RESEARCH ARTICLE

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Attenuation of N-Nitrosodiethylamine -Induced Hepatocellular Carcinoma by Piceatannol and/or Cisplatin: The Interplay between Nuclear Factor (Erythroid Derived 2)-like 2 and Redox Status

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Abstract

Background: The natural compound's alternative and complementary uses have increased hopes for hepatocellular cancer treatment (HCC). **Objects:** The goal of this study was to see if Piceatannol (PIC) in combination with cisplatin has a synergistic effect on N, N-nitrosodiethylamine (DEN)-induced HCC in rats. **Methods:** Tissue antioxidant enzymes, malondialdehyde (MDA), and nuclear factor erythroid 2 related factors 2 (Nrf2) and tumor necrosis factor α (TNF-α) gene expression were all measured. Nuclear Factor Kabba B (NF-κB) was also tested, as well as hepatic caspase 3 and NAD (P) H quinone oxidoreductase 1 (NQO1). Liver specimens were subjected to histopathological analysis. **Results:** When compared to the HCC group, piceatannol and/or cisplatin caused a significant improvement in liver function tests, as well as a significant modulation in Nrf2 gene expression and antioxidant enzyme activities, as well as a significant decrease in tissue MDA, TNF-α, NF-κB levels, NQO1 activity, and prompt and caspase-3 activities. When the PIC and/or cisplatin combination was compared to each of these compounds alone, the results were substantial. **Conclusion:** PIC in combination with cisplatin has been shown to have a synergistic anticancer impact through modulating Nrf2 and redox state. In addition, adding PIC to an HCC therapy plan that includes chemotherapeutic medicines may boost the efficacy of cisplatin while reducing its negative effects.

Keywords: HCC- Piceatannol- Cisplatin- Nrf2- NFκB- NQO1- TNF-α

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Introduction

Liver cancer spreads in the late years among the entire world's population. Hepatocellular carcinoma accounts for almost 90% of liver cancers (Locatelli et al., 2012). There are many risk factors shared in the incidence of HCC, as viral infections including virus B (Tang et al., 2018), virus C (Neamatallah et al., 2019), and virus D (Puigvehí et al., 2019) are associated with some molecular alterations, or non-viral infections such as non-alcoholic steatohepatitis (Llovet et al., 2021) which is associated with metabolic syndromes such as diabetes mellitus, chemicals, and alcohol (Donato et al., 2002).

Nrf2 is a cap 'n' collar basic leucine zipper transcription factor that binds specifically to the antioxidant response element (ARE) at the promoter of its target genes, (Raghunath et al., 2018) which regulates a broad spectrum of genes involved in redox homeostasis, for example, NADP/NADPH quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HO-1) and glutathione glutamate-cysteine ligases (Rangasamy et al., 2004). Under normal growing conditions, Nrf2 interacts with the Kelch-like ECH-associated protein 1 (KEAP1) protein in the cytoplasm, which ensures Nrf2 posttranslational degradation through the ubiquitin-proteasome system. Under stressful conditions, including metabolic stress such as an imbalance in redox homeostasis, Nrf2 dissociates from KEAP1, enters the cell nucleus, and drives gene transcription to restore the cellular redox homeostasis. Redox imbalance is a critical factor contributing to aging and age-related diseases, including liver cancer (Wang et al., 2019), Nrf2 has been shown to promote healthy aging in various model organisms (Li et al., 2018).

In the process of carcinogenesis, the promotion of reactive oxygen species (ROS) and oxidative stress disrupts redox balance, which leads to the activation of anti-oxidative repair mechanisms (Kumar et al., 2016). Nrf-2, as one of the classical transcription factors, is activated by the oxidative stress injury implicated in the initiation of cancer (Orrù et al., 2018). It translocates

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from the cytoplasm to the cell nucleus and combines with ARE to maintain cellular redox balance. Therefore, the ARE-controlled genes are activated. NF-κB activation promotes tumorigenesis in liver cancer by increasing the expression of several multifunctional antioxidative proteins such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1) (Yen et al., 2017). As a downstream molecule of Nrf-2, over-expression of HO-1 attenuated ROS generation, thus modulating oxidative stress and anti-cancer effects (Sangnam et al., 2010). Taken together, inhibition of Keap-1, NF-κB and induction of Nrf-2, HO-1 appears to be a potential therapeutic approach in rats with liver cancer induced by N, N-nitrosodiethylamine (DEN).

Despite continuous advances in managing chronic liver diseases, tumor detection and treatment, the prognosis of HCC remains lower in comparison to other tumors (Bray et al., 2018). The incidence of high mortality and associated side effects following chemotherapy and/ or radiotherapy increases the demand for alternative medicines for cancer treatment. Not surprisingly, many potent anti-cancer compounds have been isolated from plants, e.g., doxorubicin, taxol, etoposide, cisplatin, vinblastine, vindesine, vincristine, and topotecan (Gordaliza, 2007). This has led to the current interest in alternative medicine that will aid in therapy and help to avoid its unjustifiable use. Currently, naturally derived compounds have notable importance for their massive application as antioxidants to inhibit free radical species and protect the cellular organelles from oxidative damage (Singh et al., 2018).

Piceatannol (3,4',3',5-transtrihydroxystilbene) is a natural analogue and a metabolite of resveratrol (Piotrowska et al., 2012). It was first isolated from the seeds of Euphorbia lagascae (Ferrigni et al., 1984). In addition to grapes, the most important sources of PIC in the diet are grapes, passion fruit, white tea, and Japanese knotweed (Tyagi et al., 2012). It is an important naturally occurring polyphenolic stilbene, produced by plants in response to fungal infection, mechanical damage, and ultra-violet irradiation. It can be consumed daily with no harmful effects on the human body (Kukreja et al., 2014). Also, PIC is known as a potent scavenger of free radicals due to the hydroxyl groups in its stilbene rings (Rossi et al., 2008; Moustafa et al., 2021). Piceatannol biological activities are to induce the apoptotic mechanism in cells of many types of cancer, such as melanoma, bladder, prostate, and colorectal cancers (Moustafa et al., 2021). Furthermore, PIC works as an antitumor agent, promoting the accumulation of cells in the G2/M phase of the cell cycle. This also activates apoptosis as a result of growth inhibitory effects, so the G2/M phase of the cell cycle is blocked and the cells are prevented from entering the G1 phase (Seyed et al., 2016). It was reported that PIC controls tumor growth in different systemic malignancies, including hepatocellular carcinoma. Piceatannol proapoptotic effect occurs due to mitochondrial potential, caspase activation, and cytochrome C release (Piotrowska et al., 2012). Hence, the present study aimed to investigate whether combined PIC and cisplatin exert a synergistic effect on DEN-induced hepatic cancer and explore its underlying mechanism by examining Nrf2 and redox status, which significantly correlated to differentiation and growth of HCC.

Materials and Methods

Chemical materials

Sigma–Aldrich® (St. Louis, Missouri, USA) provided the DEN. Piceatannol was obtained from Zetpil™ Nutritionals, LLC (Florida, United States). Cisplatin [Cis-diaminedichloroplatinum (II), DDP] was purchased from a commercial pharmacy.

Animals

The present study was carried out using seventy adult male Swiss albino rats (130-150 g) obtained from Nile Company for Pharmaceuticals and Chemical Industries (Cairo, Egypt). The animals were kept in standard plastic cages in a well-ventilated room. Rats were maintained with free access to laboratory pellet chow with water ad libitum under controlled conditions of temperature (27± 2°C) and humidity (60 ± 5%) with 12 h light/ 12 h dark cycles. The experimental protocol was carried out according to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, 1985) as approved by the institutional animal care and use committee (IACUC) with No. Vet CU.

Hepatocellular carcinoma induction

For the induction of hepatocellular carcinoma, freshly prepared DEN was dissolved in 0.9% NaCl solution, and rats received daily oral administration of 20 mg DEN/Kg b.w for six constitutive weeks according to the method of Darwish and El-Boghdady (2011) with some modification.

Experimental Design

Normal group: Animals served as control

Cisplatin group: Rats received cisplatin once a week by intraperitoneal injection of 0.64 mg/kg b.w. for one month according to Medhat et al., (2017)

PIC group: According to Farrand et al., (2013), rats were given 20 mg/kg b.w of PIC orally once a day for one month.

DEN group: Rats received DEN-induced HCC.

DEN + Cisplatin group: Rats received DEN orally for six weeks, and then rats received cisplatin by intraperitoneal injection for one month as in group 2.

DEN + PIC group: Rats received DEN orally for six weeks, and then rats received PIC by oral administration as in group 3 for one month.

DEN + PIC+ Cisplatin group: Rats were given DEN orally for six weeks, then PIC was given orally as in Group 3 and cisplatin was given intraperitoneally as in Group 2 for one month before being sacrificed.

At the end of the experimental period, rats were sacrificed by cervical dislocation, and blood samples from each rat were collected into clean centrifuge tubes and centrifuged at 3,000 rpm for 20 min. For further analysis, the separated serum was frozen at -20°C. The rats were dissected and the livers were immediately excised, rinsed with ice-cold saline, blotted dry, and accurately

weighed. They were then minced and homogenized in ice-cold buffered saline (10% w/v). The homogenates were centrifuged at 3,000 rpm for 10 min at 4°C. Finally, the supernatants were subjected to biochemical analysis. Other samples of the liver tissue were stored in 10% neutral formalin for histopathological studies.

Biochemical Assay

Liver function

The activity of alanine transaminase (ALT) was measured colorimetrically in serum (Reitman and Frankel, 1957), using a kit supplied by Bio diagnostic Company, Egypt. Alkaline phosphatase (ALP) activity was quantified using kits supplied by Bio diagnostic kit Company, Egypt according to Belfield and Goldberg (Belfi and Goldberg, 1971). The activity of gamma-glutamyl-transferase (γ-GT) was determined in serum colorimetrically using the Diamond company kit. The levels of bilirubin were evaluated in serum using a kit supplied by Bio-med Diagnostic, Egypt.

Kidney function

Serum creatinine and urea were determined enzymatically using commercially available kits from Bioclin, Santa Coloma, Spain.

Determination of oxidative stress biomarkers in liver tissue homogenate

Lipid peroxidative products were measured using the thiobarbituric acid test for malondialdehyde (MDA), as described by Satoh (Satoh, 1978). Superoxide dismutase (SOD) activity was determined spectrophotometrically (Marklund, 1992) Reduced glutathione (GSH) contents were measured according to the method of Ahmed et al., (1991).

Inflammatory biomarkers

The levels of TNF-α, NF-κB and NQO1 were estimated in the liver homogenate using a rat Elisa kit from Mybiosource Company, San Diego, California, USA.

Apoptotic biomarker

The level of caspase-3 activity was estimated in the liver homogenate by a rat Elisa kit from Mybiosource Company San Diego, California, USA.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated using a QIAGEN tissue extraction kit (QIAGEN, USA) according to the instructions of the manufacturer. The amplification and analysis were performed using Applied Biosystems with software version 3.1 (StepOne™, USA). The qPCR assay with the primer sets was optimized at the annealing temperature. All complementary DNAs (cDNAs) were in duplicate and included previously prepared samples for Nrf-2 with Beta-actin (β- actin) as an internal control, and water is used as non-template control to confirm the absence of DNA contamination in the reaction mixture. The primers used were as follows: Nrf-2 forward primer: 5′-CCTCAACTATAGCGATGCTGAATCT-3′; Nrf-2 reverse primer:

5′-AGGAGTTGGGCATGAGTGAGTAG-3′; β-actin forward primer: 5′-CCCATCTATGAGGGTTACGC-3′; β-actin reverse primer: 5′-TTTAATGTCACGCACGATTTC-3′.

Histological Examination Findings

At the time of sacrifice, the liver tissues of rats from different groups were collected and fixed in 10% formalin for 48 h. Following fixation, the specimens were dehydrated in an ascending series of alcohol and then the tissue specimens were cleared in xylene and embedded in paraffin at 60°C. A section of five microns in thickness was cut by a sliding microtome. The obtained tissue sections were collected on the glass slides and stained with hematoxylin and eosin stain for histological examination according to the method described by Banchroft et al., (1996)

Statistical analysis

All data were expressed as the mean ± standard error. One-way analysis variance (ANOVA) with least significant difference (LSD) was used to test for differences in means of variables between groups. A probability of $p<0.05$ was considered significant. All data were analyzed by Statistical Package for Social Science (SPSS) version 20.0 for Windows (SPSS1 Chicago, IL, USA) software program.

Results

Effect of PIC and/ or cisplatin on DEN-induced liver and kidney injury

Given levels of liver function and kidney function parameters related to the liver and kidney injury respectively, the ALT, ALP, GGT, bilirubin, creatinine and urea levels in serum of DEN-exposed rats were measured. It was found that the treatment of rats with DEN-induced hepatic and kidney injury ends up with a significant increase in serum ALT, ALP, GGT and bilirubin along with a marked increase in the levels of creatinine and urea compared to the normal control group. However, the administration of either PIC or cisplatin injection showed a significant improvement in the liver and renal functions as compared to the DEN group (Tables 1 and 2).

Effect of PIC and/or cisplatin on DEN-induced oxidative and inflammation damage

Following our results, we discovered that DEN not only clearly increased MDA formation, NF-B and TNF- levels, but also clearly decreased SOD activity and GSH content in rats' livers when compared to normal rats. In contrast, the levels of oxidative stress biomarkers MDA, SOD, and GSH, as well as inflammatory NF-κB and TNF-α, were altered after single or combined treatments with PIC and/or cisplatin. However, the combined treatment with PIC and cisplatin showed more improvement than a single treatment (Table 3 and Figure 1).

Table 1. Effect of PIC and/or Cisplatin on Liver Functions (ALT, ALP, GGT & Bilirubin) in the Serum of Different Studied Groups

Groups	ALT (U/L)	ALP (U/L)	GGT (U/L)	Bilirubin (mg/dl)
Normal	17.0± 0.26[b]	89.2± 1.51[b]	116.5±0.76[b]	1.115±0.021[b]
CIS	15.0± 0.26[b]	86.1± 0.25[b]	111.9± 0.25[b]	0.995± 0.003[b]
PIC	14.5± 0.65[b]	86.0± 0.62[b]	113.4± 0.25[b]	1.020± 0.003[b]
DEN	75.5± 0.65[a]	198.3±3.14[a]	228.1±0.93[a]	2.100±0.050[a]
DEN+CIS	42.5± 2.20[ab]	136.3± 0.72[ab]	162.2± 0.80[ab]	1.450± 0.01[ab]
DEN+PIC	42.5± 0.90[ab]	139.0± 1.60[ab]	160.9± 2.50[ab]	1.400± 0.05[ab]
DEN+PIC+CIS	33.5± 0.39[ab]	113.± 0.33[ab]	129.1± 0.89[ab]	1.145± 0.14[ab]

Cis, Cisplatin; PIC, Piceatannol; DEN, N, N-nitrosodiethylamine; Each value represents mean ± standard error (SE) of six values (p≤ 0.01). [a] Significance vs. Normal control group.[b] Significance vs. DEN group.

Table 2. Effect of PIC and/or Cisplatin on Kidney Function (Creatinine & Urea) in Serum of the Different Studied Groups

Groups	Urea (mg/dL)	Creatinine (mg/dL)
Normal	43.5±2.194[b]	0.17±0.130[b]
CIS	37.5± 0.387[b]	0.18± 0.003[b]
PIC	40.0± 0.52[b]	0.17± 0.004[b]
DEN	105.5±1.94[a]	1.29±0.081[a]
DEN+CIS	69.5±0.65[ab]	0.57±0.020[ab]
DEN+PIC	67.0± 0.52[ab]	0.53± 0.003[ab]
DEN+PIC+CIS	48.0± 1.55[ab]	0.34± 0.04[ab]

PIC and/or Cisplatin Treatment Regulated Antioxidant Signaling Pathways in DEN-Induced HCC

Oxidative damage is also one of the major factors in DEN-induced HCC in rats. Hence, the Nrf2-mediated signaling pathway, as an important antioxidant pathway, was first taken into account to determine whether PIC and/or CIS treatment could regulate the Nrf2 antioxidative signaling pathway in DEN-induced HCC. In this study, the DEN challenge suppressed Nrf2 gene expression but strongly stimulated NQO1 protein expression; whereas these changes were completely reversed by PIC and/or CIS treatment when compared

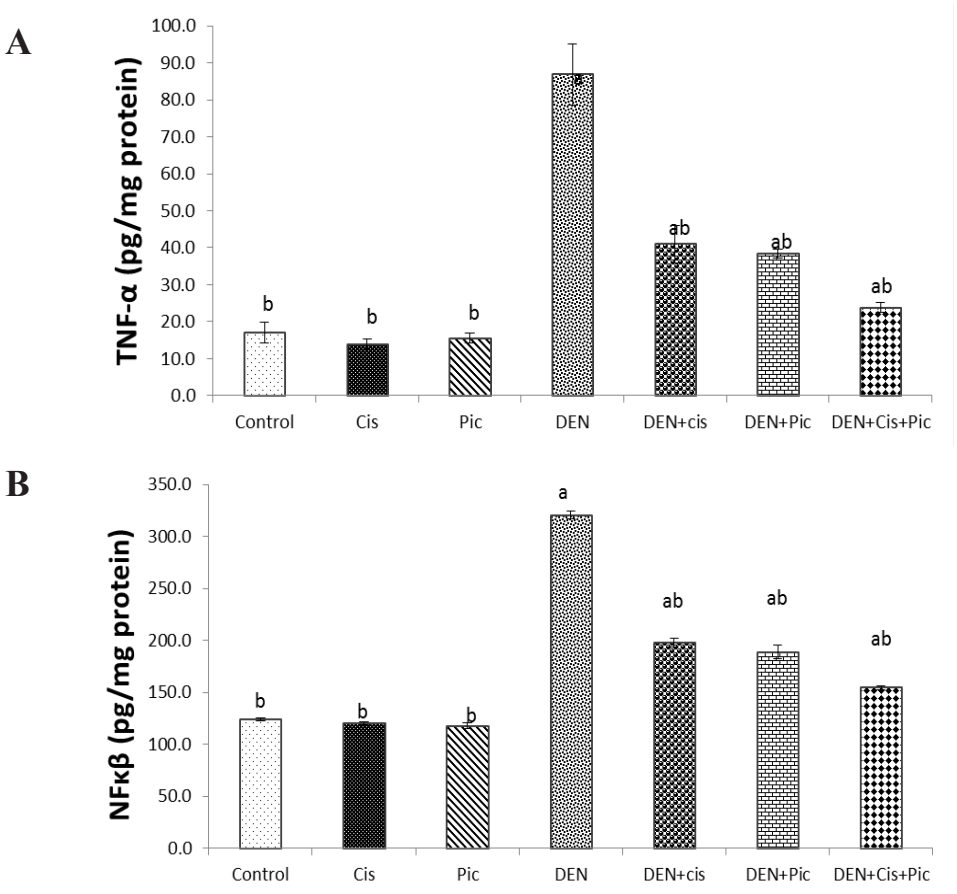

Figure 1. Effect of PIC and/or C isplatin on Inflammatory Markers (NF-κB and TNF-α) in Liver Tissue Homogenate of the Different Studied Groups. Cis, Cisplatin; PIC, Piceatannol; DEN, N, N-nitrosodiethylamine. Each value represents the mean ± standard error (SE) of six values (p≤ 0.01). a Significance vs. Normal control group. b Significance vs. DEN group.

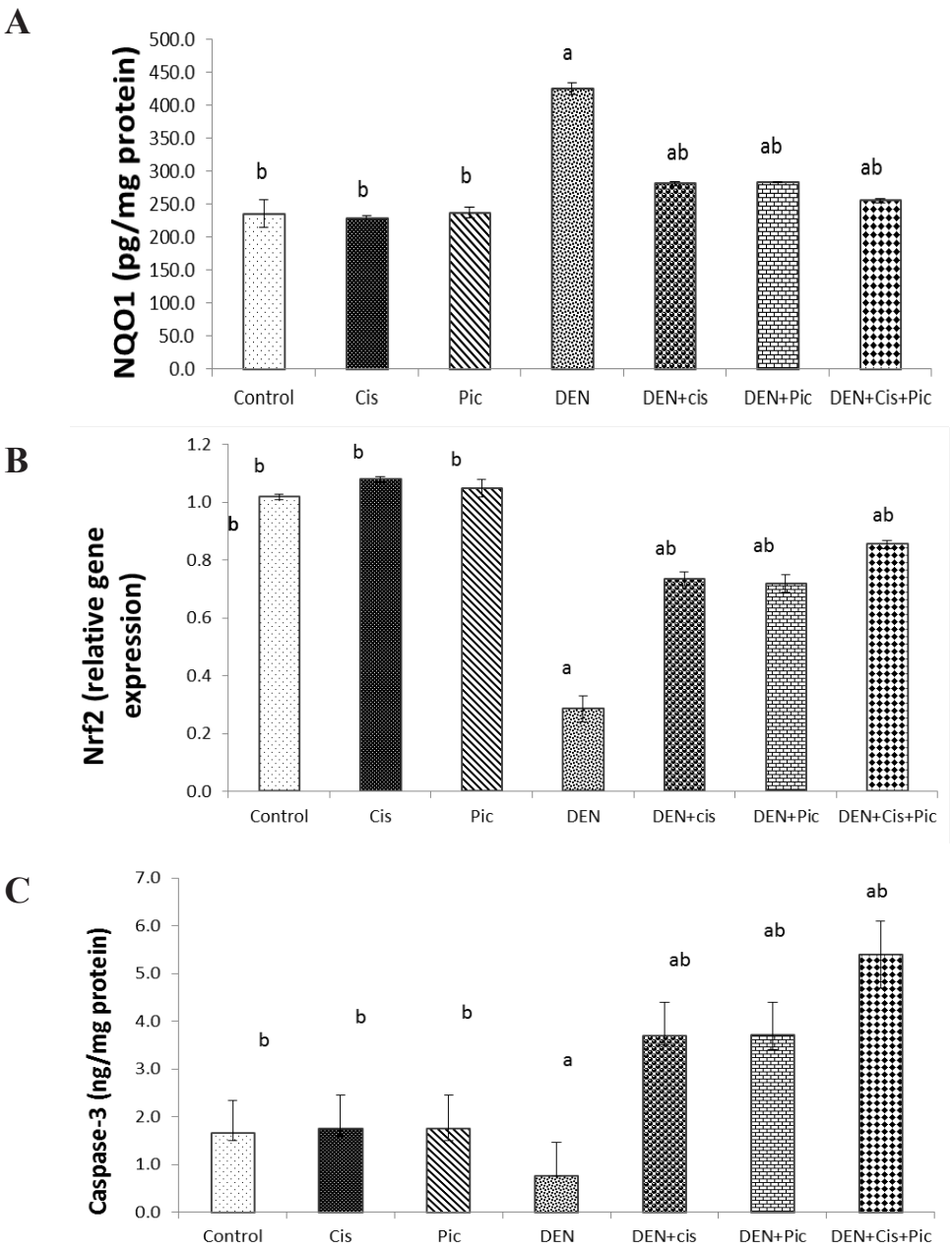

Figure 2. Effect of PIC and/or Cisplatin on Oxidative Homeostatic Markers (Nrf2 and NQO1) and Apoptotic Protein; caspase-3 in liver tissue homogenate of the different studied groups. Cis: Cisplatin; PIC: Piceatannol; DEN: N, N-nitrosodiethylamine. Each value represents mean ± standard error (SE) of six values ($p \leq 0.01$). a Significance vs. Normal control group.b Significance vs. DEN group.

with the DEN group (Figure 2A and B).

Effect of PIC and/or cisplatin on DEN-induced apoptotic dysfunction in rats

Since cell apoptosis is regarded as a crucial factor in DEN-induced liver cancer, the current study examined whether PIC and/or CIS treatment could stimulate cell apoptosis in DEN-induced HCC in rats. Indeed, DEN significantly inhibited the activity of caspase-3, whereas this effect is effectively stimulated by PIC and/or CIS treatment. However, this result suggested that PIC and/or CIS treatment could induce cell apoptosis (Figure 2C).

Histological Finding

The histological sections of the liver of the control normal rats showed preserved tissue architecture, no histopathological alteration, as well as normal histological structure of hepatic lobules and organization of hepatic cords with prominent central hepatic vein as shown in Figure 3 (A and B). The liver sections of CIS received rats one month showed histopathological alterations in the architecture of the liver tissue as revealed disorganization of hepatic plates and swelling of hepatocytes. The nuclei appeared pyknotic with condensation of nuclear chromatin. The hepatic lobule showed binucleation of hepatocytes with a frequent number of apoptotic bodies and hyperplasia of Kupffer cells with lymphocytic infiltration without any criteria of malignancy (Figure 3 C and D). The histopathological sections of the liver of rats received PIC showed the normal histological

Table 3. Effect of PIC and/or Cisplatin on Oxidative Stress Biomarkers (MDA, SOD and GSH) in Liver Tissue Homogenate of the Different Studied Groups

Groups	MDA (nmol/mg protein)	SOD (U/mg protein)	GSH (mmol/mg protein)
Normal	67.20±0.49[b]	47.8±0.44[b]	144.6±0.55[b]
CIS	61.55± 1.72[b]	49.0± 1.14[b]	150.65± 0.50[b]
PIC	60.50± 0.75[b]	49.8± 0.70[b]	147.3± 0.54[b]
DEN	257.6±0.45[a]	16.25±0.68[a]	58.75±1.64[a]
DEN+CIS	99.3±0.49[ab]	32.4±1.06[ab]	114.6±0.37[ab]
DEN+PIC	101.2± 2.35[ab]	30.3± 0.75[ab]	113.7± 0.30[ab]
DEN+PIC+CIS	85.1± 0.62[ab]	43.4± 0.98[ab]	121.75± 0.5[ab]

Cis, Cisplatin; PIC, Piceatannol; DEN, N, N-nitrosodiethylamine. Each value represents mean ± standard error (SE) of six values ($p \leq 0.01$). [a] Significance vs. Normal control group.[b] Significance vs. DEN group

structure of hepatic lobules and organization of hepatic cords (Figure 3 E and F). The liver of rats administrated with DEN showed multinodular lesions consisting of polyhedral to round hepatocytes with dense, centrally located vesicular nuclei. The neoplastic cells proliferated in a solid pattern without distinct sinusoid-like blood spaces, and only slit-like blood vessels were observed in large tumor nests. The neoplastic cells showed anisokaryosis, anisocytosis, deeply basophilic scanty cytoplasm and few mitotic Figures (Grade III) (Figure 3 G and H). The histopathological sections from the liver of rats received DEN + PIC showed necrobiotic changes

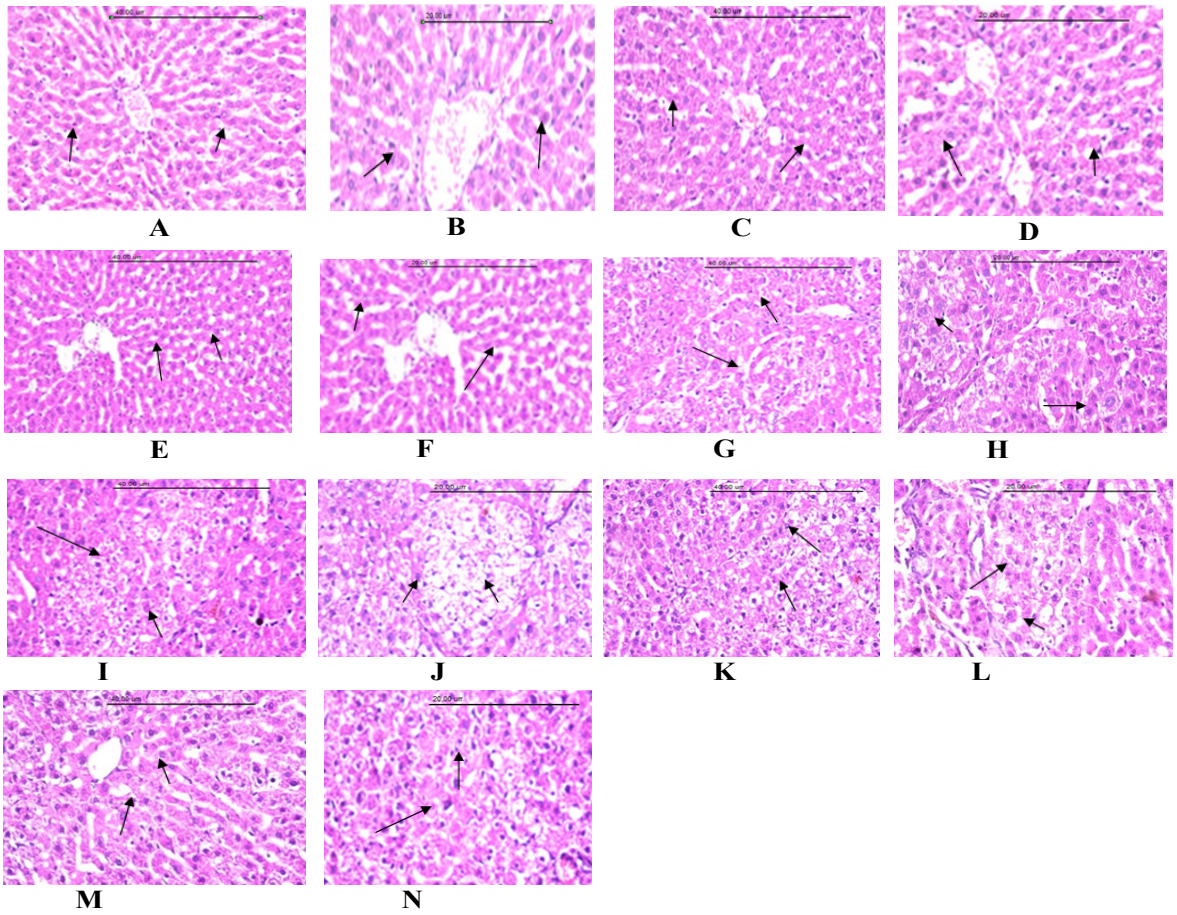

Figure 3. Photomicrographs of Liver Specimens Stained with Hematoxylin and Eosin (H&E, 200X and 400X). (A & B) section of liver of control normal animals showed normalorganization of hepatic cords with prominent central hepatic vein and normal large polygonal cells with prominent round nuclei and eosinophilic cytoplasm, (C & D) section of liver of CIS injected rat showed disorganization of hepatic plates and swelling of hepatocytes, pyknosis with condensation of nuclear chromatin, (E & F) section of liver of PIC treated rats showed normal histological of hepatic lobules and organization of hepatic cords, (G & H) Section of liver of DEN treated rats showed disorganization of hepatic cords with hyperplasia of Kupffer cells and neoplastic cells proliferated in a solid pattern, (I, J) section of liver of the DEN + PIC treated rats showing necrobiotic changes of hepatic carcinoma nodules with Focal coagulative necrosis of carcinomatous nodule, (K & L) section of liver of DEN + CIS showed multiple small degenerated nodules scatter all over the hepatic lobule with swelling and vacuolation of hepatocytes, (M &N) section of liver of the DEN + CIS + PIC treated rats showing massive necrosis of hepatic carcinoma cells with hyperplasia of Kupffer cells with nuclear pyknosis and deeply eosinophilic cytoplasm.

Table 4. The Pathological Score of All Cancer Induced Groups

Group	Grade	Pathological response	Incidence Nodular lesions	Size of nodules/x200	Number of nodules/x200
DEN	No change	Minimal necrosis	80%- 65%	12.27-15.44um	4_6
DEN+PIC	Partial	51%-99% tumor necrosis	60% -50	14.75- 15.63um	4_2
DEN+CIS	Poor	<50% tumor necrosis	75% - 60%	11.66 - 9.55um	5_3
DEN+PIC+CIS	Complete	100% tumor necrosis		Absence	

*Size and number of nodular hepatic lesions were applied by using TSV software

of hepatic carcinoma nodules with hyperplasia of Kupffer cells. Partial 51%-99% tumor necrosis was noticed. Focal coagulative necrosis of carcinomatous nodules without obvious mitotic figures was seen (Figure I and J). The histopathological sections from the liver of rats received DEN + CIS revealed multiple small degenerated nodules scatter all over the hepatic lobules. Swelling and vacuolation of hepatocytes were seen. The nuclei appeared vesiculated with prominent nucleoli with poor tumor necrosis <50%. Disorganization of hepatic plates that invaded infiltrated with lymphocytes and macrophages were seen. The hepatic lobule showed binucleation of hepatocytes with a frequent number of apoptotic bodies and hyperplasia of Kupffer cells as illustrated in Figure (K and L). On the other hand, the sections from the liver of rats that received DEN + PIC + CIS revealed massive necrosis of hepatic carcinoma cells with hyperplasia of Kupffer cells. Complete 100%-tumor necrosis was seen. The necrotic areas showed nuclear pyknosis and deeply eosinophilic cytoplasm without obvious mitotic figures as shown in (Figure M and N).

Discussion

Chemotherapeutic agents may be used in lower as well as higher doses, but the higher dosages provide a better efficacy profile, while the lower dosages give a better safety profile (Rossi et al., 2010; Al-Zubairi, 2017). Therefore, it is urgent to develop probable therapeutic agents for better efficacy as well as a safety profile to sustain the clinical benefits in cancerous patients. The present investigation was carried out to evaluate the ameliorative effects of DEN-induced hepatocellular carcinogenesis in albino Wistar rats by PIC alone or in combination with cisplatin and the role of specific cytokines and oxidative stress manifestations during cancer progression.

In the current study, various enzymes, such as ALT, ALP, and GGT, are notably increased in human serum with HCC, which is similar in DEN-exposed rats. This elevation is due to leakage from damaged or necrotic cells and can be found supported in multiple studies and can be used as evidence for HCC development in rats intoxicated with DEN (Minnady et al., 2010). Moreover, bilirubin is the catabolic byproduct of RBCs, and the elevated level of this biomarker indicates a hepatic disease state. During this study, it was observed that the level of bilirubin was elevated in the carcinogen-exposed group (Keshari et al., 2017). However, the levels of these liver biomarkers were brought down to a normal level with cisplatin, either independently or in combination with PIC. However, the tendency of PIC and cisplatin to decrease the elevated liver enzyme levels caused by DEN suggests that it might be attributed to the antitumor activity of both PIC and cisplatin.

Moreover, experimental studies indicate that the maximum increase in serum creatinine occurs following cisplatin administration (Francescato et al., 2001). It has been suggested that the increase in urea and creatinine during cisplatin toxicity is due to irreversible renal tubular damage (Dickey et al., 2008). However, in the present study, there was no significant change in creatinine, which could be due to the presence of a four-week interval between injections, which provided a chance for the creatinine level to be normalized. The redox-sensitive transcription factor Nrf2, plays a central role in the inducible expression of genes encoding detoxifying systems, including phase II drug-metabolizing enzymes; NQO1, glutathione peroxidase, ferritin, and heme oxygenase-1 (HO-1) (Jaiswal, 2004). These defense enzymes are coordinately induced through the ARE and are tightly regulated by Nrf2 (Nguyen et al., 2003). The attenuated expression of these enzymes in Nrf2-deficient rats has verified the role of Nrf2 in the regulation of many detoxifying and antioxidant enzymes under oxidative stress conditions; rendering Nrf2-deficient rats more vulnerable to carcinogen-induced toxicity and carcinogenesis (Enomoto et al., 2001). DEN administration significantly decreased hepatic Nrf2. This result was reflected by the dramatic decrease in reduced SOD and GSH content of the investigated tissues, besides the increase in oxidative stress marker MDA and inflammatory cytokine TNF-α. Similarly, a study reported that DEN down-regulates Nrf2 in the liver along with the induction of oxidative stress, inflammation, and angiogenesis (Ramos-Gomez et al., 2001). Piceatannol and/or cisplatin modulated the decrease in hepatic Nrf2. The upregulation of Nrf2 by PIC could exert an anti-inflammatory effect through the elevation of antioxidant enzymes, leading to the inhibition of NFκB signaling giving new insight into cancer prevention through upregulation of the Nrf2/ARE pathway (Chi et al., 2015).

Effects of the dietary stilbenoid piceatannol on HCC chemosensitivity in vivo were examined in this work, to strengthen a synergistic treatment combination to induce tumor cell death. ROS has a very short lifetime and a limited diffusion distance, resulting in ineffective tumor cell death and unsatisfactory treatment effects (Mahmoud et al., 2017). Effects of the dietary stilbenoid piceatannol on HCC chemosensitivity in vivo were examined in this work, to strengthen a synergistic treatment combination to induce tumor cell death. ROS has a very short lifetime and a limited diffusion distance, resulting in ineffective tumor cell death and unsatisfactory treatment effects. It can be concluded that PIC possessed promising therapeutic

potential which potentiated cisplatin effects. This positive effect was believed to be due to the improvement observed in the Nrf2 modulation and the reduction of lipid peroxidation and NF-κB which were confirmed by histological improvements. To obtain better curative success, we suggest that management methods for HCC include treatments like Piceatannol in conjunction with chemotherapy.

Author Contribution Statement

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by (Omnia Ahmed Mohamed Salama), (Fatma Saed Moawed), (Enas Mahmoud Mostafa), (Eman Ibrahim Khandil). The first draft of the manuscript was written by (Omnia Ahmed Mohamed Salama) and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

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