Antioxidant and hepatoprotective activities of grape seeds and skin against Ehrlich solid tumor induced oxidative stress in mice

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Abstract

The current study was conducted to examine the antioxidant effect of grape seeds and skin (GSE and GSK) against Ehrlich solid tumor (EST)-induced oxidative stress, hepatic dysfunction and pathological changes in the liver of albino mice. GSE and GSK were mixed with the standard diet and given to mice 14 days before subcutaneous tumor cells inoculation and continued for 30 days. EST-bearing mice showed increase of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST), elevation in lipid peroxidation (MDA) level accompanied by a decline in glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in blood and liver. Histopathologically and ultrastructurally, liver of EST bearing group showed hepatic degeneration with sinusoidal EST and lymphocytic infiltration, increase of collagen fibers, irregular nuclei, altered mitochondria and increase of secondary lysosomes. Histochemically, total protein and DNA contents were reduced in the liver of EST group. Conversely, GSE and GSK supplementation to EST bearing mice potentially recovered liver function enzymes, reduced MDA level, augmented antioxidant parameters, normalized liver protein and DNA contents and improved the pathologically examined hepatic lesions. In conclusion, GSE and GSK revealed potent antioxidant properties by augmenting the antioxidant defense system thereby protecting the liver against oxidative stress induced by Ehrlich solid carcinoma tumors.

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1. Introduction

Oxidative stress is closely related to all aspects of cancer, from carcinogenesis to the tumor-bearing state, from therapy to prevention. The tumor-bearing state is supposed to be under oxidative stress associated with active oxygen production by tumor cells and abnormal oxidation-reduction control [1]. Several studies have indicated that tumor growth can cause antioxidant disturbances and accelerates lipid peroxidation...
(Lpx) in vital organs of the tumor hosts [2–4]. The use of natural products is considered as one of the most effective approaches of cancer treatment and was evidenced to have fewer side-effects. Dietary intake of foods rich in antioxidants was used in cancer prevention [5]. One of the most notable plants is grape, which has shown promising chemopreventive and antioxidant effects in numerous in vitro and in vivo models [6,7]. Grape is one of the world’s largest fruit crops and one of the most commonly consumed antioxidants rich fruits in the world. Grape contains a lot of active ingredients; chiefly exist in grape skin and seeds, including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins, and resveratrol [8,9]. The beneficial effects of grape seeds and skin are due to their antioxidant [10–12], anticancer [8,13], antimicrobial [14], anti-inflammatory [15,16] activities and activation of apoptosis signal [14].

Our ongoing studies (not shown) indicate that GSE and GSK intake exerts a significant reduction in tumor growth of animals bearing Ehrlich carcinoma. Knowledge about the effect of GSE and GSK intake on lipid peroxidation and antioxidant status has been examined in different models such as, chemical induction by DMN [9] and high fat diet [10]; however, Studies involved oxidative stress and antioxidant status in the distant organs of tumor-bearing animals are limited. Therefore, it was of particular interest to examine the antioxidant effect of GSE and GSK against Ehrlich solid tumor-induced oxidative stress in the liver of female albino mice.

2. Materials and methods

2.1. Ehrlich ascites carcinoma cells & tumor induction

Ehrlich ascites carcinoma (EAC) cells were supplied from National Cancer Institute, Cancer Biology Department, Cairo, Egypt via a 25 g female albino mouse. They were conserved by weekly intraperitoneal inoculation of saline solution containing 10^6 cells/mouse [17]. Mice were inoculated subcutaneously with 0.2 ml of EAC, which contained 2.5 × 10^6 viable EAC cells, in the back of each mouse to produce Ehrlich solid tumors (EST).

2.2. Preparation of GSK and GSE

Grape (Vitis vinifera) skin (GSK) and seeds (GSE) were separated from the pulps manually, GSK was dried at 50 °C and GSE was dried at 70 °C in a dry oven for several hours, then grinded to powder using a grinder [9]. Equivalent amount of GSE and GSK were mixed uniformly with the standard diet powder at concentration of 10% (w/w) according to Shin and Moon [9].

2.3. Experimental design

A total of 50 adult female Swiss albino mice weighting (18–21 g) were obtained from the animal Farm of Vucsera, Helwan, Egypt. Animals were housed under a constant temperature of 25 ± 1 °C with free access to drinking water and acclimatized to laboratory conditions for one week prior to the experiment. They were fed on a standard rodent diet composed of 55% corn starch, 20% casein, 15% corn oil, 5% salt mixture and 5% vitaminized starch (Egyptian Company of Oil and Soap, Kafr Elzayat, Egypt). All experiments were carried out in accordance with the protocols approved by the Local Experimental Animal Ethics Committee. The animals were randomly divided into four groups. Group 1 (10 mice) served as untreated control (received neither EST inoculation nor GSE and GSK), Group 2 received only GSE and GSK treatment (10 mice); animals were freely fed daily the diet mixed with GSE and GSK powders at concentrations of 10% (w/w) for 44 days. Group 3 received only EST (15 mice); animals were injected subcutaneously at the back with 2.5 × 10^6 EAC cells for solid tumor induction on day 14, and left without any treatment for 30 days. Group 4 received EST inoculation plus GSE and GSK (15 mice); animals were fed daily the diet mixed with GSE and GSK at concentrations of 10% (w/w) for 44 days, on day 14, they were injected subcutaneously at the back with 2.5 × 10^6 EAC cells for solid tumor induction and the diet regime was continued for 30 days.

2.4. Blood sampling and biochemical investigations

At the end of the experiment, animals were fasted for 16 h before sampling. Whole blood was collected by heart puncture after light anesthesia using heparinized syringes. The separated plasma from heparinized blood was collected and kept at −20 °C until used for the determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities colorimetrically as described by Reitman and Frankel [18], lipid peroxidation as malondialdehyde (MDA) was determined according to the colorimetric method of Yoshioka et al. [19], catalase (CAT) according to the colorimetric method of Johansson and Borg [20] and superoxide dismutase (SOD) according to the method of Minami and Yoshikawa [21]. A portion of whole blood sample was used for estimation of glutathione (GSH) according to the colorimetric method of Beutler et al. [22].

2.5. Liver tissue sampling for biochemical investigations

After the collection of blood samples, all animals were killed by cervical dislocation and liver tissues were dissected out and carefully trimmed, weighed, and homogenized in potassium phosphate buffer solution (50 mM, pH 7.5) using a Potter-Elvehiem homogenizer to give a 10% homogenate. Homogenates were centrifuged at 1500 g for 10 min at 4 °C; supernatant was recovered, placed on ice, and immediately used for the determination of MDA, CAT, SOD, and GSH levels by the previously mentioned methods [19–22].

2.6. Histopathological examination

At the end of the experiment, liver sample from all different animal groups were obtained and fixed in 10% buffered neutral formalin. The fixed liver specimens were dehydrated in ascending series of ethyl alcohol and embedded in paraffin. Sections at 5 μm thickness were stained according to the following histological stains: H&E [23] and Masson’s Tri-chrome method [24] for collagen fibers.
2.7. Histochemical investigation

Total protein was detected using mercury-bromophenol blue stain [25] and DNA content was investigated using Feulgen reaction [26].

2.8. Electron microscopic investigation

Dissected liver samples were fixed in 4% glutaraldehyde in phosphate buffer (pH 7.2) at 4 °C and post-fixed in 1% cold osmium tetroxide in phosphate buffer at pH 7.2 for 3 h. The specimens were then dehydrated in graded ethanol and embedded in Epson-Araldite resin. Ultrathin sections were stained by uranyl acetate followed by lead citrate as described by Reynolds [27] and examined on Joel Electron Microscope (JAPAN) operating at 60 kV.

2.9. Statistical analysis

Values are expressed as mean ± SE (standard error). Biochemical parameters data were analyzed using one way analysis of variance followed by the bonferroni post hoc test for multiple comparisons. A p-value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Biochemical studies

As shown in Fig. 1, untreated EST mice showed marked increase in plasma ALT & AST levels by 79.58% for ALT and 48.98% for AST as compared with the normal control group. Supplementation with GSE and GSK 14 days prior to tumor cells inoculation and throughout the experimental period, significantly inhibited the elevated levels of ALT & AST in EST bearing animals to reach 8.49%, 5.83% of control values, for ALT & AST respectively.

As shown in Table 1, marked elevation in MDA levels and significant inhibition of GSH content, SOD and CAT activities were observed in blood and liver of EST bearing group as compared with that of control group. In contrast, supplementation with GSE and GSK 14 days prior to tumor cells inoculation and throughout the experimental period significantly reduced the elevated levels of MDA in EST bearing animals to be comparable with the control values in blood and liver tissues. In addition, GSE and GSK supplementation restored the reduced GSH content and elevated SOD and CAT activities in blood and liver tissues to reach the normal values.

3.2. Histological observations

The liver section of the control group showed the polyhedral hepatocytes with centrally located nucleus and granular cytoplasm (Fig. 2A). The hepatocytes arranged in strands alternating with blood sinusoid forming a network around central vein (Fig. 2A). Liver sections of GSE and GSK treated group showed similar structure of control liver (Fig. 2B). While liver sections of EST bearing group displayed disruption of the characteristic cord-like arrangement of the liver cells, ballooning degeneration with cytoplasmic vacuolation and intranuclear cytoplasmic inclusions, sinusoidal infiltration of clumps of Ehrlich tumor cells mixed with lymphocytes and erythrocytes with associated long-lasting as shown in Fig. 2(C&D). On the other hand, liver sections of EST plus GSE and GSK treated group appeared to be normal without any tumor cell infiltration and showed a decrease in the inflammatory cells (Fig. 2E and F). Also, the hepatocytes restored their cytoplasmic appearance and nearly normal arrangement of hepatic cords as shown in Fig. 2(E&F).

Liver tissue from the control and GSE and GSK-treated animals revealed negligible amounts of collagen fibers around blood vessels (Fig. 3A&B). Liver sections of EST bearing group showed an increase of collagen fibers around the central veins and in the blood sinusoids (Fig. 3C). However, liver sections of EST plus GSE and GSK treated group showed...
Effect of GSE liver MDA, GSH, SOD, CAT levels of different experimental groups.

Table 1. Effect of GSE & GSK intake on blood & liver MDA, GSH, SOD, CAT levels of different experimental groups.

| Parameters | Blood (μmol/gm wet tissue) | Liver (μg/gm wet tissue) |
|------------|---------------------------|--------------------------|
| Control    | 53.4 ± 1.7                | 319.1 ± 1.1              |
| GSE        | 54.8 ± 1.3                | 346.6 ± 1.9              |
| GSK        | 54.8 ± 1.3                | 346.6 ± 1.9              |
| GSE & GSK  | 54.8 ± 1.3                | 346.6 ± 1.9              |
| EST        | 53.4 ± 1.7                | 319.1 ± 1.1              |
| GSE & EST  | 54.8 ± 1.3                | 346.6 ± 1.9              |
| GSK & EST  | 54.8 ± 1.3                | 346.6 ± 1.9              |

Each value represents the mean ± SE of 5 mice/group.

Significantly different from control group at 0.01 level.

Significantly different from GSE/GSK group at 0.01 level.

Significantly different from EST group at 0.01 level respectively (% change of control group).

3.3. Histochemical observations

The protein materials in the liver cells of the control and GSE and GSK supplemented animals appeared as small bluish irregular particles which sometimes were packed closely together forming blue irregular dense bodies in the cytoplasm. The hepatocytes were limited by intensely-stained cell membranes and their nuclei contained positively stained nucleoli together with chromatin particles (Fig. 4A&B). Liver sections of EST bearing group showed reduction in total protein content and most of the hepatocytes appeared with cytoplasmic vacuolation (Fig. 4C). Contrarily, liver sections of EST + GSE and GSK treated group showed normal content of the total protein (Fig. 4D).

DNA-containing particles in the nuclei of hepatocytes of the control and GSE and GSK treated animals appeared as red dense stained particles which were distributed in the nucleoplasm or restricted to the peripheral rims of the nuclei (Fig. 5A&B). Also, the nuclei of kupffer cells were strongly stained. Examination of liver sections of EST bearing group showed reduction in the DNA content (Fig. 5C). In contrast, liver sections of EST plus GSE and GSK treated group showed normalization in the DNA content (Fig. 5D).

3.4. Ultrastructural observations

In the present investigation, the hepatic cells of the control group display large rounded nucleus with normal distribution of euchromatin and heterochromatin. Profiles of rough endoplasmic reticulum are observed in the hepatocyte cytoplasm especially around the nuclear envelope and in between the mitochondria (Fig. 6A). The mitochondria are numerous rounded and elongated profiles with membranous cristae and electron dense matrix (Fig. 6A). The hepatic sinusoids are lined with a discontinuous layer of endothelial cells. Microvilli of the hepatic cells are project into the lumen of the bile canaliculi and in the space of Disse (Fig. 6B&C). The hepatic cells, bile canaliculi and blood sinusoids of GSE and GSK supplemented animals seem to be normal as those of the control group (Fig. 6D–F). In contrast, the liver tissue of EST-bearing mice showed ultrastructural alterations including irregular nuclei with lipid droplets inclusion (Fig. 7A). In addition, mitochondria were packed close to each other. Some were large, some were small and others appeared to be branching or budding (Fig. 7A&C). Moreover, lipid droplets, glycogen particles and large number of secondary lysosomes and microbodies were obviously seen in most of the cells (Fig. 7B&C). The hepatocytes became swollen and lost their cytoplasmic density (Fig. 7B&C). In addition, the blood sinusoid was wide and continues with destructed endothelial cells and detachment of kupffer cells (Fig. 7C). However, the liver tissue of EST plus GSE and GSK exhibit remarkable improvements. The nuclei of the hepatic cells were more or less similar to those of control (Fig. 7E). The mitochondria and smooth ER were prominent in the cytoplasm of these cells in a healthy appearance and the lipid droplets were mostly disappeared (Fig. 7D&E). The bile canaliculi and blood sinusoid...
were quite normal with respect to that of control group (Fig. 7E&F).

4. Discussion

In the present work, untreated EST bearing mice showed significant elevation in plasma ALT and AST levels as compared with that of control group. These data indicate that the development of tumor in the animal body can affect many functions of vital organs such as liver function. These results correlated well with Gupta et al. [28] who recorded elevation of liver transaminases in EAC bearing mice indicating liver dysfunction. Conversely, EST plus GSE and GSK group showed significant decrease in plasma levels of ALT and AST to reach the normal values. These results suggest that grape skin and seed supplementation protects the hepatocytes from injuries and improves the liver functions of EST bearing mice. These findings are in agreement with the study of Shin and Moon [9] who found that grape skin & seeds mixed with normal food to rat, significantly inhibited the elevated levels of serum AST & ALT due to dimethylnitrosamine (DMN)-induced liver injury.

Malondialdehyde (MDA) the end product of lipid peroxidation acts as a marker of oxidative stress [10,29]. In the current study, EST bearing mice displayed significant increase in blood and liver MDA content. Previous studies demonstrated that tumor growth disrupts the antioxidant system and increases LPx in tumor host vital organs [2–4]. The generation of lipid peroxide and its increase in the mouse liver could result from a chain reaction or could be initiated by indirect mechanisms that have escaped the antioxidant capacity of the liver of EST bearing mice [3].
Fig. 3 – Liver histology of EST bearing mice and/or treated with GSE and GSK demonstrating the collagen fibers. Liver section of control mice (A) illustrating negligible amount of collagen fibers around the central vein (CV), liver section of GSE and GSK treated mice (B) showing no remarkable changes, liver sections of EST bearing mice (C) illustrating large amount of collagen fibers around central vein (CV), liver section of EST plus GSE and GSK treated group (D) showing decrease in the amount of collagen fibers around the central vein (CV) (Masson trichrome stain, X400).

Fig. 4 – Liver histochemical demonstration of the content and localization of total proteins of EST bearing mice and/or treated with GSE and GSK. Liver section of control mice (A) showing normal dense protein content with normal distribution of protein in all of the hepatocytes, liver section of GSE and GSK treated mice (B), liver sections of EST bearing mice (C) displaying reduction of total protein content, liver section of EST plus GSE and GSK treated group (D) illustrating normalization of total protein (Bromophenol blue stain, X400).
The present data reveal marked depletion in GSH content of blood and liver of EST bearing mice accompanied by significant inhibition of SOD and catalase activities. There is a close correlation between depletion of GSH and antioxidant enzymes and the increase in LPx [30]. GSH plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in protecting cells by scavenging ROS [31,32], modulating cellular redox status and acting as a cofactor for antioxidant enzymes [29,33,34]. On the other hand, the free radical scavenging system, CAT and SOD are to provide a guard against the potentially injurious reactivity of superoxide and hydrogen peroxide [35-37]. Level of GSH and antioxidant status during tumor growth was previously investigated by Navarro et al. [38]. Their study showed a reduction in blood glutathione redox (GSH/GSSG) in Ehrlich ascites carcinoma-bearing mice. They attributed this result to the oxidative stress that caused an elevation in peroxide formation by cancer cells. GSH oxidation occurred in the red blood cells leads to the release of GSSG from the different tissues to the blood stream [38,39].

Significant reduction in SOD and catalase levels in blood and liver tissue of tumor-bearing mice was detected in the present study. SOD activity was also found to decline in Ehrlich ascites carcinoma-bearing mice as reported by others [40]. They reported that such decline could be due to a loss of mitochondria that may lead to a reduction in SOD level in several tissue of the tumor-bearing animal. Similar results of SOD activity were detected by Abu-Zeid et al. [41] in plasma, lung and liver of Ehrlich carcinoma-bearing animals. It is worth mention that tumor development may lead to the degradation of antioxidant enzymes such as SOD and catalase as a result of uncontrolled oxidative damage [42].

The current study revealed that GSE & GSK intake reduced the elevated levels of LPx and increased GSH, SOD and CAT activities in EST bearing mice, which may indicate the possible antioxidant and free radical scavenging property of GSE and GSK supplementation. These results are in agreement with Chis et al. [43], Al-Sowayan and Kishore [44] and Leifert and Abeywardena [45] who reported that oral administration of proanthocyanidin extract improved SOD and CAT levels and reduced the levels of lipid peroxides and enhanced the antioxidant defense against reactive oxygen species produced under Doxorubicin treatment, thereby protecting liver cells. These results were supposedly caused by supplementation with grape seeds & skin that considered as a rich source of polyphenols, which have numerous beneficial effects on oxidative stress and protect cells and tissues from oxidative damage that could be due to their strong antioxidant activities of scavenging reactive oxygen [29].

In the present investigation, liver sections of EST bearing mice displayed hepatocyte cytoplasmic vacuolation with intranulear cytoplasmic inclusions and clumps of Ehrlich tumor cells mixed with lymphocytes and erythrocytes. Confirmation of the present result comes from previous studies [46-48]. Ehrlich tumor cells infiltrations may be due to the tumor cells proliferate and migrate into the internal organs [17,49]. Aggregations of inflammatory cells may be due to degeneration of the mitochondria or disorganization of the cytoplasm [50]. Ballooning degeneration of hepatocytes...
supposed to be caused by lysosomal enzymes and hydration [51], as evidenced in the present ultrastructural observations. In contrast, liver sections of EST plus GSE and GSK treated group showed nearly normal construction of hepatic lobules with few altered hepatic cells. Neither inflammatory cells nor Ehrlich cells could be detected and this result are agree with Sun et al. [52] who showed that grape polyphenols dietary supplementation prevented the liver injury induced by ethanol. Also, Kasdallah-Grissa et al. [53] found that resveratrol reduced hepatic tissue injury. In addition, oral intake of grape seeds attenuated histopathological changes caused by tamoxifen in the liver of rats [54]. Moreover, another study demonstrated that grape polyphenols, including resveratrol, epicatechin, and epigallocatechin, can inhibit cancer cell invasion [55]. This can be considered as functional improvement of hepatocytes, which might be due to accelerated regeneration of parenchymal cells or limited damage in the presence of GSE&GSK supplementation. Also, the current study showed that GSE and GSK intake to normal mice did not produce any detectable structural changes in the liver tissues.

Liver sections of EST bearing mice in this study showed increased collagen fibers around the central veins. Horn et al. [56] declared that the presence of collagen in the perisinusoidal space might affect the blood supply to liver cells and would reduce the exchange of metabolites, perhaps causing hepatocellular dysfunction and necrosis. Enzan et al. [57] and Foo et al. [58] attributed this finding to the activation of hepatic stellate cells. George et al. [59] contribute the

Fig. 6 – Electron photomicrograph of liver of control mice (A–C), illustrating the hepatocytes with normal large rounded nucleus (N), rough endoplasmic reticulum (RER) and mitochondria (M), normal blood sinuoids with endothelial lining cells (EN), wide space of Disse (DI), Kupfer cell (KC) and red blood cells (RBCs). Microvilli (MV) are project into the bile canaliculi (BC) which secured with the junctional complex (JC) and in the space of Disse (DI). The liver of GSE and GSK treated animals are quite normal as those described of the control group (D–F).
accumulation of collagen to the decreased synthesis of collagenolytic enzymes by the damaged hepatocytes. The presence of fibrosis suggests more advanced and severe liver injury [60]. Oxidative stress, in particular lipid peroxidation, induces collagen synthesis [61], as demonstrated in the present study. While GSE and GSK supplementation to EST bearing mice significantly reduced the collagen deposition in the liver tissue. These findings suggest that GSE and GSK have an additional protective effect against oxidant-induced production and deposition of extracellular matrix components.

Histochemically, liver sections of EST bearing group in the current study showed reduction in total proteins and DNA contents. Likewise, El-Banhawy et al. [62] indicated existence of a close parallelism between nucleic acids and the level of protein synthesis, thereby, this reduction in the protein and DNA contents could be attributed to the nuclear pathological changes which were evidenced in the present work as well as in previous studies [17,48,63]. Similarly, Salem et al. [64] showed a significant decrease in total protein and albumin level in EAC bearing mice. They attributed these results to an increase in mitotic division of neoplastic cells with high bloody fluid withdrawal and capillary permeability which enable the escape of plasma proteins into the peritoneal cavity and it may also be due to hepatic cell necrosis [65]. In addition, total proteins may decrease in animals with liver disease [66]. DNA damage has been also reported in tissues proximal and distant from non-metastasizing implanted tumors in mice due to inflammation and oxidative stress [67].
contrast, liver sections of EST plus GSE and GSK treated group showed normalization of the total protein and DNA contents. Previous studies [68,69] demonstrated that proanthocyanidin provides significantly greater protection against biochemical changes, free radicals, lipid peroxidation and DNA damage. In addition, its presumed contribution to DNA repair may be another important attribute, which plays a role in grape protective effects [44].

In the present ultrastructural findings, the liver tissue of EST bearing mice showed irregular nuclei with lipid droplets inclusion. Such result goes parallel with the previous results obtained by other investigators in benign and malignant tumors [70–72]. Intranuclear inclusions are in fact nuclear invaginations by cytoplasm. It has been suggested that these inclusions may also be formed by cytoplasmic contents being forced into the nucleus during mitosis [73] as evidenced by observation of lipid droplets in the nucleus in the present study. Rose [74] made similar observations and nicely depicted the formation of intranuclear pseudoinclusions in cells from cultures of human melanoma, osteogenic sarcoma and other tumors. In many of these pseudoinclusions, the double nuclear membrane may not be seen, possibly because of the degeneration and subsequent disintegration of the outer membrane [72,74]. In addition, mitochondria were packed close to each other; some were large, some were small and other appeared to be branching or budding. Such results are confirmed through the work of previous studies [72]. This mitochondrial pleomorphism has been observed in states of iron deficiency [75] and in both hypoxia and hyperoxia [76], which may represent the direct toxic effect of tumor on the liver. Also, large number of secondary lysosomes was obviously seen in many cells. Our findings are in accordance with Ghadiali and Parry [77] and Parry and Ghadiali [78,79] who observed a marked increase in lysosomes in the livers of tumor bearing animals. According to Telbiz et al. [80] and Abd El-Wahab and Fouda [17], activation of autophagy is frequently observed in different degenerated tissues. Lysosomal enzymes discharges have a key role in the induction of necrotic changes. On the contrary, the liver tissue of EST plus GSE and GSK group exhibited remarkable improvements. The bile canaliculi and blood sinusoid seemed to be normal with respect to that of EST bearing group. Thus, the dietary supplementation of GSE and GSK to EST bearing mice exhibited significant ameliorative potential probably by attenuating the tumor-mediated oxidative stress and preserving the structural and functional integrity of hepatocytes.

5. Conclusions

In conclusion, the present study confirmed that grape skin and seeds treated animals exhibited in vivo hepatoprotective and antioxidant effects against liver injury induced by Ehrlich solid tumor growth. The mechanism appeared mostly to be mediated by counteracting free radicals thereby reducing oxidative stress and augmenting antioxidants in Ehrlich solid tumor-bearing mice.

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