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SULFORAPHANE SUPPRESSES HEPATOCELLULAR CARCINOMA IN MICE VIA ACTIVATING p53, MITIGATING OXIDATIVE STRESS, AND INHIBITING ANGIOGENESIS

Background. Hepatocellular carcinoma (HCC) is the most common primary liver malignancy. Sulforaphane (SF) is a natural isothiocyanate that exhibits anticarcinogenic activity against various cancer cells in vivo and in vitro, with no observed side effects. It also has a prophylactic effect against early stages of some cancer models, including HCC. The study **aimed** to assess the therapeutic anticarcinogenic effect of SF in experimental murine HCC in vivo and to estimate possible underlying mechanisms of its effects on cell proliferation, angiogenesis, and antioxidant status. **Materials and Methods.** HCC was induced in mice by a single intraperitoneal dose of 100 mg/kg of diethylnitrosamine, followed by 22 weekly intraperitoneal doses of 0.5 mg/kg of carbon tetrachloride. After the induction of HCC, SF (2 mg/kg body weight) was given orally from weeks 25 to 28. Then we conducted histopathological examination, biochemical analysis of blood sera, and immunohistochemical analysis of marker expression in liver sections. **Results.** SF improved liver structure and function in HCC-bearing mice and survival rate, significantly reduced the expression of tumor marker alpha-fetoprotein and the number of hepatic nodules, and downstaged HCC. These changes were accompanied by an increase in total antioxidant capacity, expression of p53 and Bax, as well as a significant decrease in lipid peroxidation and immunoreactivity of PCNA, VEGF, and Bcl-2 in liver tissues. **Conclusion.** SF decreased the number of tumor nodules and ameliorated HCC by activating p53 expression, inducing apoptosis, and inhibiting proliferation, oxidative stress, and angiogenesis. These findings support further translational evaluation of SF as a potential therapeutic agent for HCC.

Keywords: hepatocellular carcinoma, sulforaphane, Bax, Bcl-2, VEGF, PCNA, p53, oxidative stress, mice.

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and accounts for more than 80% of liver tumors worldwide [1, 2]. It is the sixth most common cancer and the third most common cause of cancer-related death worldwide [2, 3]. Most HCC patients are diagnosed at the advanced stages of the disease, when surgical options are no longer feasible [4]. Fewer than one-third of these patients benefit from chemotherapy [5], which remains the primary treatment option

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for advanced HCC [6]. However, drug resistance within six months of initiating the regimen [5, 7], intolerable adverse effects, and high cost remain the obstacles to effective chemotherapy [8]. These challenges have encouraged many researchers to discover and develop natural product-derived substances for treating cancer with higher therapeutic efficacy, lower cost, and fewer side effects compared to chemical drugs [9, 10]. Sulforaphane (SF) is one of the most extensively studied isothiocyanates and has been frequently examined for its anticancer effects [11—13]. SF is widely recognized as a promising natural chemopreventive agent both in vitro and in vivo against many cancers, including cervical, breast, bladder, renal cell, lung, prostate, and colon cancers [14—18], as well as hepatic cancer [17, 19]. SF also exhibits apoptotic, antioxidant [20], and anti-inflammatory effects [21], supporting its potential as a candidate in cancer chemoprevention [22, 23]. In addition to its putative beneficial pharmacological effects and efficacy, SF is non-toxic and has no side effects [24—26].

Despite the extensive published work on the beneficial effects of SF, studies carried out on its effectiveness against HCC are rare. Previous studies evaluating the anticarcinogenic effect of SF in liver cells were conducted mostly in vitro [27, 28]. However, those carried out in experimental animals studied the prophylactic effects on the early stages of HCC [29] or transplanted tumors in nude mice [30]. Therefore, the present work was designed to evaluate the anticarcinogenic effect of SF against advanced HCC in vivo and estimate possible underlying mechanisms of cell proliferation, angiogenesis, and antioxidant status.

Materials and Methods

Chemicals. Diethylnitrosamine was sourced from Sigma–Aldrich (USA) at a concentration of 0.95 g/mL (Sigma N0258-1G). Carbon tetrachloride was acquired from Research Lab (Egypt), identified by serial #02076 and batch #02076170415. DL-SF, with a purity of ≥90% (HPLC), in liquid form, was procured from Sigma–Aldrich (USA) under the code S4441-5MG, with CAS number 4478-93-7. Kits for assessing serum alanine aminotransferase (ALT; catalog # 264001), aspartate aminotransferase (AST; catalog # 260001), albumin (Alb; E-EL-R0362), and total protein (TP; catalog # MBS9389057) were obtained from SPINREACT, S.A./S.A.U. (Spain). The serum AFP ELISA kit was sourced from CUSABIO (USA). Liver malondialdehyde (MDA; Cat. # MD2529) and total antioxidant capacity (TAC; Cat. # LS-F14457) were analyzed by the manufacturer's protocols using commercially available kits. All the other chemicals utilized were of analytical grade.

Animals. Forty male albino mice, aged six weeks and weighing 19—25 g, were sourced from the Biological Unit of Theodore Bilharz Institute in Giza, Egypt. These mice belonged to the CD1 strain. Before commencing the experiments, a one-week acclimatization period was allowed in the laboratory setting. Throughout the study, the mice had ad libitum access to water and standard meal pellets. The animal experiments adhered strictly to the guidelines outlined in the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1985).

Induction of HCC. The method for inducing HCC followed the protocol described by Uehara et al. [31]. Initially, a single intraperitoneal injection of diethylnitrosamine (DEN) at a dose of 100 mg/kg, freshly prepared and diluted in sterile 0.9% sodium chloride saline, was administered. Subsequently, to potentiate the carcinogenic effects of DEN, rats received biweekly intraperitoneal injections of carbon tetrachloride (CCl_4) for 22 weeks. CCl_4 was administered at a dose of 0.5 mL/kg, diluted in sterile corn oil, following the approach outlined by Dapito et al. [32] and Tolba et al. [33].

Experimental design. The mice were randomly divided into three groups: a negative control group receiving the vehicle ($n = 10$), an HCC control group ($n = 15$), and a group treated with SF (2 mg/kg body weight) ($n = 15$), which commenced treatment from week 25 until week 28 following HCC induction. This dose and schedule were selected based on preliminary dose-finding studies identifying 2 mg/kg as the minimum effective dose, consistent with previous literature demonstrating that SF at this dose exerts anticarcinogenic effects without observable toxicity in murine models [34]. Treatment initiation after HCC establishment was designed to model a therapeutic, rather than purely preventive, intervention.

Sample collection. After the 28-week experiment, the mice were weighed before being euthanized via isoflurane inhalation. Following a 12- to

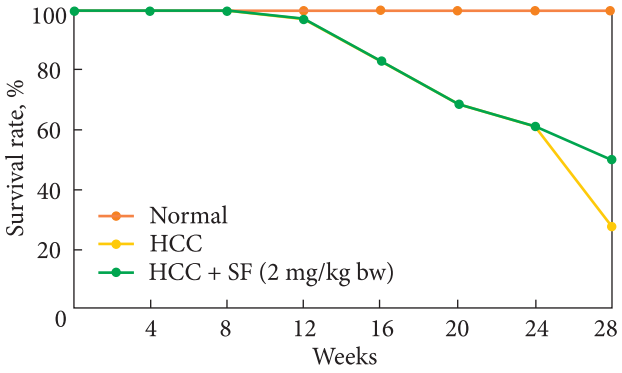

Fig. 1. Effect of SF on survival of mice with HCC (Kaplan–Meier plots)

14-h fasting period, blood samples were collected from the retroorbital vein of each mouse. These samples were allowed to clot at room temperature and then centrifuged at 6000 *g* for 15 min at 4 °C to obtain sera, which were subsequently stored at –20 °C for future biochemical analyses. After blood sampling, the animals were humanely sacrificed, and their livers were promptly excised, washed with saline, and dried with blotting paper. Liver weights were recorded, and any visible lesions were noted. Liver sections were preserved in a 10% neutral buffered formalin solution for histological and immunohistochemical examinations.

Biochemical assays. Serum levels of ALT, AST, total bilirubin, and albumin, as well as MDA and total antioxidant capacity (TAC), were determined following the guidelines provided by the respective kit manufacturers. Quantification of serum alpha-fetoprotein (AFP), a specific tumor marker for HCC, was performed using the enzyme-linked immunosorbent assay (ELISA) technique.

Histopathological estimation. The left median lobe liver samples were fixed in 10% neutral buffered formalin for 24 h. Subsequently, they underwent overnight rinsing under running tap water and were then dried before being embedded in paraffin wax. Transverse sections, 5 μm thick, were prepared and stained using hematoxylin and eosin according to the method described by Gamble [35].

Immunohistochemistry. Hepatic tissue sections were processed conventionally for analysis. The following antibodies were employed: monoclonal mouse p53 antibody (IR616) at a dilution of 1:100 (DO-7, Dako, Denmark), rabbit monoclonal Bcl-2 antibody at a dilution of 1:250 (E17, ab32124, Abcam, UK), rabbit at polyclonal Bax a dilution of 1:250 (Sc-526, Santa Cruz Biotechnology, USA), rabbit monoclonal PCNA antibody (EPR3821) at a dilution of 1:100 (Abcam, UK), and mouse monoclonal VEGF antibody (A17877) at a dilution of 1:150 (AB clonal, China). Immunohistochemical (IHC) analysis was performed following the instructions provided with each kit. Staining

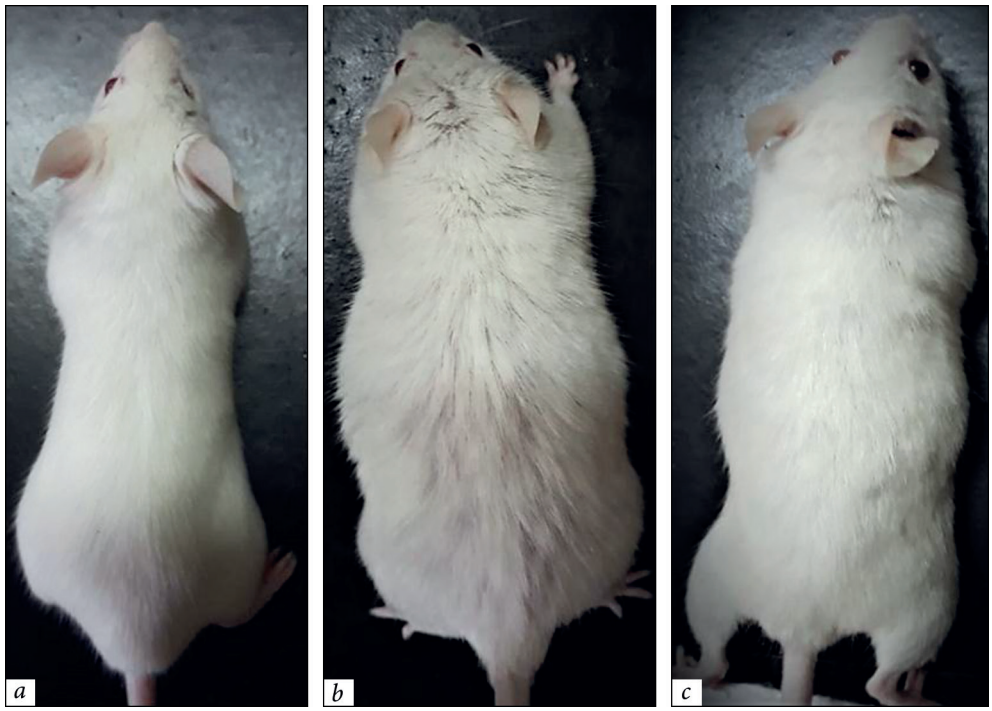

Fig. 2. Effect of SF on general appearance of experimental mice. *a* — normal; *b* — HCC control; *c* — HCC animals treated with SF

intensity was assessed semi-quantitatively using a scale ranging from 0 to 3: 0 indicating no staining, 1 indicating weak staining, 2 indicating moderate staining, and 3 indicating strong staining.

Statistical analysis. The mean values and standard errors of the means were used to represent numerical data. The statistical analyses were conducted using GraphPad Prism software (version 5.0, GraphPad Software, USA). Data were initially subjected to one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons using Tukey's test to compare between groups. A significance level of $p < 0.05$ was considered statistically significant.

Results

Survival rate. No mortality was observed among mice in the negative control group throughout the experiment. In contrast, the HCC control group exhibited a substantial mortality rate, reaching 72.2% by week 28. However, HCC animals treated with SF from week 25 to week 28 demonstrated a lower mortality rate of 50% compared to the HCC control group (Fig. 1). The Kaplan–Meier survival analysis showed that SF treatment significantly improved survival compared to the HCC control group, with 50% of SF-treated mice surviving to week 28 compared to only 27.8% of HCC controls (log-rank test, $p < 0.05$). Mortality was primarily attributed to tumor progression based on clinical signs (ascites, weakness, hair loss, and weight loss), with no toxicity-related deaths observed.

Clinical symptoms. Mice in the negative control group displayed activity and had white, soft fur, maintaining a steady increase in body weight over the 28-week experiment period (Fig. 2, *a*). Conversely, the HCC control mice showed signs of physical inactivity, with light, rough fur that appeared slightly yellowish. Ascites and soft, yellowish stool were also observed in this group (Fig. 2, *b*). Additionally, the weights of mice in the HCC control group significantly decreased compared to those in the negative control group (Fig. 3). In contrast, SF-treated HCC mice exhibited activity, had white, soft fur, and showed slight abdominal edema (Fig. 2, *c*). Interestingly, the weights of mice in this group did not significantly decrease compared to the HCC control group. Fig. 3 illustrates the average body weight changes observed during the 28-week study across the groups.

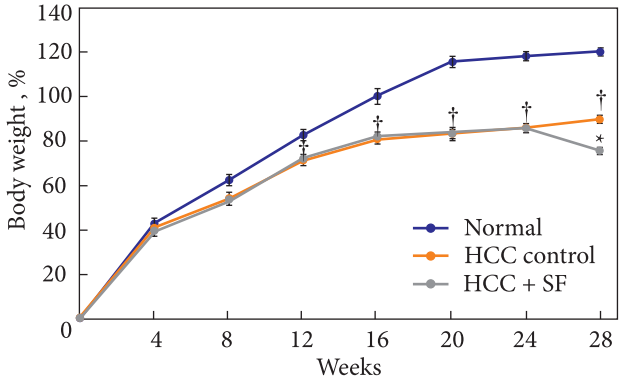

Fig. 3. Effect of SF on body weight change in HCC mice. The differences are significant compared to the normal group: † $p < 0.05$. The differences are significant compared to the HCC control group: * $p < 0.05$

Gross morphology of the liver. As depicted in Fig. 4, in the negative control group, the livers displayed a normal appearance, size, and color, devoid of any tumor signs. Conversely, the livers from the HCC control group exhibited hepatomegaly (as indicated in Table 1), characterized by a light brown color and rough surface. The liver surfaces exhibited the emergence of multiple nodules of varying sizes. However, treatment of HCC mice with SF re-

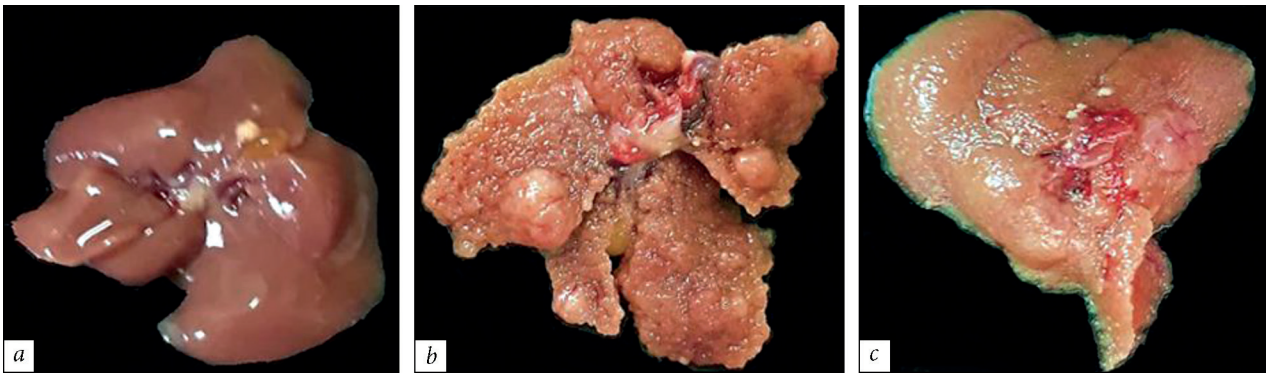

Fig. 4. Effect of SF on liver gross morphology in experimental mice: *a* — negative control group, *b* — HCC control group, *c* — HCC SF-treated group

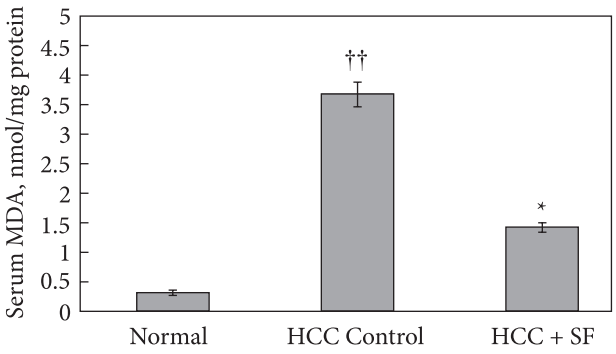

Fig. 5. Effect of SF on MDA in normal and HCC-bearing mice. The differences are significant compared to the normal group: †† $p < 0.001$. The differences are significant compared to the HCC control group: * $p < 0.05$

sulted in a significant reduction in liver relative weight. Furthermore, the liver surface of the HCC SF-treated group displayed a finer granular appearance with few small nodules (Fig. 4).

Biochemical parameters. HCC mice exhibited a notable rise in the serum levels of AST, ALT, total bilirubin, and AFP compared to the negative control group (Table 2). Conversely, albumin levels were significantly reduced in HCC mice compared to the negative control. Treatment of HCC mice with SF resulted in a significant elevation in albumin levels compared to the HCC control group. Furthermore, serum levels of ALT, AST, total bilirubin, and AFP were significantly reduced in the SF-treated HCC group compared to the HCC control group (Table 2).

Total antioxidant capacity and lipid peroxidation. The HCC control group exhibited a notably significant increase in hepatic MDA levels compared to the negative control group, while in the SF-treated group, there was a significant decrease in MDA levels compared to the HCC control group (Fig. 5). Additionally, HCC mice displayed a highly significant decrease in liver TAC concentration compared to the negative control group, whereas treatment with SF significantly increased liver TAC concentration compared to the HCC control group (Fig. 6).

Liver histology. The evaluation of hematoxylin and eosin-stained liver sections from a negative control mouse revealed normal architecture characterized by indistinct polygonal lobules (Fig. 7, *a*). Each lobule exhibited a central vein and radiating hepatic strands separated by sinusoids, containing one- to two-cell-thick hepatocytes. The hepatocytes were polygonal in shape with granular and eosinophilic cytoplasm, often with rounded, binucleated nuclei. The sinusoids were lined with endothelial cells and contained scattered Kupffer cells (Fig. 7, *g*). Conversely, the liver architecture in the HCC control group exhibited significant alterations, including the emergence of nodules and new blood vessels. The hepatic tissue lacked well-defined lobules and cell plates, being invaded by

Table 1. **Effect of SF onrelative liver weight and hepatic nodules in HCC-bearing mice**

Group	Liver relative weight	Nodular count			
		Total nodules	< 2 mm	2—5 mm	> 5 mm
Normal	3.94 ± 0.09	0	0	0	0
HCC control	8.94 ± 0.47††	26.44 ± 6.44††	15.89 ± 4.1††	9.22 ± 2.62††	1.22 ± 0.4††
HCC + SF	5.8 ± 0.24**	9.44 ± 0.51*	7.0 ± 0.4	2.67 ± 0.63*	0.33

Notes: The differences are significant compared to the negative control group: †† $p < 0.001$. The differences are significant compared to the HCC control group: * $p < 0.05$, ** $p < 0.001$.

Table 2. **Effect of SF on serum ALT, AST, albumin, total bilirubin, and AFP in HCC-bearing mice**

Group	ALT (IU/mL)	AST (IU/mL)	Albumin (μg/mL)	Total bilirubin (μmol/mL)	AFP (ng/mL)
Negative control	52.60 ± 6.94	83.8 ± 2.36	152.09 ± 11.61	7.48 ± 0.85	1.9 ± 0.51
HCC control	116.71 ± 9.45†	127.6 ± 13.08†	55.14 ± 7.22††	39.5 ± 2.91††	14.17 ± 1.68†
HCC + SF	75.5 ± 4.6*	80.4 ± 6.46*	119.95 ± 8.7**	12.98 ± 2.01**	6.75 ± 0.77*

Notes: The differences are significant compared to the negative control group: † $p < 0.05$, †† $p < 0.001$. The differences are significant compared to the HCC control group: * $p < 0.05$, ** $p < 0.001$.

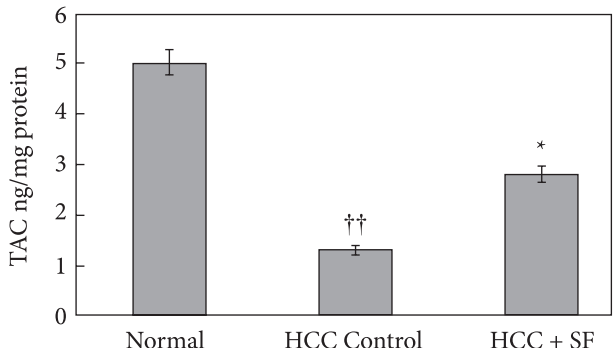

Fig. 6. Effect of SF on TAC in normal and HCC-bearing mice. The differences are significant compared to the normal group: †† $p < 0.001$. The differences are significant compared to the HCC control group: * $p < 0.05$

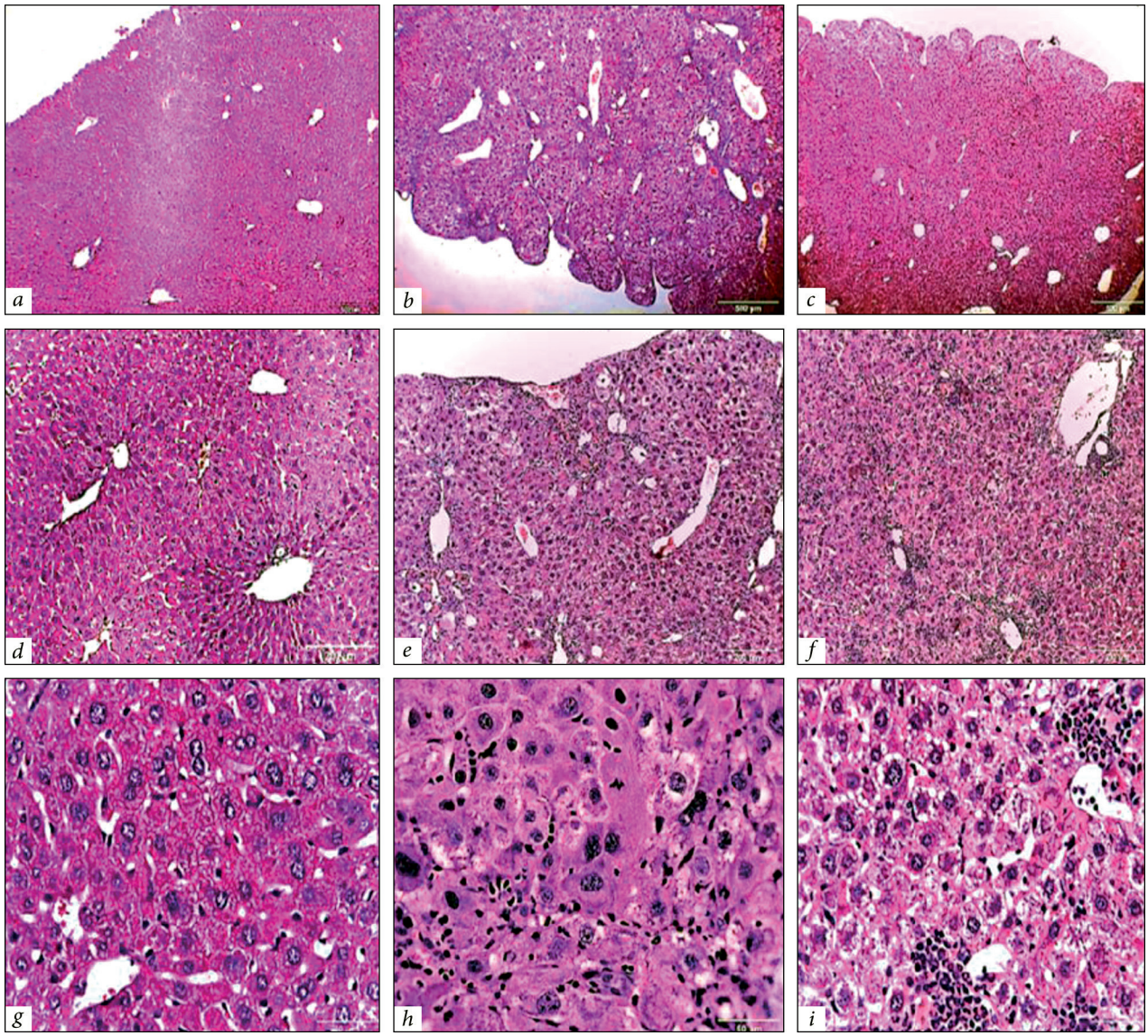

Fig. 7. Effect of SF on liver pathology in HCC-bearing mice: *a*, *d*, *g* — normal mice; *b*, *e*, *h* — HCC control; *c*, *f*, *i* — HCC treated with SF 2 mg/kg

unpaired arteries (Fig. 7, *b*). The hepatic strands appeared disorganized and collapsed, with compressed sinusoids, portal veins, and inflammatory infiltrates (Fig. 7, *e*). Hepatocytes displayed well-differentiated features with eosinophilic cytoplasm, pleomorphic nuclei often exhibiting irregular membranes, dividing nuclei, and prominent nucleoli. Sinusoids appeared obliterated (Fig. 7, *h*). In contrast, the liver in the HCC group treated with SF displayed a partially restored architecture, with peripheral nodules

observed. The hepatic tissue appeared divided into indistinct lobules, with portal veins exhibiting various orientations and compressed central veins (Fig. 7, *c*). Normal lobules with dilated portal veins and intense inflammation were observed, while central veins were challenging to identify (Fig. 7, *f*). Hepatocytes exhibited granulated cytoplasm, slightly compressed sinusoids, inflammatory infiltrate, and occasional apoptotic hepatocytes (Fig. 7, *i*).

Immunohistochemical study. IHC studies were conducted to assess the expression of p53, Bax, Bcl-2, PCNA, and VEGF in hepatic tissues, aiming to evaluate the effects of SF treatment (Fig. 8, Table 3). In the negative control group, p53 expression was barely detectable in the nuclei of hepatocytes, while a mild expression of p53 was observed in hepatocytes of the HCC-SF-treated group, compared to the dense expression in the HCC group (Fig. 8, Table 3). The liver sections obtained from the negative control and HCC mice displayed weak to moderate Bax reactions, respectively, whereas in the HCC-SF-treated group, the expression of Bax was strong (Fig. 8, Table 3). Conversely, Bcl-2 expression was weak in the HCC-SF-treated group and moderate in HCC mice (Fig. 8, Table 3). Strong positive nuclear expression of PCNA was observed in the HCC control group, while the immunoreaction was weak in the negative control group and mild in the HCC SF-treated group (Fig. 8, Table 3). VEGF expression was restricted and weak along the sinusoids and portal vessels in the negative control group. In contrast, VEGF was diffused in strong positive immunoreactivity along with the hepatocytic cytoplasm in the HCC control group. Weak immunoreactivity was observed in the HCC SF-treated group along the sinusoids, portal vessels, and some hepatocytes (Fig. 8, Table 3).

Discussion

The current study investigated the potential anticarcinogenic effects of SF against experimental HCC. The findings revealed that SF enhanced survival rates, significantly reduced nodular counts and AFP levels, improved hepatic biomarkers, and mitigated the histopathological manifestations of HCC. Although the precise mechanisms underlying SF's antitumor effects on HCC remain partially elucidated, previous research suggests that SF exerts inhibitory effects on cancer cell growth and metastasis through various pathways. These pathways include anti-inflammatory, proapoptotic, and cell cycle-arresting actions [36—39]. SF has been shown to induce DNA fragmentation and activate caspase families, indicating that its antiproliferative activity primarily involves apoptosis [40]. Moreover, SF has been demonstrated to suppress cell proliferation and growth by inhibiting transforming growth factor-beta-induced epithelial-mesenchymal transition in HCC [19].

SF is well known for its antioxidant activity [39, 41] and ability to safeguard DNA from degradation caused by highly reactive electrophiles, achieved by enhancing the antioxidant system's activity and inhibiting inflammation [24, 42]. Its antioxidant properties are associated with several pathways, including attenuation of inflammation by reducing mRNA expression of interleukin-6 and interleukin-1 in liver tissue [43—46]. This effect is mediated through the inhibition of nuclear factor kappa B and the upregulation of transcription of Nrf2, a critical factor in maintaining cellular health and protection against toxic chemicals and lifestyle-related factors [36, 42, 47, 48]. SF's impact on the Keap1/Nrf2/ARE pathway contributes to its protective properties in various disease models, including liver diseases and cancer [24, 49—52].

Beyond Nrf2, a variety of transcriptional factors are affected by SF through oxidative stress modulation, including NF-κB, activator protein 1, p53, hypoxia-inducible factor 1-alpha, peroxisome proliferator-activated receptor-γ, and β-catenin/Wnt [53]. As an electrophile, SF can react with protein thiols to form thionoacyl adducts, affecting cysteine residues in the Keap1 protein. This interaction disrupts Nrf2-Keap1 binding and allows nuclear accumulation of Nrf2 [54]. The resulting Nrf2 activation enhances the transcription of antioxidant response elements, explaining the observed reduction in oxidative stress markers in our study. Concurrently, SF inhibits NF-κB activation [36, 42] and reduces VEGF expression [40], contributing to the anti-inflammatory and anti-angiogenic effects observed in our HCC model.

A balanced interpretation of SF's antioxidant effects requires consideration of the dual role of reactive oxygen species (ROS) in cancer biology. SF exhibits a seemingly contradictory dual role: it induces mitochondrial dysfunction and apoptosis in cancer cells while protecting normal cell mitochondria from oxidative damage [55]. This biphasic behavior, mediated partly through the Nrf2-dependent mito-

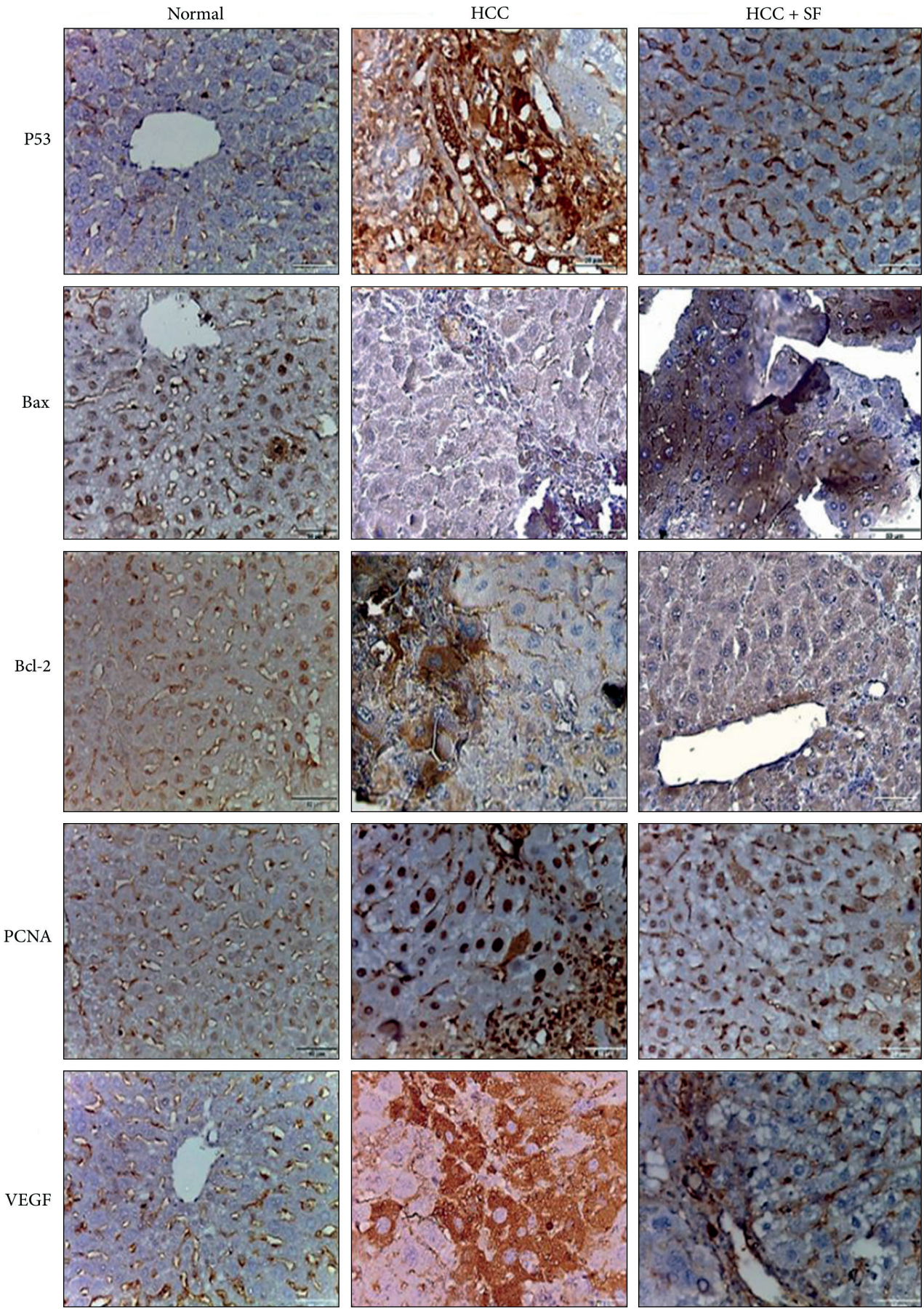

Fig. 8. Immunostaining for p53, Bax, Bcl-2, PCNA, and VEGF in the liver of normal and HCC-bearing mice

chondrial biogenesis and differential ROS thresholds for cell death [55], suggests that the antitumor effects observed in our HCC model cannot be attributed solely to ROS scavenging. Furthermore, SF has been shown to inhibit HCC cell proliferation and epithelial-mesenchymal transition through ROS-dependent pathways [19], indicating that the therapeutic window for antioxidant-based strategies requires careful evaluation in future dose-response studies. Our findings suggest that treatment of HCC-bearing mice with SF restored the reduced TAC activity and prevented lipid peroxidation of hepatocyte cell membranes, indicating SF's antioxidant potential against HCC-related oxidative stress. This aligns with prior studies by Chi et al. [53] and Kikuchi et al. [42], which demonstrate SF's effectiveness as an antioxidant by reducing MDA levels and inducing antioxidant enzymes such as glutathione S-transferase, superoxide dismutase, glutathione, and glutathione peroxidase. The antioxidant effect of SF may prompt apoptosis in HCC animals [10, 56—58], suggesting a potential link between SF's anticarcinogenic effect and apoptosis induction. This notion is supported by increased IHC expression of p53 and Bax, along with a decrease in Bcl-2 observed in SF-treated mice in our study, consistent with findings by Kaboli et al. [59]. Additionally, SF downregulates PCNA expression in HCC mice, similar to the previous reports by Kaboli et al. [59] and Rai et al. [60]. Earlier studies have indicated SF's anti-angiogenic effect on HCC by reducing VEGF levels [40, 59]. Furthermore, Liu et al. [61] demonstrated that SF interferes with endothelial cell proliferation, migration, and tube formation, inhibiting the pro-angiogenic effect of HepG2 cells both in vitro and in vivo. SF affects the interaction between HCC cells and endothelial cells by inhibiting signal transducer and activator of transcription HIF1-alpha/VEGF signaling in cancer cells, thereby suppressing HCC-induced angiogenesis and exerting an anti-tumor effect. Our study also showed a decrease in the IHC expression of VEGF in HCC animals treated with SF, further supporting SF's anti-angiogenic activity.

Table 3. **Effects of SF on the IHC expression of marker proteins in hepatic tissues of experimental animals**

Protein	Experimental groups		
	Negative control	HCC	HCC + SF
p53	0.48 ± 0.09	2.13 ± 0.15†	0.64 ± 0.13*
VEGF	0.26 ± 0.08	2.09 ± 0.18†	0.81 ± 0.16*
PCNA	0.39 ± 0.09	2.81 ± 0.07†	1.64 ± 0.14†
Bax	0.59 ± 0.11	1.61 ± 0.17†	2.48 ± 0.15†
Bcl-2	0.29 ± 0.08	1.90 ± 0.13†	0.87 ± 0.19†

Notes: Data are expressed as mean ± SEM (n = 6/group). 0 — negative; 1 — weak; 2 — moderate; 3 — strong reaction. The mean value was significantly different from that of the negative control group: † $p < 0.05$, or from the HCC control group: * $p < 0.05$.

In our study, SF demonstrated a hepatoprotective effect by reducing elevated serum levels of AST, ALT, and total bilirubin while increasing albumin levels after 30 days of therapy in mice with induced HCC. These results align with prior investigations conducted by Lee et al. [37], Abdelhamid et al. [62], and Guan et al. [39]. SF also improved liver architecture and reduced the number of nodules in HCC mice, aligning with the findings from Abdelhamid et al. [62], who observed an enhanced liver architecture following SF treatment in DEN-induced hepatic damage, attributing it to the suppression of thiobarbituric acid reactive substances and induction of hepatic phase 2 antioxidant enzymes, including glutathione S-transferase, along with the modulation of inflammatory pathways.

Although SF-treated mice exhibited lower body weight compared to the untreated HCC control mice in our experiment, they were more active. This decrease in body weight may be attributed to SF's upregulation of lipid metabolism-related enzymes/proteins, inducing adipocyte lipolysis and inhibiting adipocyte differentiation, as suggested by Du et al. [63]. Ferramosca et al. [64] and Du et al. [63] further demonstrated SF's ability to improve lipid profiles and decrease body weight, liver weight, and lipid profile parameters such as total cholesterol, triglycerides, low-density lipoprotein, and cholesterol in rodents.

One of our study limitations is that the IHC data are semi-quantitative; quantitative methods such as Western blotting or qPCR would provide more precise validation of the observed changes in p53, Bax, Bcl-2, PCNA, and VEGF expressions. Additionally, only a single dose of SF (2 mg/kg) was used. Future studies incorporating multiple dosing regimens are needed to characterize dose-response relationships and identify an optimal therapeutic dose of SF for HCC.

In summary, SF exhibited an anticarcinogenic effect in the HCC model, characterized by tumor re-

gression, increased survival rate, suppression of AFP, improvement of liver biomarkers, and downstaging of HCC pathology. SF's anticarcinogenic effect was mediated by restoring antioxidant capacity, activating p53 expression, increasing Bax levels, and suppressing PCNA, VEGF, Bcl-2, and oxidative stress. SF, as a natural product with antioxidant and angiogenic properties, holds promise as a treatment candidate for HCC. Clinical translation of these findings requires consideration of SF's limited oral bioavailability and appropriate human equivalent dosing. However, further studies are warranted to fully elucidate the mechanisms underlying the anticarcinogenic and liver-regenerating effects of SF at the molecular level.

Credit authorship contribution statement

Kholoud A. Omarn and Yomna I. Mahmoud conceived and designed the study. Kholoud A. Omarn did the laboratory work and wrote the manuscript. Yomna I. Mahmoud reviewed the manuscript. Asmaa A. Mahmoud analyzed the data and reviewed the manuscript. Nagui H. Fares is the main supervisor and reviewed the manuscript. This study was performed as part of Kholoud A. Omarn's dissertation for the degree of PhD in histology. All authors read and approved the final manuscript.

Ethical approval

Animal handling procedures were conducted following the ethical guidelines set by the Ethical Committee of Ain Shams University (REC), under approval number ASU-SCI/ZOOL/2024/5/1. These procedures were also compliant with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The research was carried out at the Faculty of Science, Ain Shams University, Abbassia, Cairo, Egypt.

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Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request. All data analyzed during this study are included in this article.

Declaration of competing interests

The authors declare no conflicts of interest.

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REFERENCES

1. Sun LY, Zhang KJ, Xie YM, et al. Immunotherapies for advanced hepatocellular carcinoma. *Front Pharmacol.* 2023;14:1138493. https://doi.org/10.3389/fphar.2023.1138493
2. Kinsey E, Lee HM. Management of hepatocellular carcinoma in 2024: The multidisciplinary paradigm in an evolving treatment landscape. *Cancers (Basel).* 2024;16(3):666. https://doi.org/10.3390/cancers16030666
3. Calderon-Martinez E, Landazuri-Navas S, Vilchez E, et al. Prognostic scores and survival rates by etiology of hepatocellular carcinoma: A review. *J Clin Med Res*. 2023;15(4):200-207. https://doi.org/10.14740/jocmr4902
4. Allaire M, Bruix J, Korenjak M, et al. What to do about hepatocellular carcinoma: Recommendations for health authorities from the International Liver Cancer Association. *JHEP Rep*. 2022;4(12):100578. https://doi.org/10.1016/j.jhepr.2022.100578
5. Anwanwan D, Singh SK, Singh S, et al. Challenges in liver cancer and possible treatment approaches. *Biochim Biophys Acta Rev Cancer*. 2020;1873(1):188314. https://doi.org/10.1016/j.bbcan.2019.188314
6. Jung KH, Rumman M, Yan H, et al. An ethyl acetate fraction of Artemisia capillaris (ACE-63) induced apoptosis and anti-angiogenesis via inhibition of PI3K/AKT signaling in hepatocellular carcinoma. *Phytother Res*. 2018;32(10):2034-2046. https://doi.org/10.1002/ptr.6135
7. El-Serag HB, Marrero JA, Rudolph L, et al. Diagnosis and treatment of hepatocellular carcinoma. *Gastroenterology*. 2008;134(6):1752-1763. https://doi.org/10.1053/j.gastro.2008.02.090
8. Ren F, Li J, Yuan X, et al. Dandelion polysaccharides exert anticancer effect on hepatocellular carcinoma by inhibiting PI3K/AKT/mTOR pathway and enhancing immune response. *J Funct Foods*. 2019;55:263-274. https://doi.org/10.1016/j.jff.2019.02.034

9. Kefayat A, Ghahremani F, Safavi A, et al. Spirulina extract enriched for Braun-type lipoprotein (Immulina®) for inhibition of 4T1 breast tumors' growth and metastasis. *Phytother Res*. 2020;34(2):368-78. https://doi.org/10.1002/ptr.6527
10. Omran KA, Mahmoud YI, Mahmoud AA, et al. Possible ameliorating effect of isoquercetin on diethylnitrosamine-induced hepatocellular carcinoma in albino mice. *Egyptian J Basic Appl Sci*. 2022;9(1):264-279. https://doi.org/10.1080/2314808X.2022.2079843
11. Russo M, Spagnuolo C, Russo GL, et al. Nrf2 targeting sulforaphane: A potential therapy for cancer treatment. *Crit Rev Food Sci Nutr*. 2018;58(8):1391-1405. https://doi.org/10.1080/10408398.2016.1259983
12. Lee YR, Chen M, Lee JD, et al. Reactivation of PTEN tumor suppressor for cancer treatment through inhibition of a MYC-WWP1 inhibitory pathway. *Science*. 2019;364(6441):eaau0159. https://doi.org/10.1126/science.aau0159
13. He L, Jiang H, Li Y, et al. Sulforaphane-enriched extracts from broccoli exhibit antimicrobial activity against plant pathogens, promising a natural antimicrobial agent for crop protection. *Biomolecules*. 2024;14(3):352. https://doi.org/10.3390/biom14030352
14. Clarke JD, Hsu A, Yu Z, Dashwood RH, Ho E. Differential effects of sulforaphane on histone deacetylases, cell cycle arrest and apoptosis in normal prostate cells versus hyperplastic and cancerous prostate cells. *Mol Nutr Food Res*. 2011;55(7):999-1009. https://doi.org/10.1002/mnfr.201000547
15. Peng X, Zhou YA, Tian HU, et al. Sulforaphane inhibits invasion by phosphorylating ERK1/2 to regulate E-cadherin and CD44v6 in human prostate cancer DU145 cells. *Oncol Rep.* 2015;34(3):1565-1572. https://doi.org/10.3892/or.2015.4098
16. Cheng YM, Tsai CC, Hsu YC. Sulforaphane, a dietary isothiocyanate, induces G2/M arrest in cervical cancer cells through cyclin B1 downregulation and GADD45β/CDC2 association. *Int J Mol Sci*. 2016;17(9):1530. https://doi.org/10.3390/ijms17091530
17. Wang DX, Zou YJ, Zhuang XB, et al. Sulforaphane suppresses EMT and metastasis in human lung cancer through miR-616-5p-mediated GSK3β/β-catenin signaling pathways. *Acta Pharmacol Sin*. 2017;38(2):241-251. https://doi.org/10.1038/aps.2016.122
18. Su X, Jiang X, Meng L, et al. Anticancer activity of sulforaphane: The epigenetic mechanisms and the Nrf2 signaling pathway. *Oxid Med Cell Longev*. 2018;2018:5438179. https://doi.org/10.1155/2018/5438179
19. Wu J, Han J, Hou B, et al. Sulforaphane inhibits TGF-β-induced epithelial-mesenchymal transition of hepatocellular carcinoma cells via the reactive oxygen species-dependent pathway. *Oncol Rep*. 2016;35(5):2977-2983. https://doi.org/10.3892/or.2016.4638
20. Bessler H, Djaldetti M. Broccoli and human health: Immunomodulatory effect of sulforaphane in a model of colon cancer. *Int J Food Sci Nutr*. 2018;69(8):946-953. https://doi.org/10.1080/09637486.2018.1439901
21. Shao D, Gao Z, Zhao Y, et al. Sulforaphane suppresses H_2O_2-induced oxidative stress and apoptosis via the activation of AMPK/NFE2L2 signaling pathway in goat mammary epithelial cells. *Int J Mol Sci*. 2023;24(2):1070. https://doi.org/10.3390/ijms24021070
22. Zhang Y, Tang L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. *Acta Pharmacol Sin.* 2007;28(9):1343-1354. https://doi.org/10.1111/j.1745-7254.2007.00679.x
23. Oshi H, Rani I, Sharma V, et al. Sulforaphane: A natural organosulfur having potential to modulate apoptosis and survival signalling in cancer. *Discov Oncol*. 2025;16(1):2049. https://doi.org/10.1007/s12672-025-02795-7
24. Guerrero-Beltrán CE, Calderón-Oliver M, Pedraza-Chaverri J, et al. Protective effect of sulforaphane against oxidative stress: Recent advances. *Exp Toxicol Pathol*. 2012;64(5):503-508. https://doi.org/10.1016/j.etp.2010.11.005
25. Jaman MS, Sayeed MA. Ellagic acid, sulforaphane, and ursolic acid in the prevention and therapy of breast cancer: Current evidence and future perspectives. *Breast Cancer*. 2018;25(5):517-528. https://doi.org/10.1007/s12282-018-0866-4
26. Ghazizadeh-Hashemi F, Bagheri S, Ashraf-Ganjouei A, et al. Efficacy and safety of sulforaphane for treatment of mild to moderate depression in patients with history of cardiac interventions: A randomized, double-blind, placebo-controlled clinical trial. *Psychiatry Clin Neurosci*. 2021;75(8):250-255. https://doi.org/10.1111/pcn.13276
27. Liu P, Wang W, Tang J, et al. Antioxidant effects of sulforaphane in human HepG2 cells and immortalised hepatocytes. *Food Chem Toxicol*. 2019;128:129-136. https://doi.org/10.1016/j.fct.2019.03.050
28. Dos Santos PW, Machado AR, De Grandis RA, et al. Transcriptome and DNA methylation changes modulated by sulforaphane induce cell cycle arrest, apoptosis, DNA damage, and suppression of proliferation in human liver cancer cells. *Food Chem Toxicol*. 2020;136:111047. https://doi.org/10.1016/j.fct.2019.111047
29. Chen YJ, Myracle AD, Wallig MA, et al. Dietary broccoli protects against fatty liver development but not against progression of liver cancer in mice pretreated with diethylnitrosamine. *J Funct Foods*. 2016;24:57-62. https://doi.org/10.1016/j.jff.2016.03.028
30. Sato S, Moriya K, Furukawa M, et al. Sulforaphane inhibits liver cancer cell growth and angiogenesis. *Arch Cancer Res*. 2018;6(4):23. http://hdl.handle.net/10564/3555
31. Uehara T, Ainslie GR, Kutanzi K, et al. Molecular mechanisms of fibrosis-associated promotion of liver carcinogenesis. *Toxicol Sci*. 2013;132(1):53-63. https://doi.org/10.1093/toxsci/kfs342
32. Dapito DH, Mencin A, Gwak GY, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell.* 2012;21(4):504-516. https://doi.org/10.1016/j.ccr.2012.02.007

33. Tolba R, Kraus T, Liedtke C, et al. Diethylnitrosamine (DEN)-induced carcinogenic liver injury in mice. *Lab Anim.* 2015;49(1 Suppl):59-69. https://doi.org/10.1177/0023677215570086
34. Saleh DO, Mansour DF, Hashad IM, et al. Effects of sulforaphane on D-galactose-induced liver aging in rats: Role of keap-1/nrf-2 pathway. *Eur J Pharmacol.* 2019;855:40-49. https://doi.org/10.1016/j.ejphar.2019.04.043
35. Gamble M. The hematoxylins and eosin. In: Bancroft JD, Gamble M, editors. *Theory and Practice of Histological Techniques*. 6th ed. Philadelphia: Churchill Livingstone/Elsevier; 2008. p. 121-34. https://doi.org/10.1016/B978-0-443-10279-0.50016-6
36. Abdel-Hamid NM, Abass SA, Mohamed AA, et al. Herbal management of hepatocellular carcinoma through cutting the pathways of the common risk factors. *Biomed Pharmacother*. 2018;107:1246-1258. https://doi.org/10.1016/j.biopha.2018.08.104
37. Lee C, Yang S, Lee BS, et al. Hepatic protective effects of sulforaphane through the modulation of inflammatory pathways. *J Asian Nat Prod Res*. 2020;22(4):386-396. https://doi.org/10.1080/10286020.2019.1581174
38. Ruhee RT, Ma S, Suzuki K. Protective effects of sulforaphane on exercise-induced organ damage via inducing antioxidant defense responses. *Antioxidants.* 2020;9(2):136. https://doi.org/10.3390/antiox9020136
39. Guan Z, Zhou L, Zhang Y, et al. Sulforaphane ameliorates the liver injury of traumatic hemorrhagic shock rats. *J Surg Res*. 2021;267:293-301. https://doi.org/10.1016/j.jss.2021.05.004
40. Jeon YK, Yoo DR, Jang YH, et al. Sulforaphane induces apoptosis in human hepatic cancer cells through inhibition of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4, mediated by hypoxia inducible factor-1-dependent pathway. *Biochim Biophys Acta Proteins Proteom*. 2011;1814(10):1340-1348. https://doi.org/10.1016/j.bbapap.2011.05.015
41. Holanda G, Castelo-Branco M, Valença S, et al. Albumin and sulforaphane in modulation of oxidative stress in intestinal ischemia and reperfusion: An experimental study. *J Am Coll Surg*. 2020;231(4):e16. https://doi.org/10.1016/j.jamcollsurg.2020.08.039
42. Kikuchi M, Ushida Y, Shiozawa H, et al. Sulforaphane-rich broccoli sprout extract improves hepatic abnormalities in male subjects. *World J Gastroenterol*. 2015;21(43):12457-12467. https://doi.org/10.3748/wjg.v21.i43.12457
43. Choudhury D, Das A, Bhattacharya A, et al. Aqueous extract of ginger shows antiproliferative activity through disruption of microtubule network of cancer cells. *Food Chem Toxicol.* 2010;48(10):2872-2880. https://doi.org/10.1016/j.fct.2010.07.020
44. Baenas N, Silván JM, Medina S, et al. Metabolism and antiproliferative effects of sulforaphane and broccoli sprouts in human intestinal (Caco-2) and hepatic (HepG2) cells. *Phytochem Rev*. 2015;14(6):1035-1044. https://doi.org/10.1007/s11101-015-9422-4
45. Wang CZ, Qi LW, Yuan CS. Cancer chemoprevention effects of ginger and its active constituents: Potential for new drug discovery. *Am J Chin Med*. 2015;43(7):1351-1363. https://doi.org/10.1142/S0192415X15500767
46. Khazaei S, Esa NM, Ramachandran V, et al. In vitro antiproliferative and apoptosis inducing effect of Allium atroviolaceum bulb extract on breast, cervical, and liver cancer cells. *Front Pharmacol.* 2017;8:5. https://doi.org/10.3389/fphar.2017.00005
47. Lu Y, Zhang Y, Lou Y, et al. Sulforaphane suppresses obesity-related glomerulopathy-induced damage by enhancing autophagy via Nrf2. *Life Sci*. 2020;258:118153. https://doi.org/10.1016/j.lfs.2020.118153
48. Mandlik DS, Mandlik SK. Herbal and natural dietary products: Upcoming therapeutic approach for prevention and treatment of hepatocellular carcinoma. *Nutr Cancer*. 2021;73(11-12):2130-2154. https://doi.org/10.1080/01635581.2020.1834591
49. Piao CS, Gao S, Lee GH, et al. Sulforaphane protects ischemic injury of hearts through antioxidant pathway and mitochondrial KATP channels. *Pharmacol Res*. 2010;61(4):342-348. https://doi.org/10.1016/j.phrs.2009.11.009
50. Shan Y, Wang X, Wang W, et al. p38 MAPK plays a distinct role in sulforaphane-induced up-regulation of ARE-dependent enzymes and down-regulation of COX-2 in human bladder cancer cells. *Oncol Rep*. 2010;23(4):1133-1138. https://doi.org/10.3892/or_00000742
51. Cui W, Bai Y, Miao X, et al. Prevention of diabetic nephropathy by sulforaphane: Possible role of Nrf2 upregulation and activation. *Oxid Med Cell Longev*. 2012;2012:821936. https://doi.org/10.1155/2012/821936
52. Kim HV, Kim HY, Ehrlich HY, et al. Amelioration of Alzheimer's disease by neuroprotective effect of sulforaphane in animal model. *Amyloid.* 2013;20(1):7-12. https://doi.org/10.3109/13506129.2012.751367
53. Chi X, Zhang R, Shen N, et al. Sulforaphane reduces apoptosis and oncosis along with protecting liver injury-induced ischemic reperfusion by activating the Nrf2/ARE pathway. *Hepatol Int*. 2015;9(2):321-329. https://doi.org/10.1007/s12072-014-9604-y
54. Keum YS. Regulation of the Keap1/Nrf2 system by chemopreventive sulforaphane: Implications for posttranslational modifications. *Ann N Y Acad Sci*. 2011;1229:184-189. https://doi.org/10.1111/j.1749-6632.2011.06092.x
55. Negrette-Guzmán M, Huerta-Yepez S, Tapia E, et al. Modulation of mitochondrial functions by the indirect antioxidant sulforaphane: A seemingly contradictory dual role and an integrative hypothesis. *Free Radic Biol Med*. 2013;65:1078-1089. https://doi.org/10.1016/j.freeradbiomed.2013.08.182
56. Anand T, Muthia A, Mohan SC. Ethanolic extract of *Canthium coromandelicum* leaves exhibits antioxidant, anti-inflammatory and apoptotic activity in DEN-induced hepatocellular carcinoma. *Res J Phytochem*. 2017;11(1):1-10. https://doi.org/10.3923/rjphyto.2017.1.10

57. Abolmaesoomi M, Aziz AA, Junit SM, et al. Ficus deltoidea: Effects of solvent polarity on antioxidant and antiproliferative activities in breast and colon cancer cells. *Eur J Integr Med*. 2019;28:57-67. https://doi.org/10.1016/j.eujim.2019.05.002
58. El-Far YM, Khodir AE, Noor AO, et al. Selective cytotoxic activity and protective effects of sodium ascorbate against hepatocellular carcinoma through its effect on oxidative stress and apoptosis in vivo and in vitro. *Redox Rep*. 2020;25(1):17-25. https://doi.org/10.1080/13510002.2020.1739870
59. Kaboli PJ, Khoshkbejari MA, Mohammadi M, et al. Targets and mechanisms of sulforaphane derivatives obtained from cruciferous plants with special focus on breast cancer – contradictory effects and future perspectives. *Biomed Pharmacother*. 2020;121:109635. https://doi.org/10.1016/j.biopha.2019.109635
60. Rai R, Gong Essel K, Mangiaracina Benbrook D, et al. Preclinical efficacy and involvement of AKT, mTOR, and ERK kinases in the mechanism of sulforaphane against endometrial cancer. *Cancers (Basel)*. 2020;12(5):1273. https://doi.org/10.3390/cancers12051273
61. Liu P, Atkinson SJ, Akbareian SE, et al. Sulforaphane exerts anti-angiogenesis effects against hepatocellular carcinoma through inhibition of STAT3/HIF-1α/VEGF signalling. *Sci Rep*. 2017;7:12651. https://doi.org/10.1038/s41598-017-12855-w
62. Abdelhamid MS, Sherif MH, Ali EMM, et al. The extracted sulforaphane from cabbage ameliorates liver functions in rats with diethyl nitrosamine induced hepatotoxicity. *Biochem Lett*. 2021;17(1):30-39. https://doi.org/10.21608/blj.2021.180497
63. Du K, Fan Y, Li D. Sulforaphane ameliorates lipid profile in rodents: An updated systematic review and meta-analysis. *Sci Rep*. 2021;11:7804. https://doi.org/10.1038/s41598-021-87367-9
64. Ferramosca A, Di Giacomo M, Zara V. Antioxidant dietary approach in treatment of fatty liver: New insights and updates. *World J Gastroenterol*. 2017;23(23):4146-4157. https://doi.org/10.3748/wjg.v23.i23.4146

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СУЛЬФОРАФАН ПРИГНІЧУЄ ГЕПАТОЦЕЛЮЛЯРНУ КАРЦИНОМУ У МИШЕЙ ШЛЯХОМ АКТИВАЦІЇ P53, ЗМЕНШЕННЯ ОКСИДАТИВНОГО СТРЕСУ ТА ІНГІБУВАННЯ АНГІОГЕНЕЗУ

Стан питання. Гепатоцелюлярна карцинома (ГЦК) є найпоширенішим первинним злоякісним новоутворенням печінки. Сульфорафан (SF) — це природний ізотіоціанат, який проявляє активність проти різних типів злоякісних клітин in vivo та in vitro без особливих побічних ефектів. Він також має профілактичний ефект стосовно ранніх стадій розвитку пухлин на деяких моделях раку, включаючи ГЦК. **Метою** дослідження було оцінити терапевтичний протираковий ефект SF на експериментальній моделі індукованої ГЦК у мишей in vivo та дослідити можливі основні механізми його впливу на клітинну проліферацію, ангіогенез та антиоксидантний статус. **Матеріали та методи.** ГЦК індукували в мишей одноразовою внутрішньочеревною дозою 100 мг/кг діетилнітрозаміну, з наступними 22 щотижневими внутрішньочеревними дозами 0,5 мг/кг чотирихлористого вуглецю. Після індукції ГЦК SF (2 мг/кг маси тіла) застосовували перорально з 25-го по 28-й тиждень. **Результати.** SF дещо нормалізував структуру та функцію печінки в мишей з індукованою ГЦК, покращивши виживаність, значно зменшивши експресію пухлинного маркера альфа-фетопротеїну та кількість пухлинних фокусів у печінці, при цьому ГЦК набувала ознак, що відповідали меншому ступеню прогресії. Ці зміни супроводжувалися збільшенням загальної антиоксидантної здатності, імуноекспресії p53 та Bax, а також значним зниженням перекисного окислення ліпідів та імунореактивності PCNA, VEGF та Bcl-2 у тканинах печінки. **Висновок.** SF зменшив кількість пухлинних фокусів у печінці, спричинивши морфологічні зміни, що відповідали меншому ступеню прогресії ГЦК, активуючи білок-супресор пухлинного росту p53, індукуючи апоптоз та пригнічуючи проліферацію, оксидативний стрес та ангіогенез. Все це свідчить про необхідність подальшої трансляційної оцінки SF як потенційного терапевтичного засобу для ГЦК.

Ключові слова: гепатоцелюлярна карцинома, сульфорафан, Bax, Bcl-2, VEGF, PCNA, p53, оксидативний стрес, миші.