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The Anticancer Impact of Anthraquinones and Sorafenib on Rat Liver Cancer

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ABSTRACT

Background: Hepatocellular carcinoma (HCC) is the primary malignant tumor of the liver and is a deadly and complex cancer mostly associated with inflammation and oxidative stress in hepatic tissue. An essential first-line treatment for advanced HCC is sorafenib which can significantly improve overall survival. However, due to acquired resistance against sorafenib and the heterogeneity of HCC, there is still opportunity for HCC progress. As a result, sorafenib and other medication combinations may work in concert, offering a novel approach to treating HCC.

Objectives: This study was aimed to investigate the impact of the combinations of sorafenib and free anthraquinones fraction, containing aloe-emodin, emodin, chrysophanol and physcion, isolated from Rhubarb officinale rhizome on the chemically induced HCC in rats.

Material and methods: sixty male albino rats (*Rattus Albinus*) of average body weight 130±20 g were used in this study. they were divided into 5 groups (n=12/group): normal control group, diethylnitrosamine (DEN)+ carbon tetrachloride CCL4 induced HCC (HCC group); and 3 treated groups with anthraquinones (Anth group), sorafenib (Sor group), and both (Anth+Sor).

Results: The HCC group of rats exhibited the most deteriorated effects, including elevated serum alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), declined antioxidant activity of glutathione peroxidase (GPX); upregulated expression of the genes related anti-apoptosis (survivin), autophagy (beclin 1, Bec1), angiogenesis (vascular endothelial growth factor, VEGF); and downregulated expression of genes related to apoptosis (p21 and p53). After Anth and Sor were administered, all the negative effects caused by HCC were reversed, with the combined group (Anth+Sor) which showed the greatest improvement.

Conclusion: It could be concluded that administration of anthraquinone could improve the therapeutic potential of sorafenib on HCC.

Keywords: sorafenib, Anthraquinones, Hepatocellular carcinoma, Rhubarb officinale rhizome

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most prevalent primary form of liver cancer and, after stomach and lung cancers, the third most lethal form of cancer worldwide. Hepatocarcinogenesis is mainly dependent on angiogenesis from the beginning. Vascular endothelial growth factor (VEGF) is a crucial proangiogenic factor that is generated in dysplastic nodules and rises as HCC develops [(1)]. Providing nutrients and oxygen to neoplastic cells requires forming a new vascular network once the tumor is established.

According to Liu Y *et al.* [(2)], VEGF binds to its receptors, VEGFR1 and VEGFR2, and then transduces its actions by activating many signaling pathways that are engaged in the migration, proliferation, and invasion of electric cells. Stresses potentially compromise cell division and DNA replication fidelity and trigger the p53 pathway's reaction. Through post-translational changes, the p53 protein receives a stress signal.

Consequently, a program of cell cycle arrest, cellular senescence, or apoptosis is started by activating the p53 protein, a transcription factor [(3)]. Many different cancer types express survivin, an inhibitor of the apoptosis protein. It seems that survivin is also a caspase inhibitor13 since it can be co-immunoprecipitated with caspases-3, -7, and -9 and inhibits apoptosis caused by overexpressing these caspases. Moreover, it prevents cell death by interfering with the processing of caspase-9, the primary inhibitor of the intrinsic route of apoptosis [(4)].

Two strictly regulated biological processes in cells, autophagy, and apoptosis, are essential for preserving tissue homeostasis and growth. One possible target for investigating the relationship between autophagy and apoptosis is the link between the autophagy protein Beclin 1 and the anti-apoptotic protein Bcl-2. Pro- and anti-apoptotic Bcl-2 family proteins are distinguished by the presence of the Bcl-2 homology (BH) domain. Bcl-2 attaches to pro-apoptotic proteins exclusive to BH3 and prevents them from becoming pro-apoptotic. A BH3 domain has also been discovered in Beclin 1. The BH3 domain of Beclin 1 cannot interact with Bcl-xl if it is removed or if Bcl-xl's BH3 receptor domain is altered[(4).]

One of the most important active ingredients in rhubarb is anthraquinones. Nearly all species of Rhubarb contain high concentrations of free anthraquinones, emodin, chrysophanol, particularly rhein, aloe-emodin, and physcion, which have

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significant roles in hepatoprotection in addition to having potent anti-tumor, anti-inflammatory, and cardiovascular protective properties [5].

Emodin targets mitochondria, which may cause apoptosis. A family of cysteine proteases known as caspases must get active for apoptosis to happen. This will cause many substrates to be cleaved, leading to cellular death and disintegration. Although cytochrome c released from the mitochondria during mitochondrial-mediated apoptosis activates caspases, other intracellular signaling pathways govern this process. The equilibrium between anti - and pro-apoptotic proteins in the mitochondrial membrane controls mitochondrial-mediated apoptosis. Interestingly, emodin (at 50 and 100 µM) was discovered to localize GI cancer cells' mitochondria. The process of mitochondrial-mediated cell death involves the suppression of Bcl-2. This anti-apoptotic protein allows pro-apoptotic proteins, including PARP, Bak, and Bax, to disrupt the mitochondrial membrane potential (MMP). Apoptosis is caused by the activation of caspase -9 and -3 by the release of cytochrome c, which is triggered by the loss of MMP. Therefore, it has been shown that emodin and its derivatives decrease Bcl-2, interfere with MMP, and enhance Bax, PARP, and cytochrome c release, as well as cleave caspase -9 and -3, causing colon, pancreatic, and liver cancer cells to undergo apoptosis [6].

Sorafenib inhibits multiple tyrosine kinases, such as VEGFR3 and VEGFR2. Also inhibited are the platelet-derived growth factor receptor beta (PDGFR-ß), the c-KIT protein, the FLT3 kinase, and the Raf kinase. They are important components in the cascade of the Ras/Raf/mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK)/ERK signaling pathways. SOR also inhibits the phosphoinositide-3 kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR), which regulates basic cellular functions. HCC cells are strongly pro-apoptotic and anti-proliferative when treated with SOR. A strong endoplasmic reticulum (ER) stress is likely to cause sequential autophagy and apoptosis pathways to be activated by tumor-derived xenograft mouse models and hepatoblastoma (HB) and HCC cell lines [7].

The purpose of this research was to examine the effects of sorafenib in conjunction with the free anthraquinones fraction extracted from the rhizome of Rhus officinale, which includes the compounds aloe-emodin, emodin, chrysophanol, and physcion, on rats with chemically induced HCC.

MATERIALS AND METHODS

Chemicals

Carbon tetrachloride (CCl4) and diethylnitrosamine (N-nitrosodiethylamine; DEN; purity, 99.0%) were acquired from Sigma-Aldrich Company, St. Louis, USA. Sorafenib (SOR) (Nexavar) was acquired from Bayer AG Pharmaceutical Chemicals Co., Germany,

Isolation of total anthraquinones from Rhubarb:

To increase the amount of free aglycone, 2 Kg of the powder was subjected to acid hydrolysis by heating with 4L of 10% HCl, under reflux for 2 hours. The powder was filtered and allowed to dry before being subjected to exhaustive (i.e., stopped to respond to Borntrager's test) extraction with methylene chloride, and methylene chloride was evaporated under vacuum. The residue was dissolved in 1 litre10% Na_2CO_3 thrice and concentrated under vacuum. Then, the PH was adjusted to 6 by stepwise adding diluted hydrochloric acid to give a heavy yellowish brown precipitate, which was collected and dried under vacuum to give 20 g of yellowish brown powder [8].

Thin layer chromatography (TLC) analysis of the methylene chloride fraction using light petroleum-ethyl acetate-formic acid (75:25: 1) as mobile phase showed 4 major spots at Rf (0.3-0.7), indicating the presence of the 4 major anthraquinone aglycones.

Animals and experimental design

Sixty male albino Wistar rats weighing between 170 and 200 grams were used in this study. They were obtained from the animal house of the Egyptian Organization for Biological Products and Vaccines (Cairo, Helwan, Egypt). Rats were housed in conventional settings with a typical light/dark cycle and a 25 ± 3 °C temperature. They were also given unrestricted access to food and water. Before the experiment had begun, the animals were given a week to adapt to their new environment. The Animal Care and Use Committee of the Science Department, Al-Azhar University, Cairo, Egypt, accepted the ethical procedures and regulations that were followed throughout the conduct of this research. The Al-Azhar University Ethics Board gave its approval to the research.

The rats were divided randomly into five groups (n=12). **Group I (normal control):** Saline was administered orally to the control group animals until the trial's completion (no treatment). **Group II (HCC group):** A single intraperitoneal (IP) dosage of 200 mg/kg of DEN dissolved in 0.9% normal saline at a final 1 ml/kg volume was given to rats with HCC. IP treatment of CCl4 solution (CCl4/olive oil; 1:1; 1 ml/kg) was started two weeks after the DEN challenge and continued for six weeks [9].

After the detection of HCC development, treatment with sorafenib and anthraquinones started. The dose of herbal fraction (200mg/kg dissolved in 20 ml of 0.5% CMC)[10] and sorafenib (30mg/kg) [11]. These treatments

were orally administrated by stomach tube for thirty days for the following 3 groups. **Group III (**HCC+SOR **treated group):** HCC rats treated by SOR. **Group IV (**HCC+ANTH **treated group):** HCC rats treated by ANTH. **Group V (**HCC+SOR+ANTH **treated group):** HCC rats treated by SOR and ANTH

A cervical dislocation was used to kill the rats under light ether anesthesia, and their livers were weighed (absolute liver weight). The liver was washed in saline for RNA extraction and quickly frozen in liquid nitrogen.

Evaluation of Serum Biochemical Parameters.
At the time of sacrifice, blood samples were collected in serum tubes (vacutainers) and centrifuged for 5 minutes at 3000g to obtain serum. As part of the study, serum levels of liver injury biomarker Alkaline Phosphatase (ALP) and using commercially available assays, γ-glutamyl transferase (GGT) for liver cancer has been determined.

Evaluation of Antioxidant Biomarkers:
Homogenization of liver tissue was conducted in cold PBS, followed by centrifugation for 15 minutes at 5000 g at 4°C. In accordance with the previous descriptions, commercial kits (Biodiagnostics Co., Cairo, Egypt, and Randox Laboratories Ltd., Crumlin, UK) and an automated system were used and measured the concentration of the antioxidant enzyme GPX in the supernatants [12].

Molecular Analysis by Real-Time PCR.
The relative expression of candidate genes was analyzed using real-time PCR. Total RNA was isolated from liver tissue using a RNeasy Mini kit from Qiagen. Nanodrop analyses and 1% agarose gel electrophoresis were used to determine the integrity and purity of RNA. Secondly, QuantiTect Reverse Transcription Kit was used to convert 4 mg of the RNA into cDNA. This method also produces cDNA, which is used for real-time PCR reactions. The qPCR master mix QuantiTect SYBR Green was used, along with gene-specific primers that were designed online using Primer 3 using the published rat sequence (Table 1). Our reaction cycles and StepOnePlus real-time PCR system (Applied Biosystems, USA) were the same as those explained previously [13]. Normalization was conducted between the quantities of target critical thresholds (Ct) and the internal controls (β actin) as previously described [14].

Table 1 Primers used for real-time PCR.

Gene	Forward primer	Reverse primer.
apoptosis P21	GAGGCCTCTTCCCCATCTTCT	AATTAAGACACACTGAATGA AGGCTAAG
P53	GTTCCGAGAGCTGAATGAGG	TTTTATGGCGGGACGTAGAC
Anti Apoptosis Survivin	GAGCAGCTGGCTGCCTTA	GGCATGTCACTCAGGTCCA
Angiogenesis VEGF	GATCATGCGGATCAAACCTCACC	CCTCCGGACCCAAAGTGCTC
Autophagy becline	GGTTGCGGTTTTTCTGGGAC	TTGATGGAATAGGAGCCGCC
Housekeeping β actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

Ethical approval

Al-Azhar University Ethics Board gave the study its approval. The Animal Care and Use Committee, Faculty of Science, Al-Azhar University, Cairo, Egypt, approved the ethical norms and guidelines used in this work. All the experimental procedures conformed to "Guide for the care and use of Laboratory Animals" for the use and welfare of experimental animals, published by the US National Institutes of Health (NIH publication No. 85–23, 1996).

Statistical analysis

A one-way ANOVA was performed in GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA), followed by Tukey's Honestly Significant Difference (HSD). The significance level was set at P <0.05.

RESULTS

As regard the effect of treatment by ANTH and/or SOR on body weight, liver weight) on HCC rats, compared to the final body weight of the normal control group of rats, there was a significant reduction in the body weight of rats following the administration of DEN+CCL4. Treatment by ANTH and/or SOR showed significant weight gain when compared to HCC group Table (2).

There was a rise in liver weights of HCC rats compared to normal rats. However, treatment by ANTH and/or SOR decreased the level compared to HCC untreated rates (Table 2). There was no significant difference between ANTH and SOR treatment.

Effect of treatment by ANTH and/or SOR on liver enzyme ALP (Alkaline phosphatase), γ-glutamyl transferase (GGT), and activities of antioxidant enzyme (GPX) on HCC rats.

The ALP (Alkaline phosphatase) and (GGT) level were increased in HCC rats as compared to normal rats. Treatment of HCC-induced rats by ANTH and/or SOR showed a significant reduction of ALP and GGT levels. The antioxidant enzymes, GPX Table (3) were reduced in the tumor-bearing rats (HCC) group compared to normal rats. However, HCC rats treated by ANTH and/or SOR had reversed antioxidant status as compared to HCC rats. A combination of ANTH and SOR showed better results than ANTH or SOR alone, with no significant difference between the combination of (ANTH + SOR) and control groups.

Upregulation Effect after treatment by ANTH and/or SOR on the relative expression of (apoptotic genes) P21, P53, and (anti-apoptotic genes) survivin genes in treated groups.

Results revealed a significant upregulation of relative expression of the P21 gene and P53gene Table (4) after HCC treatment by ANTH and/ or SOR compared to HCC rats. However, this expression was not significantly downregulated in HCC rats compared to normal control rats. Moreover, there are significant differences in the relative expression of these genes in HCC rats applied by ANTH or SOR. Interestingly, treatment by a combination of ANTH and SOR showed the most upregulation for these genes. The survivin gene was increased in HCC rats as compared to normal rats. Treatment of HCC-induced rats by ANTH and/or SOR showed a significant reduction of Survivin levels. The combination between ANTH and SOR showed better results than ANTH or SOR alone, while no significant difference exists between ANTH and SOR results in treated groups.

Down regulation effect after treatment by ANTH and/or SOR on the relative expression of angiogenesis gene VEGF and autophagy (Becline 1).

Results revealed a significant downregulation of the relative expression of VEGF and Becline 1. gene Table (5) after HCC treatment by ANTH and/ or SOR compared to HCC. However, this expression was significantly upregulated in the HCC group compared to normal control. Interestingly, treatment by a combination of ANTH and SOR showed the most downregulation for these genes. Moreover, no significant differences exist between the combination of (ANTH +SOR) and control groups.

Table (2): Effect of ANTH and/or SOR treatment on body weight and liver weight in HCC rats

Group	Body weight (Mean ± SEM)	Liver weight (Mean ± SEM)
Control	185.25 ± 3.99	5.012 ± 0.244
HCC	147.85 ± 2.95##	7.16 ± 0.281##
Anth	172.06 ± 4.16**	6.08 ± 0.285*
Sor	169.55 ± 3.37**	6.20 ± 0.187
Anth+Sor	176.65 ± 3.46**	6.012 ± 0.239*

####P < 0.0001 vs. normal group; *, **, ***, **** at P < 0.05, 0.01, 0.001, 0.0001 vs. HCC group. Data was presented as mean ± SEM (n=5).

Table (3): Effect of treatment by ANTH and/or SOR on liver enzyme ALP (Alkaline phosphatase), Liver cancer marker (GGT), and activities of antioxidant enzyme (GPX) on HCC rats.

Group	ALP(U/L) (Mean± SEM)	GGT (U/L) (Mean± SEM)	GPX (U/gt) (Mean± SEM)
Control	129.09± 3.8	8.450± 0.72	138.22± 5.0
HCC	235.22± 5.600####	33.12± 2.02####	69.54± 2.44####
Anth	174.36± 4.540****	20.38± 1.01****	105.190± 3.510****
Sor	160.27± 3.52****	15.63± 0.91****	85.83± 2.76
Anth+Sor	140.74± 2.43****	10.05± 0.85****	124.27± 3.83****

####P < 0.0001 vs. normal group *, **** at P < 0.05, 0.0001 vs. HCC group. Data was presented as mean ± SEM (n=5).

Table (4): Effect of treatment by ANTH and/or SOR on the relative expression of (apoptotic genes) P21, P53, and (anti-apoptotic genes) Survivin genes in treated groups.

Group	**P21 (Mean± SEM)**	**P53 (Mean± SEM)**	**Survivin (Mean± SEM)**
Control	1.00± 0.08	1.00± 0.08	1.00± 0.06
HCC	0.21± 0.02	0.52± 0.02	3.23± 0.15####
Anth	2.55± 0.14****	3.51± 0.16****	1.99± 0.12****
Sor	4.41± 0.21****	4.29± 0.24****	2.08± 0.13****
Anth+Sor	9.19± 0.42****	6.87± 0.28****	1.26± 0.09****

####P < 0.0001 vs. normal group; ****P < 0.0001 vs. HCC group. Data was presented as mean ± SEM (n=5).

Table (5): Effect of ANTH and/or SOR treatment on the relative expression of angiogenesis gene VEGF and autophagy (Becline 1).

Group	**VEGF (Mean± SEM**	**Bcline 1 (Mean± SEM**
Control	1.00± 0.090	1.00± 0.07
HCC	8.750± 0.430#	3.89± 0.17##
Anth	4.110± 0.250*	3.530± 0.15
Sor	3.01± 0.16***	2.16± 0.11**
Anth+Sor	1.31± 0.10***	1.84± 0.10**

####P < 0.0001 vs. normal group; ****P < 0.0001 vs. HCC group. Data was presented as mean ± SEM(n=5).

DISCUSSION

Liver cancer still has a high recurrence rate, and very few effective treatments are available currently. The only systemic medication that the FDA has authorized for people with advanced HCC is sorafenib. Sorafenib's direct inhibition of tumor cells and its influence on angiogenesis are both quite significant. Numerous papers outlined Sorafenib's mechanisms. Sorafenib causes tumor cells to undergo apoptosis and inhibits angiogenesis and multiplication of tumors [15].

Nevertheless, sorafenib has only a very little therapeutic impact, and taking it has been linked to serious negative side effects. Furthermore, a significant percentage of individuals experience treatment resistance. Therefore, creating novel, efficient treatments for HCC is difficult. In the meantime, it is essential to identify new approaches for enhancing the effectiveness of Sorafenib therapies in conjunction with other medications to minimize its unfavorable side effects while simultaneously increasing its efficacy [7]. The primary active components of rhein, rhubarb, chrysophanol, emodin, and other substances have a wide range of pharmacological actions, little toxicity and adverse effects, and promising applications [16].

This work focuses on combining sorafenib with the isolation of four free anthraquinones from the Rhubarb rhizome (physcion, emodin, chrysophanol, and aloe-emodin) to treat HCC by improving the drug's therapeutic efficacy and sensitivity. First, we used DEN and CCL4 to generate HCC in the current research. Tumor promoters such as CCl4 help to guarantee that liver cancer develops more quickly than DEN alone, which takes a long period to produce hepatocarcinoma. Through indirect methods, CCl4 increased the proliferation of hepatocytes [17]. The mortality rate, changes in liver weight, body weight, serum ALP level, and liver cancer marker (GGT) were used to track the course of HCC. All groups showed no mortality except the HCC group. As

might be expected, the HCC group had the lowest body weight and the largest liver weight in relation to the other groups. In the current investigation, the group treated with DEN exhibited elevated levels of the cancer marker GGT in their blood and hepatic cellular dysfunction, as shown by an increase in the liver enzyme ALP [18]. discovered comparable outcomes for rats exposed to DEN and carbon tetra chloride (CCL4)-induced HCC, respectively. Hepatocyte injury and dysfunction are indicated by elevated liver enzyme levels and decreased protein content. An elevated level of ALP in blood may indicate liver injury, even though the enzyme is uniformly distributed mostly in the tissues of the kidney, liver, and bones. ALP is one of the best tests for liver function available for identifying hepatic impairment. Leaky tight connections may be the cause of the method by which alkaline phosphatase enters the bloodstream as a consequence of bile canaliculi leaking into the hepatic sinusoids. Patients with cirrhosis have been observed to have elevated blood levels of intestinal alkaline phosphatase [17]. This might be explained by oxidative stress brought on by DEN, which damages liver tissue and impairs liver function [19].

In accordance with Zhang *et al.* [20], who found that DEN may promote HCC via interactions with crucial macromolecules such as lipids, antioxidant enzymes, DNA, and enzymes of the DNA repairing system, the present research also revealed a substantial drop in GPX, a prominent antioxidant enzyme. Furthermore, [21] discovered that CCl4 biotransformation with the aid of cytochrome P-450 typically resulted in the conversion of two metabolites linked to lipid peroxidation, ROS generation, and a reduced in the enzymatic activities of SOD, CAT, GPX and GST: trichloromethyl proxy free radical (CCl3OO*) and trichloromethyl free radical (CCl3*).

Gamma-glutamyl transferase (GGT) is a glycoprotein enzyme that is normally employed as a biomarker for alcohol abuse and liver damage. It is present on the cell membranes of numerous human tissues. However, it is most often seen in hepatocytes. The primary role of GGT is to catabolize glutathione extracellularly, resulting in the generation of reactive oxygen species. During regular metabolism, glutathione is essential for shielding cells from the oxidants that follow. Transferring a glutamyl residue to an acceptor is the process that GGT catalyzes, which aids in maintaining sufficient glutathione levels. GGT buildup in the liver and excessive GGT secretion into the blood may be caused by bile duct obstruction or liver injury. Consequently, an increased GGT level in serum may be suggestive of hepatic or biliary injury [22].

Our study's data showed that rats intoxicated with DEN had much higher GGT activity than rats in normal health. This might be explained by the quick turnover of cancer cells, which releases the GGT enzyme into the bloodstream. I agree with [23] that the injection of DEN seemed to induce damage to the plasma membrane, as shown by the considerable drop in the liver activity of GGT and the observed rise in enzyme activity in serum.

On the other hand, HCC rats given ANTH and/or SOR had significantly reduced liver weight, ALP, and GGT. The HCC group's elevated blood levels of these enzymes, which signify liver failure, were predicted. ANTH and/or SOR therapy, however, reduced these increased levels to levels like the normal control group. Conversely, the administration of ANTH and/or SOR resulted in a rise in body weight and GPX levels that were like those of the normal control group, with the combination-treated group showing the greatest benefit. All of these results point to the fact that cotreatment with ANTH and SOR has a greater therapeutic impact against HCC than either treatment alone.

The role of rhubarb in liver protection has been studied extensively in recent years. The effects of rhubarb anthraquinones on liver damage and protection are biphasic. Anthraquinones significantly reduced liver cell damage and liver fibrosis. Additionally, hepatic encephalopathy, liver fibrosis, and intrahepatic cholestasis are studied in relation to raspberries and their free anthraquinones [16].

Serum levels of total alkaline phosphatase, direct bilirubin, bilirubin, alkaline phosphatase, total bile acid, and γ-glutamyl transferase showed protection against α-naphthylisothiocyanate-induced liver injury with cholestasis against Rhein, physione, aloe-emodin, emodin, and to a lesser extent, chrysophanol [24].

The protective effects of emodin on pancreatic and liver cancer in vivo have been shown in several recent investigations. One day after the subcutaneous injection of HepG2 cells, daily dosing of emodin (1 and 10 mg/kg) slowed tumor development and decreased mortality in a dose-dependent manner [6].

In (EAC) Ehrlich ascites carcinoma mice applied with (Amy) amygdalin and/or (Sor) sorafenib, Attia observed a substantial reduction in the serum of liver enzymes like ALT, GGT and AST; the greatest impact was seen in animals treated with both Sor IP and Amy [25].

Compared to normal control rats, the livers of HCC rats showed a substantial downregulation of the pro-apoptotic genes P21 and P53. After ANTH and SOR were administered, either alone or in combination, this decreased expression was markedly raised, with the combined group showing the highest outcomes. Conversely, the anti-apoptotic marker survivin was much lower in HCC rats treated with ANTH and SOR. I concur with Mohamed's findings [26] that the pro-apoptotic genes caspase 9, p53, and caspase 3 were significantly downregulated in the livers of HCC rats compared to

normal control rats. The DEN group substantially upregulated the anti-apoptotic gene survivin [18].

In the liver of treated rats, there was a considerable rise in apoptotic markers such as p53 and caspase-3 proteins, together with a decreased amount of anti-apoptotic Bcl-2 protein, indicating damage to DNA and genotoxicity. These results may indicate that rats given DENA/CCl4 had a higher incidence of HCC [27].

Emodin, an anthraquinone derivative, has been shown in several studies to exhibit anti-cancer and apoptosis-promoting activities against pancreatic cancer in mice via inhibition of Akt activation. Emodin inhibits the expression of survivin and increases apoptosis in gallbladder carcinomas produced by cisplatin in a way that relies on ROS. In human lung adenocarcinoma A549 cells, emodin generates ROS and activates the ATM-p53-Bax-dependent signaling pathway. In human renal proximal tubule HK-2 cells, emodin induces apoptosis via the caspase-3-dependent pathway and inhibits the proliferation of human prostate cancer LNCaP cells via the androgen receptor and p53-p21 pathways [28].

The anthraquinone chemicals found in rhubarb prevent HCC from proliferating and growing. HepG2 cell growth was inhibited by emodin, which also caused ATP synthesis to malfunction and a marked reduction in mitochondrial membrane potential. These events ultimately led to the opening of mitochondrial permeability transport pores, which allowed calcium ions to exit the cell and activated the caspase protein family, which in turn caused apoptosis. Aloe-emodin, a further bioactive component of rhubarb with anti-cancer properties, inhibited HepG2 and Hep3B cells' ability to proliferate by blocking the p21-dependent and p53-induced apoptotic pathways [5].

Prostate cancer cell lines undergo apoptosis when exposed to sorafenib. All three of the studied cell lines saw a dose-dependent reduction in cell viability after a 72-hour incubation period with sorafenib. Independent techniques showed that sorafenib caused apoptosis in all cell lines in a dose-dependent manner; this included an increase in pan-caspase activity and a decrease in mitochondrial membrane potential [29].

The angiogenesis gene VEGF and autophagy were significantly upregulated in the livers of HCC rats (Becline 1). After ANTH and SOR were administered, either alone or in combination, this expression was dramatically decreased, with the combined group showing the highest outcomes.

The hepatic angiogenic factors NO, Il-4, Il-8, VEGF, and PDGF were increased after the DEN/CCl4 challenge. The two most significant angiogenic factors that are prognostic, aggressive, and regulated are PDGF and VEGF, both of which show promise as treatment targets for HCC. According to [9], VEGF is associated with increased dysplastic grades and vascular invasion in HCC via its receptors.

According to El-Magd M *et al.*, the DEN group had the highest and most significant mRNA levels of the angiogenesis gene (VEGF) and inflammation-related genes (IL1β, NFκB) [18].

A key player in the process of tumor angiogenesis is VEGF, and studies have shown that sorafenib inhibits both receptor tyrosine kinases (FMS tyrosine kinase 3) and serine/threonine kinases (c-RAF and b-RAF) as well as VEGF receptors 2 and 3. In a mouse model, sorafenib was also shown to dramatically reduce the proliferation and migration of cancer cells [30].

An oral multikinase inhibitor called sorafenib is used worldwide to treat advanced or metastatic HCC. According to [31], sorafenib completely inhibits tumor development in mice and reduces tumor angiogenesis in a mouse HCC xenograft model.

Angiogenesis and the epithelial-mesenchymal transition (EMT) are essential components promoting tumor development, metastasis, and invasion. Tumor EMT is a process wherein epithelial cells take on characteristics of mesenchymal stem cells, with increased extracellular matrix (ECM) synthesis and improved cell migration capabilities to promote growth and metastasis. Five One Furthermore, angiogenesis mediated by vascular endothelial growth factor (VEGF) is necessary for the creation of new blood vessels in order to support ongoing development and metastasis. It has been suggested that imodin (1–80 mg/kg) may stop tumor development by suppressing angiogenesis and blocking epithelial-mesenchymal transition. Emodin (40 mg/kg, p.o.) effectively inhibited Wnt/β-catenin signaling in vivo, preventing colon cancer cell motility and EMT. According to [6], emodin (5–20 μM) also decreased colon tumor cell invasion and migration in vitro via lowering VEGF.

In HCC cells, like Hep3B, HepG2, SK-HEP-1, Huh7, and PLC/PRF5, the combination of sorafenib (5 mg/kg, i.p.) with emodin (10 mg/kg, i.p.) substantially suppressed tumor development and cell proliferation. Additionally, emodin synergistically boosted sorafenib effectiveness to result in larger tumor suppression. Animal models xenografted with SK-HEP-1 or HepG2 cells were sufficiently inhibited by the combination of emodin and sorafenib after tumors were established, suggesting that emodin used as a complementary therapy can enhance the effectiveness of standard therapies [32].

The highly controlled process known as autophagy, or "self-digestion," is crucial for maintaining cellular homeostasis and is involved in replacing damaged cellular organelles. Autophagy is a coin with two sides because, depending on the cellular context, it may either inhibit apoptosis and become a mechanism for cell survival or cause cell death [33].

Autophagy may play different functions as a tumor progresses and is likely involved in promoting and preventing cancer. Maintaining normal cell function prevents tumors from starting in normal liver tissue and aids in the survival of carcinoma cells in the tumor microenvironment, especially during chemotherapy. Basal autophagy serves as a tumor suppressor in the dysplastic phase of hepatocytes by eliminating mutant cells and recently damaged mitochondria, preserving genomic integrity. Unbalanced autophagy, on the other hand, will support HCC cell survival under a variety of stress circumstances after a tumor has grown, which in turn will increase tumor development [34].

In HCC, sorafenib-induced autophagy functions as a chemoresistance mechanism. Sorafenib will kill more malignant cells, and its anti-proliferative efficacy will increase when the autophagic important genes Beclin 1 or Atg5 are reduced [35].

CONCLUSION

It could be concluded that administration of anthraquinone could improve the therapeutic potential of sorafenib for treating rats with DENA/CCl4-induced HCC. Apoptosis is induced, oxidative stress is inhibited, autophagy is inhibited, and angiogenesis is inhibited.

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