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Research Article

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Anticancer properties of resveratrol on chemically induced hepatocellular carcinoma in rats: Inhibition of metastasis and angiogenesis

Abeer H. Abdel-Halim[1], Amal A. Fyiad[1], Mamdouh M. Ali[*1] and Saeed M. Soliman[2]

[1]*Biochemistry Department, Division of Genetic Engineering and Biotechnology, National Research Centre, Dokki, Giza, Egypt*
[2]*Radiation Biology Department, National Centre for Radiation Research, Cairo, Egypt*

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the major health burdens and the fifth most common cancer worldwide. Although there are several drugs available to control cancer growth, there are fewer drugs presently available to specifically inhibit the metastasis of cancer cells or prevent its angiogenesis. The present study aimed to investigate the protective or therapeutic effect of resveratrol (natural phytoalexin) on diethylnitroseamine (DENA) induced hepatocarcinogenesis in rats. Rats were injected with a single dose DENA (200mg/kg b.w, i.p) only or with pre and post treatment of low and high doses of resveratrol (300mg/kg b.w and 450mg/kg b.w) respectively by feeding for 9 months. To elucidate the mechanism by which resveratrol exerts its function as anticancer agent, the following parameters were determined: aspartate and alanine aminotransferase (AST, ALT), alkaline phosphatase (ALP), total bilirubin as liver function test; vascular endothelial growth factor (VEGF) as a marker for angiogenesis and heparanase, elastase and matrixmetalloproteinases(MMPs) as markers of metastasis. Histopathological investigation was also confirmed. All parameters are elevated in DENA group, which confirmed by histopathological analysis where hepatic hemorrhages associated with necrosis, hyperchromatism, hyperplasia, proliferating hepatocytes were noticed. While the administration of resveratrol improved all biochemical and histopathological changes in post-treated groups more than pre-treated one, indicating that resveratrol may be a potent anticancer agent and its therapeutic effect is more potent than protective effect.

Keywords: Hepatocellular carcinoma, Chemoprevention, Resveratrol, Angiogenesis, Metastasis.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequent tumors representing the fifth commonest malignancy worldwide and the third cause of mortality from cancer[1]. The vast majority of HCC cases is attributed to underlying hepatitis B and hepatitis C virus infection, but several other risk factors, e.g., excessive alcohol consumption, obesity, iron overload as well as environmental and chemical carcinogens are also involved in its etiology[2,3].The survival of patients with clinically apparent HCC is generally limited a few months. The majority of patients with symptoms of HCC have tumors that are not amenable to curative treatment because of the extensive involvement of the liver, invasion of the hepatic or portal vein and the presence of metastases [4,5]. In view of the limited treatment by surgical resection or liver transplantation and negative prognosis of liver cancer, preventive control approaches, notably chemoprevention has been considered to be the best strategy in lowering the present prevalence of the disease [6].

Tumor growth and expansion requires an ability not only to proliferate, but also to down-modulate cell death and activate angiogenesis to produce a tumor neovasculature. Metastasis of cancer cells to distant sites is one of the major deciding factors in cancer outcome [7,8]. In fact, prognosis of cancer is mainly determined by the invasiveness of the tumors and its ability to metastasize. Thus, the promotion of apoptosis with antimetastasis and antiangiogenesis targeting strategies is one of the important focuses in current cancer therapy [9]. The development of such novel, effective and less or no toxic compounds with multiple mode of action for targeted cancer therapy has become an innovative approach and efforts have been directed towards discovering such anticancer agents endowed with cytotoxic action [10,11].

It has been demonstrated that dietary factors contribute to as much as one-third of potentially preventable cancers [2]. Epidemiological and current laboratory studies suggest consumption of certain types of fruits and vegetables, containing phytochemicals, is associated with reduced cancer risk, where they can function as chemopreventive and/or adjuvant chemotherapeutic agents [12]. One such phytochemical is resveratrol (3, 4, 5-trihydroxystilbene). It is a stilbene-type aromatic phytoalexin predominantly found in grapes, peanuts, berries, turmeric, and other food products [13]. Resveratrol exists as two geometric isomers, trans and cis forms. The trans- and cis-resveratrol can be either free or bound to glucose [14]. The trans-form can undergo isomerization to the cis-form when exposed to ultraviolet irradiation, a process called photoisomerization[15].Resveratrol has been reported to exhibit several physiological activities including anti-aging, anticancer, antiviral, anti-diabetic, anti-inflammatory, skin-, neuro- and cardio-protectiveactivities *in vitro*and in experimental animal models, as well as in humans [16]. Resveratrol has elicited much attention as a potential anticancer agent since its inhibitory effect on carcinogenic processes at the initiation, promotion, and progression stages [17]. Subsequently, numerous studies have illustrated resveratrol's capacity to modulate a multitude of signaling pathways associated with cellular growth and division, apoptosis, angiogenesis, invasion, and metastasis [18].

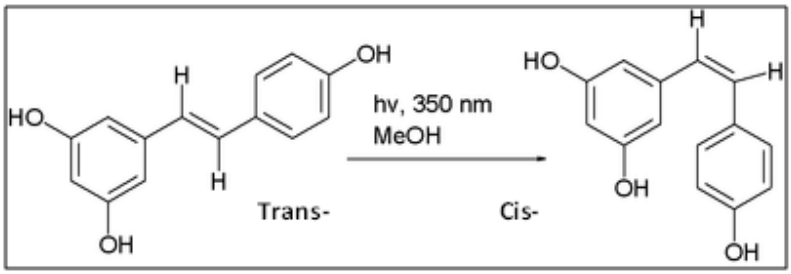

Fig. 1. Structure of trans and cis– resveratrol

However, despite the identification of numerous molecular targets, the underlying mechanisms involved in the anticancer activities of resveratrol are not completely understood. So, we demonstrate in this study a novel antiangiogenic and antimetastatic-related mechanism for resveratrol-induced inhibition of liver tumor growth.

EXPERIMENTAL SECTION

2.1 General

Trans-resveratrol(98% purity) was purchased from Shenzhen Sungening bio-technologyCo., Ltd.(Shen Zhen City 518116, China). All other chemicals and solvents used in this study were of the highest purity and analytical grade, and purchased from Sigma-Aldrich chemic (Deisenhofer, Germany).

2.2 Animals and treatment

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland. Healthy, and according to approval from the Committee for Animals Care at the National Research Centre, Egypt.Eighty of adult male Sprague-Dawley rats with body weights 120-150 g, bred in the Animal House Colony of The National Research Centre, Dokki, Giza, Egypt. Animals were allowed 7 days for acclimatization at 24ºC with 12 hr light – dark cycle and fed a standard laboratory diet and water *ad libitum*before the experiment. After one week of acclimation, the animals were then divided into eight groups of rats each of ten. Group 1: (control group) rats fed a plain chow diet for 9 months. Group 2: (low dose resveratrol group) rats fed resveratrol at a low dose of (300 mg/kg b.w) for 9 months. Group 3: (high dose resveratrol group) rats fed resveratrol at a high dose of (450 mg/kg b.w) for 9 months. Group 4: (diethylnitroseamine treated group) rats received a single dose of

diethylnitroseamine (DENA) intraperitoneally (i.p)at a dose of (200 mg/kg b.w),two weeks later rats were given a single dose (2 ml/kg b.w) of carbon tetrachloride (CCL_4) by oral cavage. Group 5: (low dose resveratrol pre-treated group) rats fed resveratrol at a low dose of (300 mg/kg b.w) for 4 weeks before being injected with a single dose of DENA i.p.(200 mg/kg b.w), after 2 weeks rats were given a single dose of CCL_4 (2 ml/kg b.w) as previously mentioned, followed by feeding the rats with resveratrol (300 mg/kg b.w) for 9 months. Group 6:(high dose resveratrol pre-treated group) rats fed resveratrol at a high dose of (450 mg/kg b.w) for 4 weeks, then complete as mentioned in group 5. Group 7: (low dose resveratrol post-treated group) rats received (i.p) a single dose of DENA at a dose of (200 mg/kg b.w), after 2 weeks the rats were given a single dose of CCL_4 orally at a dose of (2 ml/kg b.w), followed by feeding rats with low dose of resveratrol (300 mg/kg b.w) for 9 months. Group 8:(high dose resveratrol post-treated group) the same as group 7, but rats fed high dose of resveratrol (450 mg/kg b.w) for 9 months.

At the end of the treatment protocol, food was withheld for 16-18 hr., animals were anesthetized with ether and blood samples were drawn from the retro-orbital plexus of the individuals of all groups. Samples were left to clot then centrifuged at 3000 rpm for 15 min to separate the sera and stored at -20°C until analysis. This was then used to determine ALT, AST and ALP activities and total bilirubin level spectrophotometrically according to the manufacturer's instructions, using reagent kits obtained from Biomerieux (France).After blood collection, all animals were sacrificed by decapitation and their livers were rapidly excised, weighed, washed with saline, and a portion of it was preserved in 10% formalin in saline and subjected to histopathological analysis. The remaining part of liver immediately homogenized in ice-cold 10% sucrose buffer using Omni tissue master homogenizer. The homogenate was centrifuged at 1700 rpm at 4ºC and the resulting supernatant was stored at -70 ^{o}C for biochemical analysis.

2.3 Histopathological analysis

Samples of the liver from all animals were fixed in 10% neutral formalin and embedded in paraffin wax. Sections (4 μm thickness) were stained with hematoxylin and eosin (H&E) and examined microscopically for detection of histopathological alteration[19].

2.4 Estimation of VEGF concentration

VEGF concentration was determined in serum using ELISA kit obtained from Koma Biotech Inc., Korea. This technique depends on binding VEGF antigen to a specific immobilized antibody. The formed immune complex binds to avidin-peroxidase conjugate, and a color developed in proportion to the amount of VEGF bound which was measured spectrophotometrically at 450 nm.

2.5Determination of heparanase activity

Heparanase activity was determined in serum using ELISA kit obtained from Glory Science Co., Ltd (Del Rio, TX 78840, USA) according to the manufacturer's instructions. The kit assay rat heparanase level in serum using purified rat heparanase to coat microtiter plate wells, makes solid-phase antibody, then add rat heparanase to wells containing enzyme labeled to form antibody-antigen-enzyme-antibody complex, after washing completely add substrate solution which becomes blue color at enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid and the color change is measured spectrophotometrically at 450 nm. The concentration of serum heparanase in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.6. Determination of elastase activity

The elastase activity is determined in serum by its catalytic effect on the N-succinyl-trialanyl-*p*-nitroanilide substrate releasing *p*-nitroaniline (*p*NA) which is measured photometrically at 405 nm[20].

2.7 Estimation of MMP-2 and MMP-9 levels

Serum MMP-2 and MMP-9 levels were determined using ELISA kit purchased from Glory Science Co., Ltd (Del Rio, TX 78840, USA) using purified MMP-2 or MMP-9 to coat microtiter plate wells, makes solid-phase antibody, then add MMP-2 or MMP-9 to wells containing enzyme labeled to form antibody-antigen-enzyme-antibody complex, after washing completely add substrate solution which becomes blue color at enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid and the color change is measured spectrophotometrically at 450 nm. The concentration of Serum MMP-2 and MMP-9 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.8 Estimation of sirtuin concentration

Sirtuin concentration was determined in tissue homogenate using ELISA kit obtained from Glory Science Co., Ltd (Del Rio, TX 78840, USA). The kit assay rat sirtuin level in tissue homogenate using purified rat sirtuin to coat microtiter plate wells, makes solid-phase antibody, then add rat sirtuin to wells containing enzyme labeled to form antibody-antigen-enzyme-antibody complex, after washing completely, add substrate solution where a blue colour is obtained. The reaction is terminated by the addition of a sulphuric acid and the color change is measured spectrophotometrically at 450 nm. The concentration of rat sirtuin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.9 Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Results of biochemical studies were statistically analyzed using one-way analysis of variance (ANOVA). In case of the significant F-ratio, *Posthoc Bonferroni's* test for multiple comparisons was done. All statistics were done using SPSS (Version 17) for Windows (Chicago, IL, USA). Differences were considered significant at a *P* value less than 0.05.

RESULTS AND DISCUSSION

Therapeutic intervention for HCC remains elusive and there is a strong rationale to justify a chemopreventive approach for the treatment of this disease [21]. HCC is characterized by active angiogenesis and metastasis, which account for rapid recurrence and poor survival [22].Angiogenesis is an essential component of the metastatic pathway. These vessels provide the principal route by which tumor cells exit the primary tumor site and enter the circulation [23].One of the key events in tumor metastasis is the adherence of tumor cells to blood vessel walls, followed by penetration of the vessel wall and the subsequent emigration of tumor cells into tissues. These events require localized degradation of the inter-endothelial cell junctions and sub-endothelial matrix constituents [24]. Therefore the present study aimed to investigate the protective or therapeutic effect of resveratrol on liver carcinogenesis as antiangiogenic and antimetastatic agent in experimental animals.

The activities of liver enzymes (AST, ALT and ALP) and bilirubin level of all experimental groups were given in (Table 1). The results revealed that the activities of these enzymes and the level of bilirubin were significantly higher in DENA group compared to control group, this results were in parallel withthe finding of Gaskill et al. [25] whomentioned that the levels of hepatic enzymes elevated in liver damage due to tissue necrosis or membrane damage and subsequent leakage of enzymes into the serum. Feeding rats with resveratrol alone didn't effect on the previous parameters which indicate the safety of using resveratrol. While there was a significant decrease in the activities of liver enzymes and bilirubin level in pre-treated and post-treated groups compared to DENA group. While, the ameliorative effect of post-treatment was more pronounced than that of pre-treatment.

Table 1.The effect of resveratrol on liver function in all groups

Group	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	Bilirubin (mg/dl)
- Control	54.48±1.40	38.90±1.03	100.62±3.53	1.33±0.30
- High dose resveratrol	56.10±2.10	39.86±2.51	101.67±5.17	1.30±0.10
- Low dose resveratrol	53.66±3.11	38.24±1.58	100.66±3.28	1.29±0.13
- DENA	132.18±6.77[a]	105.32±6.70[a]	250.46±8.56[a]	3.52±0.23[a]
- Low resveratrol pre-treated	91.41±5.97[a,b]	80.81±3.09[a,b]	150.36±6.53[a,b]	2.73±0.31[a,b]
- High resveratrol pre-treated	82.35±4.00[a,b]	71.93±4.44[a,b]	141.89±6.89[a,b]	2.33±0.16[a,b]
- Low resveratrol post-treated	71.72±2.82[a,b]	52.42±2.03[b]	122.75±6.00[b]	1.93±0.14[b]
- High resveratrol post-treated	74.26±0.32[a,b]	54.00±0.27[b]	128.29±0.24[a,b]	1.96±0.16[b]

Values are expressed as mean ±SE (n=10), a: DENA group was compared to the control group. b: resveratrol post and pre-treated groups were compared to DENA group, significant at P<0.05.

Hepatic histopathological features of control and experimental groups of rats were illustrated in (Fig. 2A-K). In this study, damage to hepatic structure integrity induced by DENA is further supported by our histopathological examination, where hepatic hemorrhage associated with necrosis, hyperchromatism, hyperplasia, proliferating hepatocytes were noticed. Treatment with resveratrol alone at high or low doses showed normal liver tissue as in the control group depicts the non-toxic property of resveratrol. The post-treatment with resveratrol after administration of DENA showed that the hepatocytes thereby preserving near normal architecture. While the pre-treatment with resveratrol before DENA administration showed dense lymphocytic infiltration of portal tract by inflammatory cells, moderate necrosis, moderate ballooning degeneration of hepatic cells, enlarged nuclei with increase number and

prominence of nucleoli. These results indicate that the post-treatment with resveratrol was more effective than pre-treatment (Figure 2).

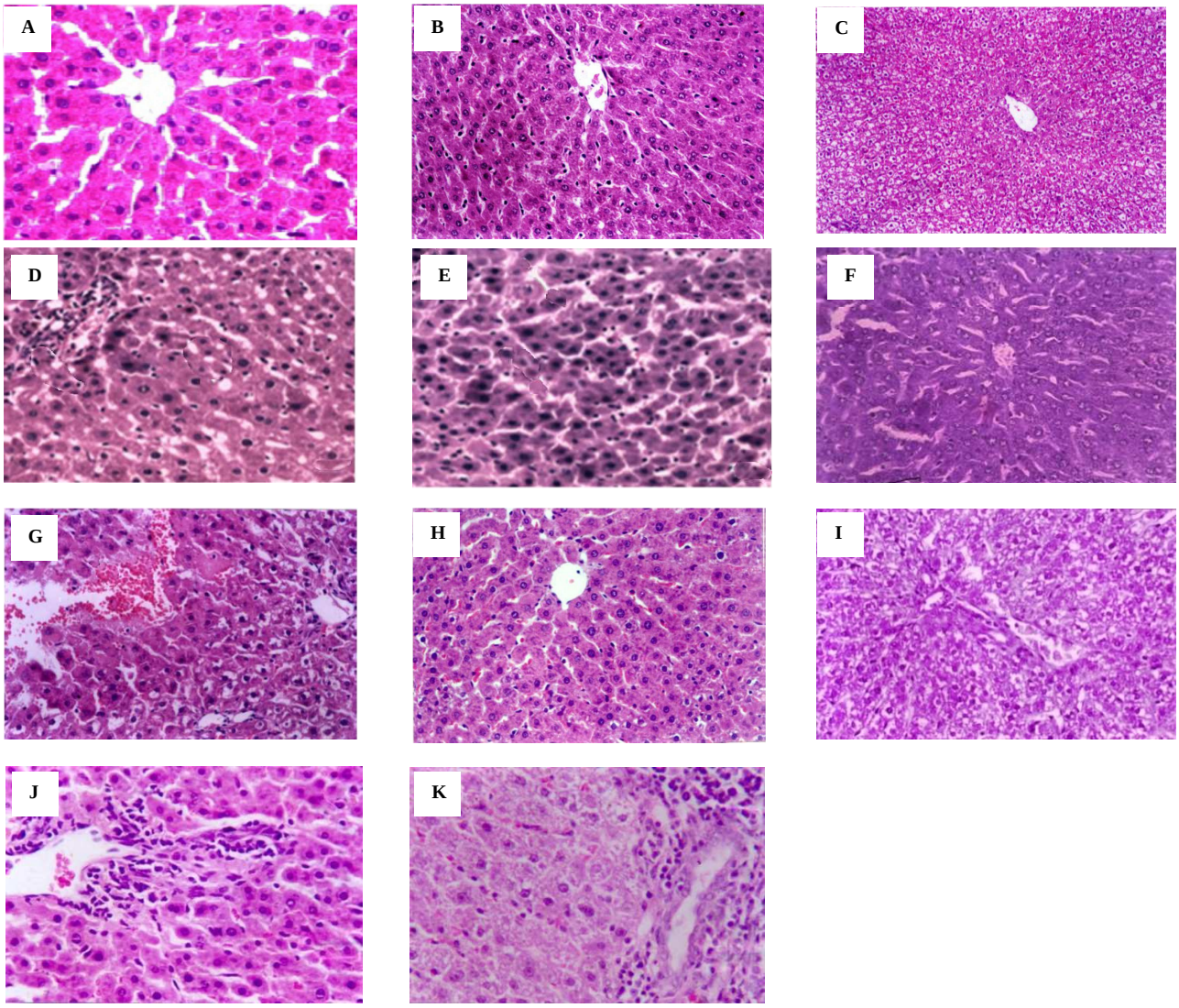

Fig 2.Normal liver tissue showed hepatic lobule with normal architecturebrought out central vein and cells with preserved granulated cytoplasm and small uniform nuclei and nucleolus (A). Liver of rats treated with high (B) and low (C) doses resveratrol, showing normal histological structure. DENA treatment showed loss of architecture with many different histological patternsis extremely variegated may be seen from hyperchromatic and irregular nuclei with fibrosis, fatty infiltration and thin walled sinusoids and disarrangement withdegenerationof hepatocytes. The cells are slightly larger, have more irregular and malignant nucleus, mitotic,granular cytoplasm with neoplastic cells (D and E). hyperchromatism, hyperplasia, proliferating hepatocytes(F), focal hepatic haemorrhages and necrosis(G). Liver of the DENA post-treated with high (H) and low (I) doses of resveratrol, showed improvement in the hepatic pattern as previously seen in normal control. Liver of rats pre-treated with high (J) and low (K) doses of resveratrol before DENA sowed dense inflammatory cells infiltration of portal tract, moderate basemel necrosis of liver cells (moderate activity) (J), and dense lymphocytic infiltration of portal tract by inflammatory cells, moderate necrosis, moderate ballooning degeneration of hepatic cells, enlarged nuclei with increase number and prominence of nucleoli (K)(H&E, 200×)

Tumor angiogenesis performs a critical role in tumor progression through which the tumor establishes an independent blood supply, consequently facilitating tumor growth and favoring the transition from hyperplasia to neoplasia [26]. This process is regulated through a balance of pro- and anti-angiogenic factors. VEGF seems to be the most potent and predominant angiogenic cellular factor sustaining tumor growth [27]. The present study showed that although he level of VEGF was significantly higher in DENA group as compared to control group, treatment withresveratrol decreased the level of VEGF in pre-treated and post-treated groups compared to DENA group indicating that resveratrol improved angiogenesis. But, the significant improvement of resveratrol post-treated group was more pronounced than that of pre-treated group(Table 2).

Heparanase is a heparan sulfate (HS) degrading endoglycosidase participating in extracellular matrix degradation and remodeling [28]. Heparanase seems to modulate two critical systems involved in tumor progression, namely VEGF expression and epidermal growth factor receptor (EGFR) activation. Neutralizing heparanase enzymatic and non-enzymatic functions is therefore expected to profoundly affect tumor growth, angiogenesis, and metastasis where heparanase induction correlated with increased tumor metastasis, vascular density, and shorter postoperative survival of cancer patients, thus providing a strong clinical support for the prometastatic and proangiogenic functions of the enzyme and positioning heparanase as an attractive target for the development of anticancer drugs [24, 29]. In addition, elastase is another broad-range proteolytic enzyme thought to be a tumor promoter involved in increasing tumor cell invasiveness by facilitating cell motility and transendothelial migration as it has the ability to degrade the basement membrane and extracellular matrixglycoproteins such as elastin, fibronectin, as well as adhesive molecules and junctional cadherins[30]. Moreover, elastase considered to be protease that is able to degrade insoluble elastin, a structural component of elastic tissues such as blood vessels, skin, lung, liver and breast tissues [31].

As shown in Table 2,the results showed that the activities of heparanase and elastase enzymes were significantly increased in DENA group as compared to control group. While the treatment with resveratrol decrease heparanase and elastase activities in pre and post-treated groups compared to DENA group which indicate that resveratrol inhibit metastasis. Otherwise, the results indicated that, the post-treatment with resveratrol showed an improvement in both activities than pre-treatment and the activities of both enzymes were not affected when resveratrol was taken alone. In consistence with our results, Elkin et al. [32] showed that expression of heparanase correlates with the metastatic potential of tumor cells, and treatment with heparanase inhibitors markedly reduces the incidence of metastasis in experimental animals. Furthermore, Taniguchi et al. [33] postulated that increased elastase destroy the barrier between the tumor and the local circulatory system, either lymphatic or hematogenous, and result in at least loco-regional metastases.

Table 2.The effect of resveratrol on VEGF level and the activity of heparanase and elastase in all groups

Group	VEGF (Pg/ml)	Heparanase (U/ml)	Elastase (U/ml)
- Control	198.9±22.03	2.20±0.20	0.21±0.03
-High resveratrol	189.35±5.44	2.00±0.21	0.22±0.02
- Low resveratrol	190.05±9.05	2.30±0.17	0.23±0.02
- DENA	435.25±40.25[a]	6.12±0.54[a]	1.01±0.11[a]
- Low resveratrol pre-treated	300.97±12.17[a,b]	4.60±0.19[a,b]	0.62±0.05[a,b]
- High resveratrol pre-treated	285.66±15.87[b]	4.00±0.34[a,b]	0.51±0.03[a,b]
- Low resveratrol post-treated	223.31±13.92[b]	2.82±0.27[b]	0.33±0.04[b]
- High resveratrol post-treated	252.94±15.24[b]	2.97±0.23[b]	0.39±0.03[b]

Values are expressed as mean ±SE (n=10), a: DENA g roup was compared to the control group. b: resveratrol post and pre-treated groups were compared to DENA group, significant at P<0.05.

MMPs are a large family of proteolytic enzymes, which are involved in the degradation of many different components of the extracellular matrix [34]. MMP-2 and MMP-9 are thought to be important in metastasis [35]. MMP-9 has been described to promote tumor malignant progression, invasion, and metastatic spread by activating tumor growth factor-β. Inhibiting the action of MMPs by synthetic and natural inhibitors represents a new therapeutic approach for the treatment of individual types of cancer and several broad-spectrum, low-molecular-weight MMP inhibitors are currently being assessed for clinical use [37]. The present study showed that MMP-2, and MMP-9 were over expressed in DENA group as compared to control group. The administration of resveratrol was significantly decreased their levels as compared to DENA group, which indicate that resveratrol had a role in improvement metastasis. The improvement of both enzymes in post-treated group was more effective than that in

pre-treated group (Table, 3). Bergers et al. [38] mentioned that MMP-9 is now regarded as a specific component of the angiogenic switch, because it renders VEGF more available to its receptors and its inhibitors impair angiogenesis and tumor growth. Moreover, MMPs are overexpressed in a variety of tumor types, and their overexpression is associated with tumor aggressiveness and poor prognosis [39].

Table 3.The effect of resveratrol on MMP-2, MMP-9 and SIRT 1 in all tested groups

Group	MMP-2 (ng/ml)	MMP-9 (ng/ml)	SIRT 1 (Pg/ml)
- Control	496.56±30.20	265.53±21.86	2.50±0.42
- High resveratrol	467.37±26.96	275.64±19.66	9.08±0.61[a]
- Low resveratrol	486.49±17.68	280.02±17.59	6.73±0.58[a]
- DENA	1467.95±98.22[a]	836.08±38.7[a]	8.68±1.00[a]
- Low resveratrol pre-treated	814.12±45.51[a,b]	480.64±26.8[a,b]	12.08±0.92[a]
- High resveratrol pre-treated	660.68±35.99[b]	427.05±25.31[a,b]	12.63±0.73[a,b]
- Low resveratrol post- treated	542.87±25.44[b]	330.84±22.57[b]	11.09±0.75[a]
- High resveratrol post- treated	551.07±37.39[b]	350.72±38.98[b]	9.42±0.78[a]

Values are expressed as mean ±SE (n=10), a: DENA group was compared to the control group. b: resveratrol post and pre-treated groups were compared to DENA group, significant at P<0.05.

Sirtuins are a highly conserved family of nicotinamide adenine dinucleotide (NAD^+)-dependent protein lysine modifying enzymes, catalyzing the deacetylation of acetyl-lysine residues of histones and other proteins [40]. Mammals have seven sirtuins, namely SIRT1-7 with diverse biological functions. They are key regulators for a wide variety of cellular and physiological processes such as cell proliferation, differentiation, DNA damage and stress response, genome stability, cell survival, metabolism, energy homeostasis, neuroprotection, organ development, aging, and cancer [41,42]. SIRT1 is described as the main target of resveratrol. However, recent reports have challenged the hypothesis of its direct activation by resveratrol [43], which may help in the treatment or prevention of obesity, and in preventing tumorigenesis and the aging-related decline in heart function and neuronal loss [41]. The present study revealed that, the level of SIRT1was significantly increased in all groups (Table 3) as compared to control group. Moreover, the pre-treatment with high dose of resveratrol was significantly increased the level of sirtuin as compared to DENA group, while post-treated group was more effective than that in pre-treated group. In addition, the level of SIRT1 was improved by the administration of resveratrol in all groups as compared with DENA group, indicating the regulation of cancer cell proliferation and DNA damage repair.In consistent with our results, Yang et al. [42] explained that Liver cancer SIRT1 expression is significantly elevated in HCC compared to non-tumor tissues, suggesting that SIRT1 may act as a tumor promoter, and the expression levels correlate with tumor grades and predict poor prognosis. Portmann et al. [44] explained that the inhibition of SIRT1 in HCC cells impairs their proliferation in vitro and tumor formation *in vivo*.

It was mentioned that resveratrol is postulated to function as a potential signaling pathway modulator and as such, is demonstrated to affect a multitude of signal transduction pathways associated with tumorigenesis and/or carcinogenesis which lead to regulation between each other [42,45]. It is likely that this collective activity, rather than just a single effect, resulting in cell cycle arrest, suppression of tumor cell proliferation, induction of apoptosis and differentiation, reduction of inflammation and angiogenesis, and inhibition of adhesion, invasion and metastasis [46]. These explanations agreed with our results which explained that although the level of sirtuin was increased in both post and pre-treated groups, there was an improvement in these groups than DENA group.

CONCLUSION

In conclusion from the present study it can be concluded that resveratrol may be considered as antiproliferative, antiangiogenic and antimetastatic agent through its effect on VEGF, heparanase, elastase, matrix metalloproteinases and sirtuin and it had therapeutic effect rather than a protective effect on DENA-induced hepatocarcinogenesis in rats.

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