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The Anticancer Effect of *Rhubarb Officinale* Fraction and Sorafenib Against Chemically Induced Hepatocellular Carcinoma in Rats

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Abstract

Background: *Hepatocellular carcinoma (HCC), the major primary malignant tumour of the liver, represents a complex and fatal malignancy driven primarily by oxidative stress and inflammation.* ***Objectives****: The aim of this study was to investigate the potential anticancer properties of four Anthraquinones (Aloe-emodin, Emodin, Chysophanol, and Physcion) isolated from the rhizome of Rhubarb and compare between the effect of sorafenib and Rhubarb Officinale fraction on DEN and carbon tetrachloride (CCl4) - induced hepatocellular carcinoma in rats.* ***Material and methods:*** *The rats were divided into 5 groups (n=12/group normal control, diethylnitrosamine DEN+ carbon tetrachloride CCL4, HCC rats +ANTH(Anthraquinones), HCC rats +SOR(Sorafenib) and HCC rats +mix of ANTH+SOR.* ***Results:*** *Rats in HCC group showed most deteriorated effect in form of increased mortality, liver cancer marker (lipid peroxidation biomarker MDA, inflammation –related genes (TNFa, ILIB, NFKB, TGFB1) and metastasis-related genes (MMP9). Liver tissues of HCC group also exhibited lower level of TIMPI and antioxidant activity (catalase (CAT). All these deleterious effects induced by DEN were reversed after administration of ANTH and SOR with best improvement for the combined group (ANTH+SOR).* ***Conclusions****: These findings reveal a better therapeutic effect for ANTH when given with SOR and we attribute this beneficial effect, at least in part, to triggering antioxidant activity, inflammation and metastasis in HCC. Therefore, combined treatment with ANTH and SOR is recommended to enhance the therapeutic potential against HCC.*

Keywords: *Sorafenib, Aloe-Emodin, Emodin, Chysophanol, And Physcion*

1. Introduction

Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer-related death worldwide. The main HCC risk factors include viral infection (especially hepatitis C and B viruses), alcohol consumption, and exposure to aflatoxin B1. HCC is the final sequelae of chronic liver disease, because chronic diseases cause slow and sustained damage to hepatocytes, along with a high incidence of genetic alterations. Once cirrhosis occurs, there is no effective HCC therapy except surgery in some selected cases. HCC treatment is difficult because of the impairment of liver function and the expression of multidrug resistance genes. Moreover, cancer cells maintain their viability through induction of inflammation. Through this mechanism, cancer cells can resist most chemotherapeutic agents and can facilitate tumor progression. Thus, an effective treatment for cancer should target this mechanism. Cancer cells can induce inflammation directly through the production of pro-inflammatory cytokines or indirectly through tumor-induced physical damage to normal tissue, which leads to hypoxia**(El-Magd et al., 2019)**.

The utmost acute hepatocarcinogensis in animals is N-nitrosamine compounds, especially diethyl nitrosamine (DEN). Diethyl nitrosamine is commonly used for HCC initiation; while CCl4 is introduced to enhance the intensity of carcinogenesis. Oxidative stress is the output of production of reactive oxygen species and hepatocellular damage could be involved in the pathogenesis of DEN-induced hepatocellular carcinoma**(Mohamed et al., 2019)**.

Anthraquinones, especially emodin, physcion, aloe-emodin and chrysophanol, are rich in almost all species of Rhubarb, which not only possess good antiinflammatory, anti-tumor and cardiovascular protective effects, but also play an important role in hepatoprotection,

anthraquinone compounds in Rhubarb is able to regulate the levels of pro-inflammation cytokines, such as tumor necrosis factorα (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), to inhibit the occurrence, invasion and metastasis of HCC .Moreover, when Rhubarb was used to treat alcoholic fatty liver disease, non-alcoholic fatty liver disease and viral hepatitis, good results were achieved through activating the manifold signal pathways in the body **(Zhuang et al., 2020).**

Many researchers are paying attention to the pharmacological effects of chrysophanol. In the past 10 years, there have been a large number of researches on pharmacological effect of chrysophanol, including neuroprotection, anticancer, antibacterial, antiviral, antioxidation and blood lipid regulation. These pharmacological effects indicate that chrysophanol has value in the prevention and treatment of certain diseases, including cancer, atherosclerosis, asthma and diabetes**(Xie et al., 2019) .**

Emodin has antibacterial, anti-inflammatory, antiviral, anti-ulcerogenic, anticancer, immunosuppressive, and chemopreventive effects. Emodin has also been reported to exert inhibitory effects on cell death in the human lung squamous carcinoma CH27 cell line, and human promyeloleukemic HL-60 cells induce apoptosis by activating the caspase-3 cascade independently of reactive oxygen species (ROS) production**(Hsu and Chung, 2012)**.

Sorafenib is a small molecule that inhibits tumor-cell proliferation and tumor angiogenesis and increases the rate of apoptosis in a wide range of tumor models. It acts by inhibiting the serine–threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors (VEGFRs) 1, 2, and 3 and platelet-derived growth factor receptor β (PDGFR-β). Cellular signaling that is mediated by the Raf-1 and vascular endothelial growth factor (VEGF) pathways has been implicated in the molecular pathogenesis of hepatocellular carcinoma, providing a rationale for investigating sorafenib for this indication. In preclinical experiments, sorafenib had antiproliferative activity in liver-cancer cell lines, and it reduced tumor angiogenesis and tumor-cell signaling and increased tumor-cell apoptosis in a mouse xenograft model of human hepatocellular carcinoma **(Llovet et al., 2008)**.

2. Materials And Methods

Chemicals

Diethylnitrosamine (N-nitrosodiethylamine; DEN; purity, 99.0%), carbon tetrachloride (CCl4), were purchased from Sigma-Aldrich Company, St. Louis., USA. Sorafenib (SOR)(Nexavar) obtained from Bayer AG Pharmaceutical Chemicals Co., Germany.

Isolation of total anthraquinones from Rhubarb:

In order 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 subjected to exhaustive (i.e stopped to response to Borntrager's test) extraction with methylene chloride, 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 of diluted hydrochloric acid to give heavy yellowish brown precipitate which was collected and dried under vacuum to give 20 gm of yellowish brown powder **(Selim et al., 2019).**

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 R_f (0.3-0.7) indicating the presence of the 4 major anthraquinone aglycones.

Animals and experimental design

Male albino Wistar rats (200 -170g) were brought from the Egyptian Organization for Biological Products and Vaccines, Cairo, Egypt. Rats were allowed free access to food and water and were maintained under standard conditions (normal light/dark cycle, and temperature 25 ± 3 °C). Animals were left to acclimatize for one week before starting the experiment. This study was conducted in accordance with ethical procedures and policies approved by animal care and use committee of faculty of science, Al-Azhar University, Cairo,Egypt. The study was approved by the Ethics Board of Al-Azhar University.

The rats were randomly divided into 5 groups (n=12/group). Animals of normal group were orally administrated saline to the end of the experiment. Rats of HCC were administered intraperitoneal (IP)

a single dose of DEN (200 mg/kg dissolved in normal saline 0.9% at a final volume of 1ml/kg. Two weeks after DEN challenging, CCl4 solution (CCl4/olive oil; 1:1; 1 ml/kg) was administered IP three times weekly for 6 consecutive weeks(Elaidy et al., 2017). After detection of HCC development, the treatment by sorafenib and anthraquinones were done. The dose of herbal fraction (200mg/kg dissolved in 20 ml of 0.5% CMC)**(Gao et al., 2016)** and sorafenib (30mg/kg)**(Yan et al., 2013)**.

These treatments were orally administrated by stomach tube for thrity days for 2 groups.The groups used in the present study were as follow:

Group I: normal control rats received only normal saline

Group II: (HCC)rats injected by DEN+CCL4)

Group III: (HCC+SOR) HCC rats treated by SOR.

Group IV: (HCC+ANTH) HCC rats treated by ANTH.

Group V: (HCC+SOR+ANTH) HCC rats treated by SOR and ANTH.

At the end of the experiment, the rats were killed by cervical dislocation under light ether anesthesia, the liver was weighed (absolute liver weight), and then the relative weight of the liver was calculated as a percentage of the absolute liver weight/final body weight. the liver was washed by saline and then divided into two parts, the first part was quickly frozen in liquid nitrogen for RNA extraction and the second was preserved in 10% formalin for histological analysis.

2.4. Evaluation of Liver Lipid Peroxidation and Antioxidant Biomarkers

Liver tissues were homogenized using cold PBS, followed by centrifugation at 5000*g* for 15 min at4°C. The supernatants were used to measure the concentration of the lipid peroxidation biomarker MDA and the activity of antioxidant enzyme CAT using commercial kits (Biodiagnostics Co., Cairo, Egypt, and Randox Laboratories Ltd., Crumlin, UK) and as previously described**(El-Magd et al., 2017)** .

2.5. Molecular Analysis by Real-Time PCR.

Real time PCR was used to evaluate the changes in the relative expression of the candidate genes. First, total RNA was isolated from liver tissue using RNeasy Mini kit (Qiagen). The integrity and purity of RNA was assessed by 1% agarose gels electrophoresis and Nanodrop, respectively. Second, a weight of 4 mg from the obtained RNA was reverse transcribed to cDNA using Quantiscript reverse transcriptase. Third, the produced cDNA was used as a template for real-time PCR reaction in the presence of QuantiTect SYBR Green qPCR Master Mix and gene specific primers, designed by the Primer 3 web-based tool based on the published rat sequence (Table 1), along with StepOnePlus real time PCR system (Applied Biosystem, USA) and reaction cycles as previously described**(Khamis et al., 2018)** . The quantities of critical threshold (Ct) of the target genes were normalized with quantities of the Ct of the internal control (*β actin*) as previously described **(El-Magd et al., 2017)**.

Table 1 Primers Eused for real-time PCR.

Gene	Forward primer	Reverse primer
Inflammation IL1β	CACCTCTCAAGCAGAGCACAG	GGGTTCCATGGTGAAGTCAAC
NFκβ	CCTAGCTTTCTCTGAACTGCAAA	GGGTCAGAGGCCAATAGAGA
TNFα	CCCAGGGACCTCTCTCTAATC	ATGGGCTACAGGCTTGTCACT
TGFβ1	AAGAAGTCACCCGCGTGCTA	TGTGTGATGTCTTTGGTTTTGTCA
Metastasis MMP9	TCGAAGGCGACCTCAAGTG	TTCGGTGTAGCTTTGGATCCA
TIMP1	CGCAGCGAGGAGGTTTCTCAT	GGCAGTGATGTGCAAATTTCC
Housekeeping β actin	AAGTCCCTCACCCTCCCAAAAG	AAGCAATGCTGTCACCTTCCC

Statistical analysis

The statistical analysis was done using one way ANOVA using Graph Pad Prism 8 (Graph Pad Software, Inc., La Jolla, CA, USA) followed by Tukey's Honestly Significant Difference (Tukey's HSD) test. Significance was declared at $P < 0.05$.

3. Results and Discussion

Effect of treatment by ANTH and/or SOR on Liver cancer marker and activity of antioxidant enzymes on HCC rats

The effect of ANTH and/or SOR on lipid peroxide (MDA) and antioxidant status in the experimental rats were shown in Table (3). The lipid peroxide level was increased in HCC rats as compared to normal rats. Treatment HCC induced rats by ANTH and/or SOR showed a significant reduction of MDA level Figure (1). The antioxidant enzyme as CAT, Table (2) Figure (1) was reduced in 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. Combination between ANTH and SOR showed better results compared to ANTH or SOR alone.

Down regulation Effect after treatment by ANTH and/or SOR on the relative expression of inflammation-related genes IL1b, NF-kB TGF-B and TNF-a genes

Results revealed a significant down regulation of relative expression of IL1b ,NF-KB ,TGF-B gene and TNF-A gene Table (3) and Figures (2)after HCC treatment by ANTH and/ or SOR compared to HCC. However, this expression was significantly up regulated in HCC group compared to normal control. Moreover, no significant difference in the relative expression of these genes in HCC rats treated by ANTH or SOR. Interestingly, treatment by combination of ANTH and SOR showed the most down regulation for these genes.

Effect of treatment by ANTH and/or SOR on metastasis-related genes (MMP9, TIMP1) on HCC rats

The effect of ANTH and/or SOR on (MMP9, TIMP1) in the experimental rats were shown in Table (4). MMP9 gene was increased in HCC rats as compared to normal rats. Treatment HCC induced rats by ANTH and/or SOR showed a significant reduction of MMP9 gene Figure (3). Moreover, no significant difference in the relative expression of these genes in HCC rats treated by ANTH or SOR. Interestingly, treatment by combination of ANTH and SOR showed the most down regulation for these genes. relative expression of TIMP1genes reduced in tumor-bearing rats (HCC) group compared to normal rats. However HCC rats treated by ANTH and/or SOR showing significantly up regulated compared to HCC rats. Combination between ANTH and SOR showed better results compared to ANTH or SOR alone.

Table (2): Effect of treatment by ANTH and/or SOR on Liver cancer marker and activity of antioxidant enzymes on HCC rats

Group	MDA (Mean ± SEM)	CAT (Mean ± SEM)
Cnt	10.29 ± 0.71	4.750 ± 0.220
HCC	$34.67 \pm 1.47^{\#\#\#\#}$	$1.03 \pm 0.07^{\#\#\#\#}$
Anth	$16.35 \pm 0.90^{****}$	$2.36 \pm 0.10^{****}$
Sor	$23.82 \pm 1.06^{****}$	$1.65 \pm 0.09^{*}$
Anth+Sor	$16.47 \pm 0.75^{****}$	$3.02 \pm 0.11^{****}$

$^{\#\#\#\#}P < 0.0001$ vs. normal group; $^{*}P < 0.05$, $^{****}P < 0.0001$ vs. HCC group. Data was presented as mean ± SEM(n=5).

Table (3): Effect of treatment by ANTH and/or SOR on the relative expression of inflammation-related genes IL1b, NF-kB, TGF-B and TNF-a genes:

Group	ILIB (Mean ± SEM)	NFKB (Mean ± SEM)	TGFB (Mean ± SEM)	TNFA (Mean ± SEM)
Cnt	1.00 ± 0.080	1.00 ± 0.07	1.00 ± 0.08	1.00 ± 0.08
HCC	$4.17 \pm 0.22^{\#\#\#\#}$	$4.86 \pm 0.23^{\#\#\#\#}$	$4.17 \pm 0.25^{\#\#\#\#}$	$6.59 \pm 0.37^{\#\#\#\#}$
Anth	$1.68 \pm 0.12^{****}$	$3.78 \pm 0.17^{***}$	$3.23 \pm 0.18^{**}$	$3.56 \pm 0.180^{****}$
Sor	$1.52 \pm 0.1^{****}$	$2.79 \pm 0.13^{****}$	$2.91 \pm 0.14^{****}$	$3.12 \pm 0.16^{****}$
Anth+Sor	$1.18 \pm 0.09^{****}$	$1.32 \pm 0.11^{****}$	$2.25 \pm 0.12^{****}$	$2.57 \pm 0.13^{****}$

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

Table (4): Effect of treatment by ANTH and/or SOR on metastasis-related genes(MMP9, TIMP1) on HCC rats:

Group	MMP9 (Mean ± SEM)	TIMPI (Mean ± SEM)
Cnt	1.00 ± 0.07	1.00 ± 0.05
HCC	2.95 ± 0.14$^{####}$	0.24 ± 0.010$^{####}$
Anth	2.10 ± 0.13****	0.55 ± 0.03****
Sor	1.83 ± 0.11****	0.74 ± 0.04****
Anth+Sor	1.23 ± 0.10****	0.82 ± 0.04****

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

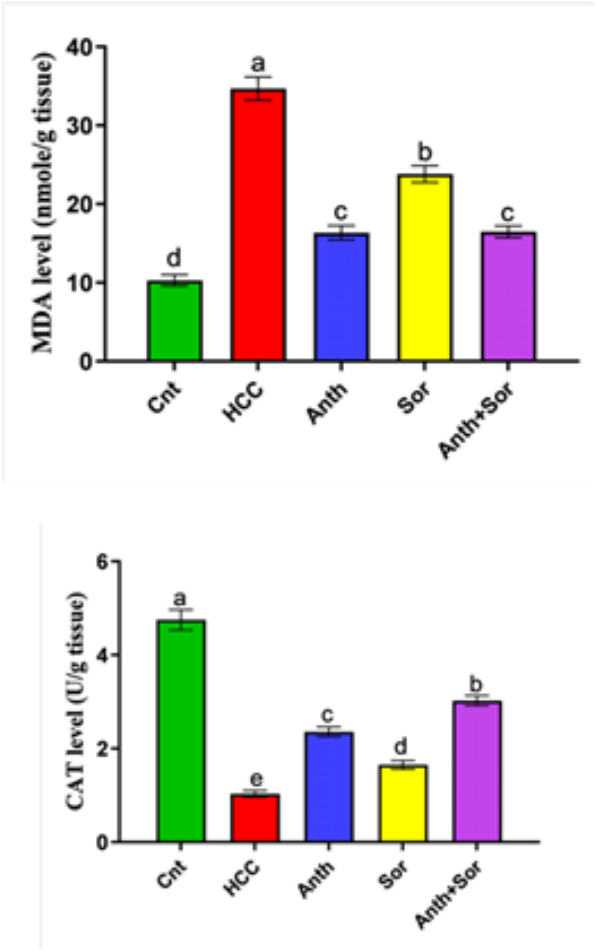

Figure (1): Effect of ANTH and/or SOR on serum levels of liver cancer markers ,oxidative stress and activities of antioxidant enzymes ..Normal control (Cnt), HCC, HCC treated by (ANTH), HCC treated by (SOR), and HCC treated by ANTH and SOR(ANTH+SOR) rats. Values are expressed as mean ± SEM (n = 7). Values carrying different lower case letter are significantly different at P <0.05

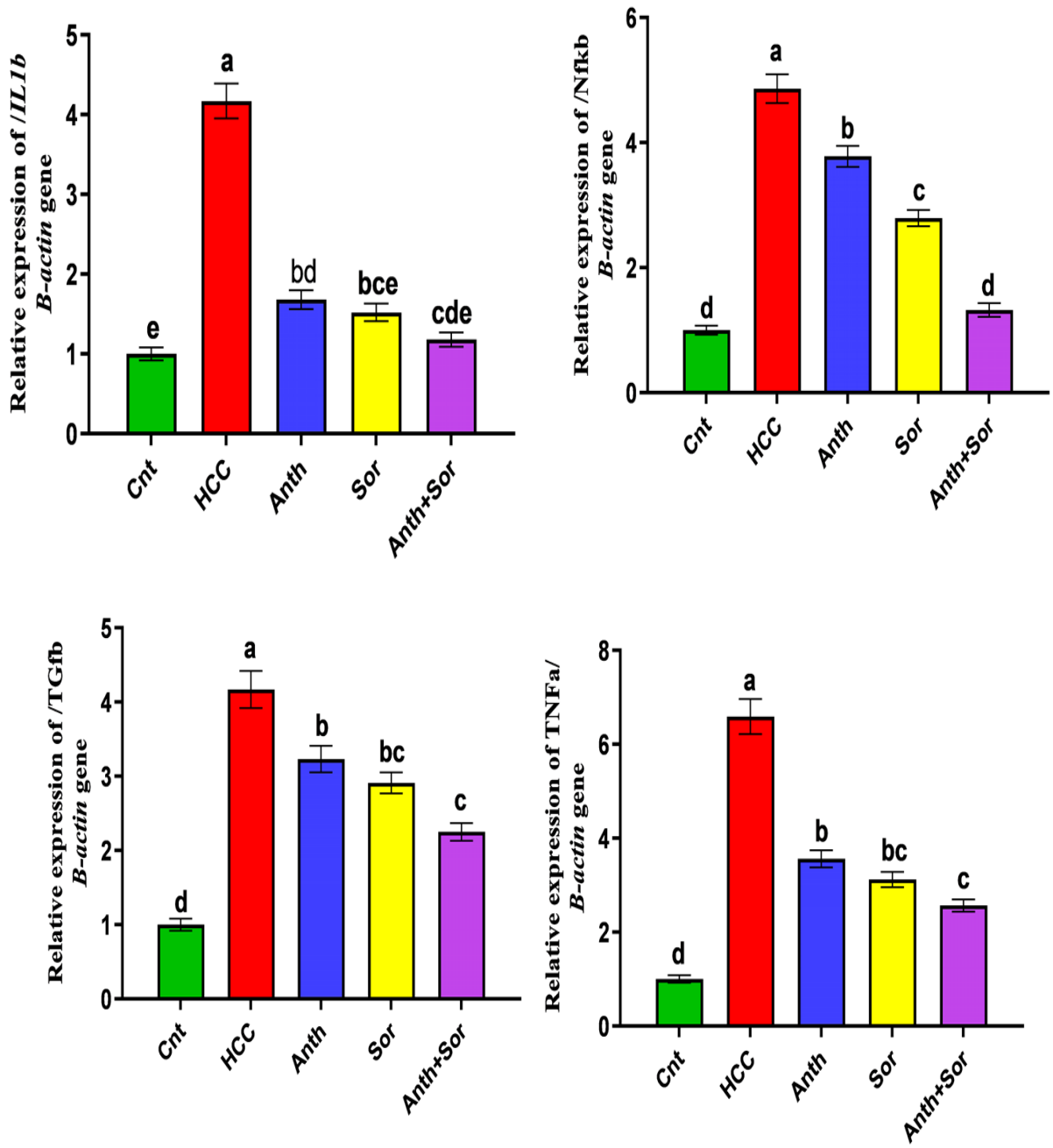

Figure (2): Effect of ANTH and/or SOR on the relative expression of inflammation-related genes IL1b, NF-kB TGF-B and TNF-a genes .Normal control (Cnt), HCC, HCC treated by (ANTH), HCC treated by (SOR), and HCC treated by ANTH and SOR(ANTH+SOR) rats. Values are expressed as mean ± SEM (n = 7). Values carrying different lower case letter are significantly different at P <0.05.

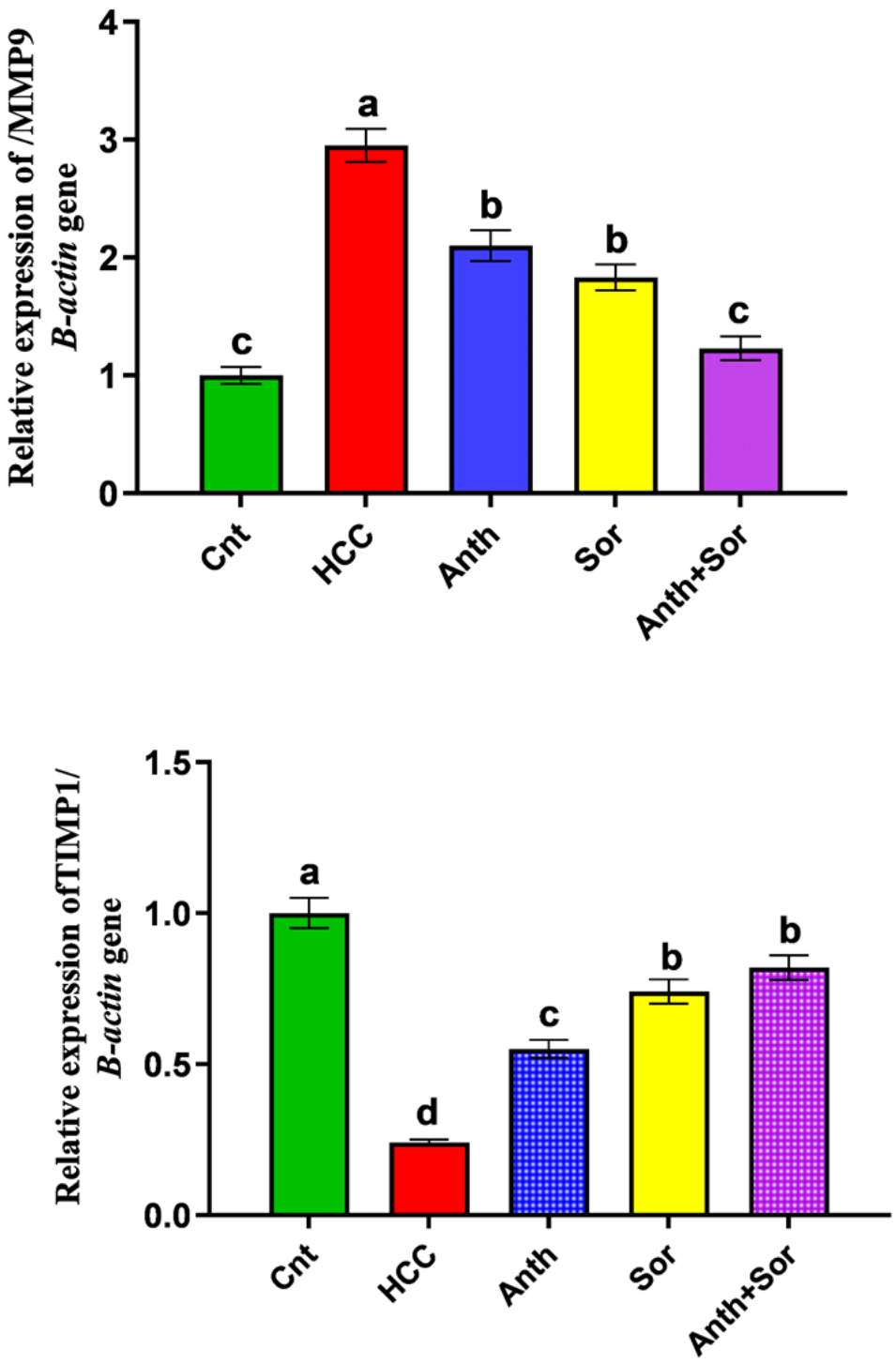

Figure (3): Effect of ANTH and/or SOR on metastasis-related genes (MMP9, TIMP1).Normal control (Cnt), HCC, HCC treated by (ANTH), HCC treated by (SOR), and HCC treated by ANTH and SOR(ANTH+SOR) rats. Values are expressed as mean ± SEM (n = 7). Values carrying different lower case letter are significantly different at P <0.05.

Discussion

Anthraquinones are one of the most pivotal active components in Rhubarb. The free anthraquinones, especially rhein, emodin, physcion, aloe-emodin and chrysophanol, are rich in almost all species of Rhubarb , which not only possess good ant inflammatory, anti-tumor and cardiovascular protective effects, but also play an important role in hepatoprotection**(Li et al., 2017).** Sorafenib is the first-line US Food and Drug Administration (FDA)-approved drug for liver cancer. Unfortunately, only a small number of patients respond to sorafenib, and its serious side effects often lead to dose reduction or treatment discontinuation. Thus, there is an urgent need for more effective treatment strategies for liver

cancer. Studies exploring sorafenib combination strategies have revealed several effective synergistic drugs. Capsaicin, Silibinin, and panobinostat were able to improve the efficacy of sorafenib to varying degrees, but were still unsatisfactory. Therefore, there is a need to find more effective combination drugs**(Zhang et al., 2022) .**

In the present study, while treatment with DEN/CCl4was effective in inducing HCC in rats, Anthraquinones administered either alone or in combination with sorafenib was effective in ameliorating the hepato-carcinogenic effect of DEN/CCl4.

a significant decrease in tissue CAT activities was detected in the current work in HCC group. It has been proposed that oxidative stress plays an important role in the progress and development of various cancer types, including liver cancer. Free oxygen radicals are primarily removed by various enzymatic antioxidants, such as SOD, CAT, GPX and GST and by various nonenzymatic antioxidants, such as glutathione(GSH), α-tocopherol and vitamin C **(Arslan et al., 2014)**. A loss in catalase activity during cancer development is associated with tumor formation and metastasis. ROS may downregulate catalase through the methylation of promoter during the development of HCC **(Hussein et al., 2016)** .

The obtained data presented in table (1) revealed that, a significant increase in tissue MDA activity. Many studies reported that serum MDA levels of primary and metastatic liver cancer patients are significantly higher than the normal group. The high lipid peroxidation in liver cancer may be due to excessive ROS production. MDA may react with the amino acid residue of proteins, resulting in their oxidative modification and causing protein breakdown in the process. Moreover, it may encourage cellular use of glutathione and increase oxidative stress by deactivating selenium dependent glutathione peroxidases **(Arslan et al., 2014)**.

In contrast, HCC rats treated with ANTH and/or SOR showed significant lower MDA. As expected, HCC group exhibited higher serum levels of these enzymes, indicating liver dysfunction. However, treatment with ANTH and/or SOR decreased this elevated level to level comparable to that of normal control group .In other hand the treatment with ANTH and/or SOR increased CAT level comparable to that of normal control group, with best effect to the combined treated group . Collectively, these findings indicate that cotreatment of ANTH with SOR gives better therapeutic effect against HCC than each alone.

Anthraquinone active components in Rhubarb can significantly reduce the levels of malondialdehyde (MDA), reactive oxygen species (ROS) and increase the activity of superoxide dismutase (SOD) in rats. This is mainly due to free anthraquinone compounds, which develop the efficacy of inhibiting hepatic oxidation and oxidative stress (OS) through increasing intracellular antioxidant components, reducing lipid peroxidation damage of cell membranes, scavenging oxygen free radicals, inhibiting intracellular ROS, and increasing the activity of hepatocyte**(Zhuang et al., 2020)**. Furthermore, Rhubarb has a significant effect on restraining the evolution of liver fibrosis and cirrhosis. The mechanism is related to the activity reduction of hepaticstellate cell (HSC)**(Wang et al., 2018).**

Oxidation of cells in the liver is generally induced by elevated levels of carbon tetrachloride(CCl4) in the body, usually manifested by an increase in serum MDA concentration and ALT levels, which reflects the degree of lipid peroxidation in the body, indirectly reflecting the degree of cell damage. Rhubarb has the capacity to scavenge free radicals, lower liver MDA level, enhance total antioxidant capacity (T-AOC), improve antioxidant damage, reduce lipid peroxidation, stabilize cell membranes, and thus benefit hepatocyte**(Wang et al., 2015)** . emodin enhanced the antioxidant status of mitochondrial glutathione in liver injury induced by CCl4, helping with relieving hepatocellular injury caused by oxidation **(Zhuang et al., 2020)**.

SOR treatment significantly reduced MDA and increased antioxidant activities of SOD and CAT. These findings are agreeing **(El-Ashmawy et al., 2017)** with which reported that SOR in rat livers exposed to DEN improved oxidative stress parameters .

Another possible way by which cancer cells maintain their high proliferative capacity and survival, is induction of inflammation**(Elgazar et al., 2018)** .

Therefore, inhibition of inflammation is an urgent need for killing cancer cells. ANTH and/or SOR have anti-inflammatory effect on HCC, expression of the inflammation-related genes (IL1b, NF-kB TGF-B and TNF-a genes) was determined. Rats with HCC exhibited the highest expression of IL1b, NF-kB TGF-B and TNF-a genes compared to other groups. In contrast, administration of ANTH and SOR alone or together significantly decreased this elevated expression, with better effect in the combined group.

Nuclear factor-kappa B (NF-κB) is considered a classical target for treating inflammatory diseases**(Kim et al., 2010)** . As a typical activator of the NF-κB signaling pathway, lipopolysaccharide (LPS) induces a large amount of inflammatory factors and aggravates inflammatory reactions, further causing inflammatory damage to living organisms **(Su et al., 2020)**.

Chr(chrysophanol) prevented inflammatory processes induced by LPS in Raw264.7 cells. Chr could potently downregulate the transcription and phosphorylation of NF-κB including p50/p105, p52/p100, RelA(p65), RelB and cRel, downregulate pro-inflammatory cytokines such as IL-1β, TNF-α and iNOS, upregulate PPAR-γ expression in Raw264.7 cells, and further attenuate LPS-stimulated inflammation**(Peng et al., 2018)**

In primary uterine and endometrium epithelial cells isolated from leptospira-infected mice, emodin (40 μg/ml) inhibited the expression of TNF-α, IL-1β, and IL-6 and suppressed the phosphorylation of p38, p65, ERK and JNK via regulating NF-κB and MAPK signaling pathways**(Zhang et al., 2017)** .

In bleomycin-induced pulmonary fibrosis rat model, emodin (20 mg/kg) alleviates fibroblast activation by repressing TGF-β1 expression and SMAD2/3 phosphorylation, and also preformed inhibitory effects on epithelial-mesenchymal transition(EMT) and ECM deposition in human alveolar epithelial A549 cells with similar mechanisms(Zheng et al., 2021).

Both emodin and chrysophanol can inhibit the expression of TNF-_, IL-1_, and vascular adhesion molecule (VCAM-1), enhance the activity of antioxidant enzymes, inhibit the excessive production of NO, scavenge free radicals, and inhibit apoptosis after cerebral ischemic injury**(Li et al., 2019).**

Selim investigated that compounds (curcumin, chrysophanol, physcion and hesperidin) were able to reduce the expression of three main pro-inflammatory mediators (TNF-_, IL-1_ and IL-6) in LPS-induced inflammation in HepG2 cell lines, in a dose dependent manner of 10 _g/mL as the highest dose **(Selim et al., 2019)**.

Our result showed that the HCC group showed the most significant and highest mRNA levels of the invasion/metastasis gene (*MMP9*) and the lowest mRNA levels for the anti-invasive gene *TIMP1*. Administration of ANTH and SOR alone or in combination with or without preconditioning significantly decreased the mRNA levels of *MMP9* and increased the mRNA levels of *TIMP1*, with the best improvement in the ANTH +SOR group compared with the HCC group.

In human cartilage tissue, rhein significantly inhibited the metastasis of chondrocytes by increasing the synthesis of tissue inhibitor of metalloproteinase-1 (TIMP-1) in cells**(Zhuang et al., 2020).**

Emodin(less than 40 μM) dose-dependently suppressed both TRAF6/HIF-1α/VEGF and TRAF6/CD147/MMP9 signaling pathways to simultaneously inhibit pro-angiogenesis and invasion capacity of human anaplastic thyroid cancer cell lines (8505c and SW1736) (Shi and Zhou, 2018). It was also demonstrated that emodin (5–200 μM) repressed MMP-2 and MMP-9 expression to restrain the migration and invasion of HCC MHCC-97H cells, by activating p38MAPK signal and inhibiting ERK/MAPK and PI3K/AKT signals**(Lin et al., 2016)**. Furthermore, as a CK2 inhibitor, emodin (20 μM) was revealed to suppress protein kinase C-induced tumor cell invasion through restraining CK2 activation and subsequently decreasing MMP-9 expression in breast cancer cells (MCF-7)**(Kim et al., 2018)** .

Aloe-emodin, which is extracted from the rhizome of Rheum palmatum, downregulates MMP-2 through a p38 Mitogen-activated protein kinase (MAPK)- Nuclear factor-kB (NF-kB)edependent pathway, thereby leading to the inhibition of invasion by nasopharyngeal carcinoma cells (NPC-TW 039 and NPC-TW 076)**(Hsu and Chung, 2012).**

Zanotto-Filho investigated that low dose sorafenib improved cyclophosphamide efficacy leading to formation of smaller and well-localized fibrotic tumors in MDA-MB231 xenografts as well as inhibited metastasis and improved survival in the 4T1 model. Alkylating agent (methyl methanesulfonate (MMS) and cyclophosphamide) exposure induced expression of various inflammation-related genes, including IL8, MMP1, CXCL2 and COX-2, thereby affecting cancer cell secretome and tumor microenvironment in vitro and in xenografts. In vitro cell culture work demonstrated that these genes exert pro-invasive and angiogenic activities in response to alkylation, and this response can be blocked by sorafenib or MEK1/2 inhibitor with only minor impact on cell viability**(Zanotto-Filho et al., 2018)**.

4. Conclusion

The present study demonstrated for the first time that administration of Anthraquinones either alone or in combination with sorafenib was effective in ameliorating the hepato-carcinogenic effect of DEN/CCl4 through inhibition of inflammation, oxidative stress, and invasion/metastasis. This treatment

strategy could also be useful in future targeting of liver disease in patients. However, additional studies are needed to confirm whether this treatment strategy is clinically relevant in human subjects.

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