid oxidation, in intestinal tissue and increased levels of two antioxidant enzymes, super oxide dismutase (SOD), and glutathione peroxidase (GSH) (42,43). GSE reversed the oxidative effect of methotrexate as demonstrated by reducing MDA levels and increasing SOD and catalase (CAT) activities in the rat liver (45).

GSE attenuated genotoxicity induced by cisplatin and decreased secondary malignancies and abnormal reproductive outcomes (40). Appropriate doses of GSE were found to have therapeutic value in preventing cisplatin induced kaolin ingestion (41).

GSE ameliorated 5-fluorouracil (5-FU) induced intestinal damage, and several indicators of disease severity in models of intestinal mucositis (46,47). GSE/GSP effectively attenuated the damage of intestinal mucosal barrier from radiation in rats. It also has a protective effect on radiation induced cellular injury in vitro (51,53). Alcaraz et al. found that the antioxidant capacity of several compounds appeared to determine the degree of protection provided by such compounds when administered before radiation. However, compounds that are soluble in lipids (e.g. alpha tocopherol) provided greater protection when administered immediately after ionizing radiation. GSE did not demonstrate protection from radiation side effects in the experiments conducted by these investigators.

DISCUSSION

The treatment of cancer patients has improved over time. For some cancers, there has been a significant improvement in the 5-year survival rate. Despite improvement in survival rate, there are still significant adverse effects associated with the use of chemotherapy. Secondary treatment related malignancies have been reported after the use of combination chemotherapy (59–61). Anticancer agents are known to have an adverse effect on the reproductive system. High intensity chemotherapy regimens have resulted in greater success rates with certain cancers while simultaneously increasing concern over the effects of anticancer drugs on germ cells (62).

GSE, which is produced as a byproduct of wine and grape juice industries, represents a rich source of proanthocyanidins (63,64). Proanthocyanidins have been reported to exhibit a wide range of biological effects including antioxidant, antiinflammatory, and anticancer effects (65,66). GSE is an excellent scavenger of hydroxyl, superoxide and other radicals (64). It also protects against lipid peroxidation in cell membranes and DNA damage caused by ROS generation (67). Attia et al (35) in their study demonstrated that proanthocyanidins were neither mutagenic nor cytotoxic at the dose tested. In addition, they were able to protect mouse somatic and germinal cells against Dox induced mutagenesis. GSE has other potential anticancer activities (e.g., inhibition of aromatase and topoisomerase II activity). In addition, gallic acid and catechins have some of these potential therapeutic activities (68,69).

A wide dose range of GSE/GSP has been studied. Doses in the research reviewed here ranged from 10 to 500 mg/kg body weight. In addition, there were several types of laboratory animals and strains used in the studies. Some of the studies reviewed omitted details about the strain, age, and weight of animals used. These factors could influence experimental results and therefore should be consistently reported (70–72). All the studies reviewed except one demonstrated that GSE/GSP produced a reduction in cytotoxicity to normal cells and tissues. This systematic review provides support for advancing GSE/GSP into clinical investigations for preventing and treating chemo/radiotherapy induced cytotoxicity. If these findings are replicated in clinical studies, GSE/GSP may become a cheaper alternative to synthetic drugs without their added side effects. The concentration of GSE/GSP used in the in vitro studies ranged from 25 μg/ml to 100 μg/ml. The authors did not state how the concentrations of GSE/GSP were determined.

There were several limitations in the quality of the studies reviewed. None of the animal studies reported on blinding, allocation concealment, power, and sample size calculation. Fourteen articles out of 33 reported on randomization; however, the method of randomization was not mentioned in the studies. Randomization reduces selection bias and increases validity of the findings. Adequate internal validity of an animal experiment implies that the differences observed between groups of animals allocated to different interventions may, apart from random error, be attributed to the treatment under investigation (73). Studies that are blinded throughout their course will not be influenced by the investigators or other persons who have knowledge of treatment assignment. This leads to the prevention of performance, detection, and attrition bias. The knowledge of treatment assignment may subconsciously or otherwise affect the supply of additional care, outcome assessment, and decisions to withdraw animals from the experiment (17). To prevent selection bias, treatment allocation should be based on randomization. Foreknowledge of treatment group assignment may also lead to selective exclusion of animals based on prognostic factors (74).

None of the studies assessed discussed how the sample size was chosen. Power analysis is a requirement in human clinical trials and is often expected by regulatory authorities in some animal studies. This analysis helps to determine the number of animals to use in an experiment in order to detect a biologically important effect when there is one (75,76). Some of the

studies reported the number of animals inconsistently between the method and result sections.

Animal research in emergency medicine has revealed that studies that did not use randomization and blinding to reduce bias when comparing two or more experimental groups were more likely to find a difference between the treatment groups (77,78). Those studies that did incorporate these measures gave a lower estimate of treatment efficacy. These findings indicate that experimental designs which minimize bias have implications for the robustness of scientific results in biomedical research, and the suitability of these animal studies for translation into clinical trials (79). We cannot rule out that some of the studies reviewed may have used randomization where appropriate but did not report it.

Dox is one of the most commonly used chemotherapeutic drugs used in the treatment of a variety of cancers. The target of Dox is the DNA of dividing cells; the drug intercalates within DNA strands causing cell cycle blockage in the G_2 phase, single-stranded breaks, and inhibition of the activity of some nuclear proteins such as DNA and RNA polymerase and DNA-topoisomerase II (80). The clinical usefulness of Dox is limited by acute and chronic cardiotoxcity (81). The cause of Dox-induced cardiotoxicity is multifactorial; however, most Dox-induced cardiotoxicity can be attributed to the formation of ROS (82). In addition, Dox administration is associated with a decrease in endogenous antioxidants, which may be responsible for the scavenging of free radicals (83,84) leading to increased oxidative stress. GSE is an excellent scavenger of hydroxyl, superoxide, and other radicals (85). It also protects against lipid peroxidation in cell membranes and DNA damage caused by ROS generation (85). Demirkaya et al (25) showed that on histology, proanthocyanidin caused a significant decrease in Dox-induced cardiac injury. In addition, the protective effect was more evident in the electron microscopic evaluation. GSP did not interfere with the antitumor effect of Dox (22, 36). Some of the side effects of drugs such as methotrexate, cisplatin, and 5-FU may also be mediated through induction of oxidative stress. GSE/GSP may have a protective effect against such oxidative damage (38,39,45,47).

Radiation induces oxidative stress through generation of ROS, resulting in imbalance of prooxidants and antioxidants in the cells culminating in cell death (86,87). ROS can induce the cellular antioxidant defense enzymes such as SOD, GSH, and CAT (88). Accumulation of ROS such as superoxide anion, hydrogen peroxide, singlet oxygen and the hydroxyl radical in the cell can overcome the natural antioxidant defenses causing damage to biological macromolecules, including nucleic acids, proteins and lipids (55). Radioprotectors were developed to minimize the effect of radiation therapy on normal tissues. The best known radioprotectors are the sulfhydryl compound products cysteine and cysteamine (89). The most effective compound tested against lethal doses of X-rays and γ rays in mice is amifostine (90). In a study conducted by Cetin et al. (55), GSE reduced the oxidative effect of radiotherapy on the rat liver. Rats receiving GSE had a low MDA level and the activity values of SOD and CAT were reversed, approaching those of the control group. GSE treatment considerably increased the formation of antioxidant product in hepatocytes. This effect may be due to the phenolic composition of GSE and its antioxidant activity (55).

The First International Symposium on Systematic Reviews in Laboratory Animal Science was held in Netherlands in 2012. The need for systematic reviews in laboratory animals was recognized and it was pointed out that conducting a systematic review in animal science involves several methodological challenges (91). The first was that a variety of animal species and strains are used in a variety of experimental designs. The large variation/heterogeneity between the animal studies impairs a reliable comparison between the study results of these individual studies. Secondly, the methodological quality of individual animal studies is usually poor and is often inadequately reported (79). Malcolm Macleod, the keynote speaker of the symposium pointed out that there is only a weak association between impact factor of journals and methodological quality in laboratory animal science (79). In addition interpreting the results of a meta-analysis can be hampered by publication bias, as negative and neutral results are less likely to be published than positive results (17,92). The speakers at this symposium felt that by increasing the number of systematic reviews in animal studies, more awareness will be generated regarding the critical assessment of the strengths and weaknesses in study design, conduct, and analysis of animal experiments (91).

There are some limitations to this review. The quality of the studies reviewed varied. About three-fourths of the studies reported randomization; however, the method of randomization was not disclosed. We were not able to determine the effect of unpublished studies in this review, hence publication bias might account for some of the effects we observed (17). Other limitations include poor or incomplete reporting of study designs. In addition, the search strategy was limited to Medline and Embase.

Four clinical trials assessing the use of GSE in cancer patients are listed on Clinicaltrials.gov (93). One trial is currently recruiting patients. Three trials have been completed with one publication (94).

The GSE/GSP used in these studies was from several sources. There is no generally accepted way of standardizing GSE. However, several manufacturers produce “standardized” GSE. The relevance of bioavailability and standardization cannot be overemphasized for future studies.

Some studies have suggested the inhibitory effect of GSE on human cytochrome P-450 CYP3A4 (95,96). This enzyme is involved in the metabolism of several drugs including chemotherapeutic drugs. The clinical relevance of these interactions should be explored in clinical studies.

CONCLUSION

Based on the findings of this review, GSE/GSP ameliorates some of the cytotoxic effects induced by chemo/radiotherapy. This effect may be mediated through GSE/GSP's antioxidant effects. However, the outcome of all the studies assessed in this review except one was positive. This increases the likelihood of publication bias. In addition, the methodological quality of the studies varied. Further, well-designed preclinical research of GSE/GSP would be desirable to better inform the design of clinical trials. However, the existing database suggests that the intervention likely can be safely combined with various cancer therapies and is sufficient to recommend a starting dose range as well as some clinical scenarios in which benefit might be seen.

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