Benha Veterinary Medical Journal 49 (2025) 76-79

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Resveratrol-induced biochemical improvements in rats with hepatocellular carcinoma caused by diethylnitrosamine/carbon tetrachloride

Hisham Fatehy EL Said[1], Hussein Abd ElMaksoud Ali[1] and Omnia Mahmoud Abdel-Hamid[1]

Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Benha University

ARTICLE INFO

Keywords

Hepatic cancer

Diethylnitrosamine (DEN)

Resveratrol (RSV)

Oxidative stress

Received 14/07/2025
Accepted 25/09/2025
Available On-Line 01/10/2025

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most prevalent causes of cancer-related death. This study attempted to investigate the impact of resveratrol (RSV) against HCC. Rats were classified into three groups. Group I (Normal control group). Group II (Diethylnitrosamine (DEN)/Carbon tetrachloride (CCL4) group): 200 mg of DEN per kg b.wt. i.p. was administered to rats once. 3 ml of CCL4 per kg b.wt. injected s.c. per week for six weeks, following two weeks of DEN application to enhance the carcinogenic impact. Group III (Resveratrol (RSV)-treated group): Rats with HCC were administered RSV at a dose of 100mg/kg body weight intraperitoneally for 4 weeks. DEN/CCL4 injection induced noticeable reduction in serum TP content and Alb level, hepatic SOD, CAT, and GSH-Px activities, along with an obvious increase in serum AST, ALT, and ALP activities and AFP level. Treatment with resveratrol in HCC-induced rats caused marked improvement in all biochemical parameters. According to the findings, resveratrol may be considered a promising option for treating hepatic cancer.

1. INTRODUCTION

There is a substantial burden of liver cancer worldwide. Based on projections for 2020, hepatic cancer ranks 3rd in terms of cancer-related death and is the 6th most prevalent cancer to be diagnosed. The two most prevalent primary liver malignancies are bile duct cancer and hepatocellular carcinoma. Liver cirrhosis is the most significant risk factor for primary hepatic cancer, irrespective of its source (Wojciechowska and Didkowska, 2022).

Diethylnitrosamine (DEN) is a nitrosamine type that is known to create DNA adducts, which in turn induce liver cancer. DEN's production of oxidative stress further complicates the underlying causes of hepatic cellular carcinoma. DEN and other recognized causative agents promote cell proliferation, which leads to hepatic cells necrosis and damage. Laboratory animals are an important research tool for studying hepatic cellular carcinoma and are widely used in cancer studies. In rat models, DEN has been demonstrated to cause early deteriorating assault, inflammatory response, and proliferation (Shetty et al., 2021). According to earlier research, CCL4 exposure significantly increased the risk of hepatotoxicity, which is characterized by fibrosis, and proliferation of the bile ducts, while the exact mechanism underlying CCL4-triggered cancer of the liver remains unclear, a multiple proof of research indicates that the cytotoxicity caused by CCL4 is dependent on the generation of highly reactive and unstable molecules, which in turn results in deteriorating assault. One of the main processes in CCL4-triggered cancer is deteriorating assault, which damages cells by binding to proteins, lipids, and DNA. Usually, the liver utilizes the route of antioxidant mechanisms to combat deteriorating assault (Wang et al., 2019).

It has long been believed that one of the most important and necessary resources for information for drug investigation and creation is naturally developed compounds. Humans have relied on such substances and/or natural remedies to stay well, avoid disease, and enhance their bodily and emotional well-being. Resveratrol (RSV), a polyhydroxylated stilbenoid, occurs naturally in several plants. RSV has a broad spectrum of bioactivities, comprising antiviral, anti-inflammatory, antifungal, and anticancer attributes. These bioactivities are mostly due to the interesting multi-phenolic hydroxyl groups present in the RSV. Because of its structural characteristics, RSV can form longer-lasting suppressed compounds with less cellular toxicity than damaging radicals while also neutralizing hazardous, highly reactive, and unstable molecules (Tallapaneni et al., 2022). The current work was performed to evaluate resveratrol's therapeutic benefits on liver cancer caused by diethylnitrosamine/CCL4 in rats.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Forty-five albino male rats (120-170 g) were purchased from the Faculty of Veterinary Medicine, Benha University. Throughout the experiment, rats were kept inside special boxes in optimal environmental and nutritional circumstances. The rats were allowed to acclimate for 2 weeks before the experiment started.

The ethical approval was obtained from the Ethics Committee in the Faculty of Veterinary Medicine at Benha University in Egypt and adhered to the established ethical standards (Approval no. BUFVTM 11-12-22).

2.2. Chemicals and natural products:

Diethylnitrosamine, carbon tetrachloride, and resveratrol were bought from Sigma-Aldrich, St. Louis, MO, USA.

2.3. Experimental design:

Following the adaptation period, rats were equally split into 3 groups

* Correspondence to: rf682023@gmail.com

Group I (normal control group): Every day of the trial, rats received an oral gavage of 1 mL of physiological saline.
Group II (HCC group): Rats received 200 mg per kg b.wt. Once an intraperitoneal injection of DEN. Following two weeks of DEN treatment, rats were given weekly 3 ml of CCL4/kg b.w. s.c. for six weeks to enhance the carcinogenic impact (Sundaresan and Subramanian, 2003).
Group III (Resvartrol treated group): Rats were treated with DEN and CCL4 injection as in group 2 and then subsequently injected with resveratrol (100mg/kg b.w./ i.p.) for four weeks (Karabekir and Özgörgülü, 2020).

2.4. Sampling:
2.4.1. Blood samples:
Samples of blood taken from the eye's retro-orbital plexus were centrifuged at 3000 rpm for 15 minutes, and all sera were preserved at -20 °C to assess the biochemical parameters.

2.4.2. Liver tissues for biochemical analysis:
To create a 10% homogenate, minced liver tissue (one g) was homogenized in nine volumes of ice-cold potassium phosphate buffer (0.05 mM, pH 7.4) and centrifuged for 15 minutes at 4°C and 6000 rpm, and the collected supernatant was used to measure the levels of biochemical markers in the homogenates.

2.5. Serum biochemical analysis
ELISA kits were used to measure serum aspartate transaminase (AST (ab263883), alanine transaminase (ALT (ab285264), alkaline phosphatase (ALP (ab287823) activities, albumin (Alb) level (ab108789, Abcam, MA, USA), alpha fetoprotein (AFP) (#MBS034337), and hepatic superoxide dismutase (SOD) (KTE101023), catalase (CAT) (MBS9712526), glutathione peroxidase (GSH-Px) (CSB-E12146r) activities. The supplier's protocols were followed during all the assays. The level of total protein (TP) content was assessed by Bradford assay kit (BioRad, USA).

2.6. Statistical analysis:
The data were examined employing a one-way analysis of variance (ANOVA), and SPSS 25 (SPSS Inc., Chicago, USA) used Duncan as the post-hoc test. P values ≤ 0.05 displayed statistically significant value in the data, which were mentioned as Mean ± SE.

3. RESULTS

3.1. Serum AST, ALT, ALP, TP and Alb:
Rats administered with DEN/CCL4 showed a substantial rise in serum AST, ALT and ALP activities as compared to a normal control group. However, RSV treated rats showed a notable decline in serum AST, ALT and ALP activities when compared to HCC group (Table 1).
When comparing to a normal control group, rats administered with DEN/CCL4 showed a substantial decline in serum TP and Alb levels. Meanwhile, RSV treated rats showed a notable rise in serum TP and Alb concentration when compared to HCC group (Table 1).

3.2. Serum AFP and hepatic SOD, CAT and GSH-Px:
Serum AFP was substantially rise in HCC group when compared with control group, while RSV treated group showed a notable decline in serum AFP level when compared with HCC group (Table 2).
Rats administered DEN/CCL4 had a notable decline in hepatic SOD, CAT, GSH-Px activities in comparison to a control group. Fortunately, rats treated with RSV revealed a notable rise in hepatic SOD, CAT activities with non-significant increase in GSH-Px activity in comparison to HCC group (Table 2).

Table 1. Impact of resveratrol on serum AST, ALT, ALP activities, TP content and Alb level in hepatocellular carcinoma-induced in rats.

Animals group	Control group	DEN/CCL4 group	RSV treated group
AST (U/L)	65.08±3.36^{b}	225.93±8.19^{a}	146.27±9.54^{ab}
ALT (U/L)	44.24±6.83^{c}	202.56±28.02^{a}	129.67±6.50^{b}
ALP (U/L)	77.44±4.17^{c}	134.48±3.75^{a}	98.91±5.10^{b}
TP (g/dL)	8.00±0.19^{a}	6.23±0.16^{c}	7.11±0.06^{b}
Alb (g/dL)	4.23±0.11^{a}	2.75±0.14^{c}	3.73±0.16^{b}

Data are presented as (Mean ± SEM)
Mean values with different superscripts letters in the same row are significantly different at P<0.05.

Table 2. Impact of resveratrol on serum AFP and hepatic SOD, CAT and GSH-Px activities in hepatocellular carcinoma-induced in rats.

Animals group	Control group	DEN/CCL4 group	RSV treated group
AFP (ng/mL)	0.33±0.12^{b}	1.92±0.25^{a}	0.73±0.02^{b}
SOD(U/mg protein)	12.04±0.61^{a}	7.62±0.77^{c}	9.96±0.09^{b}
CAT (U/mg protein)	19.33±3.15^{a}	5.87±1.40^{b}	15.27±2.97^{a}
GSH-Px(U/mg protein)	14.03±2.25^{a}	5.66±1.33^{b}	8.44±0.63^{b}

Data are presented as (Mean ± SEM)
Mean values with different superscripts letters in the same row are significantly different at P<0.05.

4. DISCUSSION

Most liver cancer cases are hepatocellular carcinoma. Globally, hepatic cancer ranks 3rd in terms of cancer-related demise. The regular intake of vegetables and fruits has an inverse correlation with malignancy and other medical conditions. The antineoplastic activity of fruits and vegetables has recently gained much attention. Biologically active components, particularly those with anti-oxidant, hepato-protective, and counter-inflammatory properties, make plants have biological value. Because of their strong anti-tumor efficacy and few side effects, natural compounds derived from plants have been considered as possible anticancer medications. Natural plant-derived products, including resveratrol, have been shown to exhibit antitumor properties (Brown et al., 2024). RSV is an extremely potent antioxidant that successfully suppresses the growth of tumors and impairments of chromosomes (Agbele et al., 2020).
DEN widely exists in the environment and is a potent carcinogen (Subramanian et al., 2007). CCL4 creates trichloromethyl radical that causes damage to cells via either covalently attaching to proteins located in cell membranes or by inducing the peroxidation of lipids (Mrwad et al., 2025).
In the current work, DEN/CCL4 administration induced substantial hepatic cells damage, as seen by raised serum ALT, AST and ALP. These results align with earlier research (Chen et al., 2025), which reported that AST, ALT, and ALP were significantly elevated in the DEN-treated rats at 12 weeks compared with those in the control group. This hepatic damage might initiate the process of neoplastic development, in addition to acutely impairing hepatic functioning (Shahin et al., 2018). Serum ALT and AST elevations are caused by hepatocyte injury leaks (Tawfik et al., 2022), while pathological changes in biliary flow cause ALP rise (Shahin et al., 2018). Hepatic carcinogenesis, necrosis, and toxicity caused an abnormality in the structural integrity of the cell membrane and the leaking and discharge of these enzymes into the blood, leading to raised ALT, AST, and ALP enzyme activities (Hussein et al., 2018). ALT and AST are health liver markers but only serve as general liver damage markers rather than specific liver function markers (Qureshi et al., 2008). As a result, whatever mechanisms are causing their declined concentration in

response to resveratrol are probably multifactorial (Wei and Yu, 2021).

RSV was able to reverse all these parameters, indicating that it can prevent an impairment of integrity of the membrane, in addition to its effects on maintaining of liver functioning (Acharya et al., 2021). RSV treated groups, when compared to HCC group, showed a notable decline in serum AST and ALT activities. These outcomes might be due to the antitumor action of resveratrol (Mrkus et al., 2019). These findings could also be the consequence of decreased cell turnover, which would minimize the amount of the enzymes released into the bloodstream (Su et al., 2019). These results suggest that resveratrol preserves the cellular structure of the liver cells membranes and, in the end, prevents these enzymes from leaking into the bloodstream (Hussein et al., 2017).

In the present study, when compared to a normal control group, rats administered with DEN/CCL4 showed a substantial decline in serum TP content and Alb level. These findings are consistent with previous study (Rezq and Elgazar, 2018), who reported that TP and Alb were significantly declined in the DEN/CCL4-treated rats when compared with control group. Scientists explained these findings to endoplasmic reticulum polysomes dissociation and destruction which play a critical role in protein production. Total protein and albumin levels declined are utilized as a biomarker of reduced protein production, which is caused by DEN/CCL4 toxins. Gani et al., (2019) observed similar results, recognizing that AFP concentration was considerably higher in the diethylnitrosamine and phenobarbital-treated group than in the control normal group. These findings supported the hepatocarcinogenic impact of DEN/CCL4.

When comparing the RSV-treated group to the HCC group, serum TP content and Alb level increased significantly. These findings could be due to the antitumor impact of resveratrol (Mrkus et al., 2019). Additionally, this correction may reflect resveratrol's capacity to restore hepatic damage brought on by DEN/CCL4. Furthermore, it might be due to the oxidative damage reduction produced by DEN/ CCL4, the plasma membrane may preserve its strength. This improvement might be the primary cause of resveratrol's antitumor effects, its capacity to inhibit the unchecked growth of tumor cells, hence reducing damaged cells brought on by DEN/CCL4 (Su et al., 2019).

Since AFP is a marker of HCC, a drop in its concentration suggests that the growth of HCC is being prevented. This was further corroborated by enzyme activity of liver function being better than in HCC rats (Serra et al., 2020). In the current work, treatment by DEN/CCL4 dramatically increased the serum AFP level as compared to the normal group. Borai et al., (2017) observed similar findings, recognizing that the DEN-treated group, AFP concentration, was substantially higher than that of normal group. According to investigations, DEN-induced hepatocarcinogens increased the level of AFP, a tumor marker commonly used to diagnose HCC, and this was linked to an increase in the growth and progression of the tumor (Murugan et al., 2015). In the same way, Hashem et al. (2016) noted that the DEN+CCL4 group exhibited a notable rise in AFP level. To diagnose and track HCC, the rise in serum AFP level might be employed as a medical diagnostic (Tork et al., 2015). Furthermore, Zaazaa et al. (2018) discovered that rats with DEN-induced HCC had higher serum AFP levels than the control group. Added to that, the most widely utilized tumor marker for the detection of hepatic cellular carcinoma (HCC) is alpha fetoprotein (AFP), a unique immune-modulatory glycoprotein that is often produced by the fetus's immature hepatocytes (oncofetal). Among patients with elevated AFP levels prior to medical treatment, AFP detection during liver cancer treatment monitoring is widely recognized. It is known that animals exposed to DEN have higher levels of AFP in their blood (Sadik et al., 2008). In the current study, when compared to the hepatic cellular carcinoma group, the RSV-treated group demonstrated a significant drop in serum AFP levels. This outcome matches the chemoprotective effect suggested by Rahman et al. (2020) study. This is comparable to the study conducted by Su et al. (2019). Additionally, resveratrol's anticancer properties may be responsible for this outcome (Mrkus et al., 2019).

Highly reactive and unstable molecules produced by DEN/CCL4 further interact with biomolecules (lipids and proteins) that interfere with organelles within cells, particularly the nucleus, resulting in damage to DNA and RNA, consequently leading to the formation of HCC (Bashandy et al., 2023). The endogenous antioxidants often eliminate these highly reactive and unstable molecules that are created (Ibrahim et al., 2015). Antioxidants thus serve as the biological cleaners by eliminating highly reactive and unstable molecules before they cause damage to DNA (Abdelhady et al., 2018). Antioxidants that are part of the first line of defense, including catalase, glutathione peroxidase, and superoxide dismutase. These antioxidant enzymes break down hydrogen peroxide, hydroperoxides, and dismutate superoxide radical into innocuous molecules (H2O2/alcohol and O2), respectively (Ighodaro and Akinloye, 2018). The current findings demonstrated that, as compared to a control group, rats given DEN/CCL4 exhibited a notable decline in SOD, CAT, and GSH-Px activities. These findings might be the result of a redox imbalance, in which these antioxidants are scavenging highly reactive and unstable molecules that DEN/CCL4 produces in excess. These outcomes aligned with earlier research (Bashandy et al., 2023).

The current study demonstrated that giving RSV boosted SOD, CAT, and GSH-Px activities in comparison to the HCC group. These results might be due to the formation of highly reactive and unstable molecules being hindered or eliminated by RSV, which would have boosted the activity of SOD, CAT, and GSH-Px. These outcomes aligned with (Yan et al., 2022). These findings suggest that RSV might strengthen antioxidant defense mechanisms. These results supported the findings of (Ghavipanjeh et al., 2024), who reported that RSV enhancing antioxidant defense mechanisms.

5. CONCLUSIONS

According to these results, resveratrol may reduce the development of hepatic cancer by preserving liver function and increasing antioxidant activity.

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