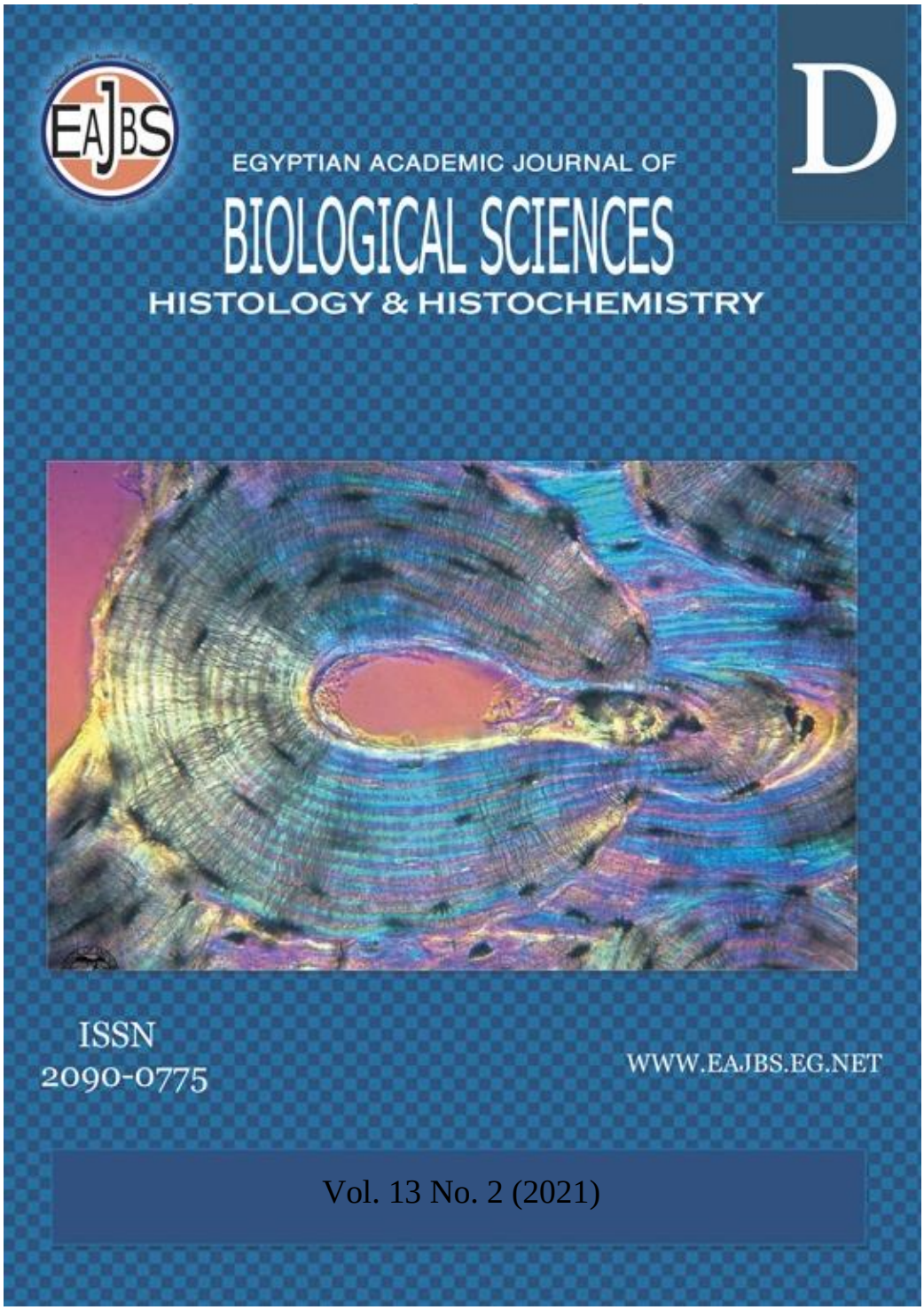

Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.13(2) pp61-68(2021)*
DOI: 10.21608/EAJBSD.2021.196336

Egypt. Acad. J. Biolog. Sci., 13(2): 61-68 (2021)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg

Amygdalin Enhances the Antitumor Effect of Sorafenib

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ARTICLE INFO
Article History
Received:29/7/2021
Accepted:3/9/2021

***Keywords*:**
Antitumor, Amygdalin, Sorafenib, EAC-bearing mice.

ABSTRACT

Sorafenib (SOR) is a potent chemotherapeutic agent used for cancer treatment, however, it has several side effects upon administration on some vital organs. Amygdalin (AMY) is a vitamin B-17 that showed several biological activities as an antioxidant, anti-inflammatory, and anticancer agent. This study was conducted to evaluate the effect of the treatment with a combination of SOR/AMY in Ehrlich ascites carcinoma (EAC)-bearing mice. Twenty-four female CD-1 mice were divided into four groups (n=6) as follows: Group 1 (Gp1) was inoculated with 2 × 10^6 EAC-cells/mouse and served as a positive control. Gp2, 3, and 4 were inoculated with the same number of EAC-cells as in Gp1, and then injected with AMY (50 mg/Kg/14 days), SOR (10 mg/Kg/14 days), and AMY/SOR intraperitoneal (i.p.), respectively. Bodyweight changes, hematological changes, and antitumor indices were evaluated post-treatments. The results showed that AMY had a slight antitumor effect against EAC-bearing mice. Compared to EAC-bearing mice treated with SOR alone, EAC-bearing mice treated with AMY/SOR showed a decrease in the final body weight, tumor volumes, tumor cells count, totally live and dead tumor cells. Furthermore, treatment with AMY/SOR ameliorated the hematological changes. In summary, co-treatment with AMY enhanced SOR antitumor efficacy in EAC-bearing mice.

INTRODUCTION

Cancer remains one of the most common diseases worldwide. Unlimited efforts were executed to find new therapies to treat cancer. Eliminating tumours by surgery, chemotherapy, radiotherapy, immunotherapy, and gene therapy is currently in use (Olga *et al.*, 2021). Even though, conventional chemotherapy is considered one of the best choices for cancer therapy, upon treatment with chemotherapeutic agents, drug resistance and side effects on vital organs were existed (El-Naggar *et al.*, 2017). To overcome the chemo-resistance and to decrease chemotherapeutic agents' side effects different protocols have been modified for treatment with different chemotherapeutic agents (Nurgali *et al.*, 2018; El-Naggar *et al.*, 2019).

Treatment with chemotherapies showed toxicities on different vital organs including the liver, heart, and kidneys (El-Sawalhi and Ahmed, 2014; Nasser *et al.,* 2021). Therefore, enhancing the antitumor efficacy of chemotherapies and reducing their toxicities is necessary.

Actually, the use of complementary and alternative medicine has increased in recent decades (Keith *et al.,* 2005). Medicinal plants have been accepted as one of the main sources for drug discovery and development. The natural antitumor agents were able to induce apoptosis in a cancer cell without many side effects (Russo, 2007). Phytochemicals are natural compounds possessing antioxidant, anti-inflammatory, and anti-tumor properties which can prove valuable in the treatment of several diseases (Sharma and Naura, 2020). Phytochemical constitutes possess anticancer properties through blockage of multiple signal transduction pathways, initiation of apoptosis, and up-regulation of anticancer immune response (Liao *et al.,* 2015; Chiang *et al.,* 2015). Flavonoid compounds such as 5,7-dihydroxy-8, methoxyflavone, fisetin, and quercetin are presented as potent sorafenib sensitizers to enhance its anticancer activity in hepatocellular carcinoma (HCC), melanoma, and glioma, respectively (Pal et al., 2015; Rong *et al.,* 2017).

Vitamin B17, otherwise known as Amygdalin (AMY) or laetrile is widely distributed in plants, especially apricot, peach, cherry, and plum, it can hydrolyze and generate prunasin and mandelonitrile under the glycosidase action, such as amygdalase and prunase, and ultimately decomposed into benzaldehyde and hydrocyanic acid (Santos Pimenta *et al.,* 2014, Jasar *et al.,* 2016). AMY itself is non-toxic, but its production HCN decomposed by some enzymes is a poisonous substance (Suchard *et al.,* 1998). B-glucosidases enzyme was found from the intestinal bacteria, it also can be found in edible plants, with the function of decomposing AMY into benzaldehyde, glucose, and hydrocyanic acid (Patil, 2020). In pre-clinical studies, Hwang et al. proved that AMY from *Prunus armeniaca* has anti-inflammatory and antibacterial activities (Hwang *et al.,* 2008; El-Naggar *et al.,* 2020). AMY is known to possess multiple pharmaceutical properties including anti-oxidant, anti-inflammatory, antitussive, anti-asthmatic, and anti-ulcerative effects (Milazzo *et al.,* 2006).

Sorafenib (SOR), an oral multi-kinase inhibitor against Raf kinase and several receptor tyrosine kinases via RAF/MEK/ERK pathway (Gahr *et al.,* 2012). SOR has been approved for the treatment of advanced HCC (Chiang *et al.,* 2012). SOR inhibits the activity of vascular endothelial growth factor receptor 2 (VEGFR2) and platelet-derived growth factor receptor (PDGFR) leading to inhibition of tumor growth and angiogenesis (Liu *et al.,* 2006). Therefore, the aim of this study is to evaluate the role of the co-treatment with AMY on the antitumor efficacy of SOR.

MATERIALS AND METHODS

Chemicals:

Sorafenib (SOR) was purchased from BAYER Company (Germany). Ehrlich ascites carcinoma (EAC) cells were obtained from the Cancer Biology Unit (CBU), Al-Kaser Al-Eini, Egypt, maintained and propagated by serial transplantation (i.p) in mice in an aseptic environment.

Experimental Animals:

Adult female CD-1 albino mice weighing 20 ± 2 g were obtained from National Research Center (NRC, Cairo, Egypt). Mice were handled according to the national ethical guidelines for the care of laboratory animals approved by the Animal Ethics Committee of Faculty of Science, Tanta University, Egypt (IACUC-SCI-TU-0000). Housing was 6/cage, in 12h/12h dark/light cycle under laboratory

condition of temperature and humidity. EAC-cells were harvested from tumor-bearing mice, then the viable and dead tumor cells were counted using the trypan blue method. EAC-cells ($2x10^6$/mouse) were inoculated for testing the antitumor efficacy of AMY and/or SOR in the EAC-mice model.

Experimental Design:

Twenty-four mice were inoculated with 1 $x10^6$ EAC-cells/mouse and then randomly divided into 4 groups (n=6) as follow: Gp1: EAC-bearing mice served as a positive control, Gp2: EAC- bearing injected with AMY (50 mg/Kg/6 days) intraperitoneal (i.p.), Gp3: EAC-bearing mice injected with SOR (10 mg/Kg/6 days) i.p. Gp4: EAC- bearing mice injected with a combination of AMY/SOR as in Gp2 and Gp3, respectively. On day 14, all groups were weighed to determine the final body weight, euthanized, and blood samples were collected for hematological and biochemical analyses. EAC-cells from different groups were harvested, washed, and processed for evaluation.

Determination of Total Body Weight Changes:

All groups of mice were weighted at the beginning (initial b.wt) and at the end of the experiment (final b.wt). The percentage of the change in the total body weight (%T.B. W) was calculated as follows: (final b.wt – initial b.wt / initial b.wt) × 100.

Determination of Hematological Parameters:

The blood samples from all groups were collected for the determination of the total red blood cells count (R.B. Cs), hemoglobin content (Hb), and the total white blood cells count (W.B. Cs) using an auto hematology analyzer (BC-3200, Mindray, China).

Statistical Analysis:

One–way analysis of variance (ANOVA) was used to assess the significant differences among treatment groups. Duncan's test was used to compare all groups against the control group to show the significant effect of treatment. The criterion for statistical significance was set at $p < 0.05$ or $p < 0.01$. All data are presented as mean ± SD.

RESULTS AND DISCUSSION

Effect of AMY or/and SOR Treatments on Body Weight Changes:

The body weight of the mice under the experimental condition was recorded on day 0 and day 14 to calculate the body weight changes (%B.wt). The results showed that there was a significant increase in the body weight changes of untreated EAC-bearing mice as compared to treated EAC-bearing mice (Table 1). EAC-bearing mice treated with AMY or SOR showed a significant decrease in % B.wt when compared to untreated EAC-bearing mice. The treatment with a combination of AMY/SOR showed a significant decrease in % B.wt when compared with EAC-bearing mice alone or EAC-bearing mice treated with AMY alone or SOR alone (Table 1).

The present data showed that the body weight change (%B.wt) of untreated EAC mice significantly increased more than its values in normal control mice because of the rapid and progressive accumulation of ascites tumor cells. These data were in agreement with the study reported by Mansour *et al.* (2019). Treatment of EAC-bearing mice with AMY or with a combination of AMY/SOR showed a significant decrease in %B.wt when compared to untreated mice. These results were agreed with Hassan *et al.* (2019).

Table 1. Change in body weight of EAC bearing mice in different groups

Groups	Change in bodyweight (%)
EAC alone	45.5 [a]
EAC/AMY	31.4 [a]
EAC/SOR	13.2 [b]
EAC/AMY/SOR	4.3 [b]

In each group results for 4 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at P ≤ 0.05. **EAC**: Ehrlich ascetic carcinoma, **AMY**: Amygdalin, **SOR**: Sorafenib.

Effect of AMY or/and SOR Treatments on The Total Ascetic Volume, Viable, And Dead Tumor Cells:

The total tumor volume in EAC-bearing mice treated with AMY alone was not decreased significantly when compared to the untreated EAC-bearing mice (P<0.0001). Moreover, treatment with SOR showed a significant decrease in ascitic volume when compared to the EAC-bearing mice alone (P<0.0001). Treatment with a combination of AMY/SOR showed a highly significant decrease in the total ascitic volume when compared to the EAC-bearing mice alone (Table 2). Total tumor cell count, live and dead EAC-cells in the untreated EAC-bearing mice were 26.86, 25.8, and 0.74 cells/ml, respectively. Compared to the EAC-bearing mice alone, treatment with AMY did not alter the total tumor cell count significantly, however, it decreased the live tumor cells and increase the dead cells (Table 2). Treatment with SOR decreased the total cell count significantly when compared to the untreated EAC-bearing mice alone. A significant decrease in the live tumor cells and a significant increase in the tumor dead cells were noticed post-treatment of EAC-bearing mice with SOR. Treatment with a combination of AMY/SOR, however, decreased the total tumor cell count when compared to EAC-bearing mice treated with SOR alone (Table 2).

The results revealed that there was a regular rapid increase in ascitic tumor volume in the untreated EAC-bearing mice. Furthermore, the treatment with AMY or SOR significantly decreased the tumor volume, viable tumor cell count. Interestingly, there was a marked significant decrease in total tumor cell count observed in the group of mice that were treated with SOR or with AMY/SOR against the control untreated EAC group. It may be concluded that AMY treatment decreased the nutritional fluid volume and arresting tumor growth. This finding was in agreement with Roy *et al.* (2017).

Table 2. Total, tumor volume, live and dead tumor cells of EAC bearing mice in different groups

Groups	Ascitic volume (mL)	Tumor cell count x 10^6/ml		
		Total	Live	Dead
EAC alone	13.25±0.27 [a,b,c]	26.68±0.91[a]	25.85±1.3[a]	0.73±0.21[a,b]
EAC/AMY	9.35±0.23 [a,b,c]	24.5±0.66[a,b,c]	15.65±0.2[a,b]	8.05±0.89[a,b]
EAC/SOR	2.67±0.15 [a,b,c]	2.75±0.79[a,b,c]	1.58±0.45[a,b]	1.27±0.45[a,b,c]
EAC/AMY/SOR	0.29±0.06 [a,b,c]	2.5±0.32[a,b,c]	1.21±0.15[a,b]	0.79±0.23[a,b,c]

In each group results for 4 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at P ≤ 0.05. The only letter (a) showing (non-significant) and the significant are expressed by dissimilar letters. **EAC**: Ehrlich ascetic carcinoma, **AMY**: Amygdalin, **SOR**: Sorafenib.

Effect of AMY or/and SOR Treatments on The Hematological Parameters:

Treatment of EAC-bearing mice with AMY did not alter the hematological parameters when compared to EAC-bearing mice alone. Treatment with SOR, however, led to a significant decrease in the total platelets and total W.B.Cs count. Interestingly, treatment with a combination of AMY/SOR increased the number of platelets and W.B. Cs count closed to the normal values (Table 3).

A significant decrease in Hb concentration, R.B. Cs, platelets, and W.B. Cs were reported in EAC-bearing mice. Typically, in cancer chemotherapy, major problems encountered are myelosuppression and anemia (Steensma, 2008). This may be due to excessive hemolysis and iron deficiency (Kumar *et al.*, 2011). AMY or SOR administration improved the Hb and R.B. Cs count of tumor-bearing animals. SOR might have a partial response to the synthesis of hemoglobin or prevented hemolysis in specific cases (Pitoia *et al.*, 2015). Our results thus suggested that the SOR is involved in low grade of anemia and RBCs count. This could be explained by the dual etiology of SOR and EAC in lessening the Hb and R.B.Cs (Sannigrahi *et al.*, 2012). The existing study signified that the SOR administration slightly returned the platelets counts to be a far from the level of the baseline. However, this is concordant with the Abou-Alfa *et al.* (2018) who concluded that the association between platelet count and outcome of patients with SOR is inconsistent and still controversial.

Table 3. Hematological parameters in different groups under the study

Groups	R.B.Cs ($\times10^6$/µl)	Hb (g/dL)	Platelets ($\times10^3$/µl)	W.B.Cs ($\times10^3$/µl)
EAC alone	7.67±0.16^{a}	10.55±0.26^{a}	661.0±11.26^{a}	12.48±0.35^{a}
EAC/AMY	6.77±0.42^{a}	11.10±0.31^{a,b}	634.8±4.53^{a}	11.45±0.28^{a}
EAC/SOR	6.62±0.11^{a}	9.20±0.22^{a}	441.3±8.54^{a,b,c}	8.40±0.22^{a,b,c}
EAC/AMY/SOR	7.32±0.12^{a}	9.40±0.17^{a}	481.5±10.31^{a,b,c}	9.14±0.30^{a,b,c}

In each group results for 4 rats are expressed by means ± SE. Small (a-c) letters showing the marked change at P ≤ 0.05. The only letter (a) showing (non-significant) and the significant are expressed by dissimilar letters. **EAC**: Ehrlich ascetic carcinoma, **AMY**: Amygdalin, **SOR**: Sorafenib.

Conflict of Interest:

All authors declare that they have no conflict of interest.

Ethical Approval:

All applicable international, national, and institutional guidelines for the care and use of animals were followed. We respected the welfare of animals and excluded situations when animals were in pain.

REFERENCES

Abou-Alfa G. K., Qian Shi, Jennifer J. Knox, Andreas Kaubisch, James Posey, Benjamin R. Tan, Petr Kavan, Rakesh Goel, Philip Edward Lammers, Tanios S. Bekaii-Saab, Vincent C. Tam, Lakshmi Rajdev, Robin Kate Kelley, Abby B. Siegel, Tyler Zemla, Imane H. El Dika, Alan P. Venook, Monica M. Bertagnolli, Jeffrey A. Meyerhardt, Eileen Mary O'Reilly2018.Platelet count at baseline (Plt) and outcomes in patients (pts) with advanced hepatocellular carcinoma (HCC) treated with sorafenib (S) in CALGB80802 (Alliance)(C8). *American Society of Clinical Oncology,* 36 (15): e16107

Chiang IT, Liu YC, Wang WH, Hsu FT, Chen HW, Lin WJ, Chang WY, and Hwang JJ. 2012.Sorafenib inhibits TPA-induced MMP-9 and VEGF

expression *via* suppression of ERK/NF-κB pathway in hepatocellular carcinoma cells. *In Vivo*. 26: 671-681.

Chiang IT, Wang WS, Liu HC, Yang ST, Tang NY and Chung JG: 2015. Curcumin alters gene expression-associated DNA damage, cell cycle, cell survival and cell migration and invasion in NCI-H460 human lung cancer cells *in vitro*. *Oncology reports*, 34: 1853-1874.

El-Naggar SA, El Tantawi HG, Mohamed. A. Ibrahim, Abozer Y. Elderdery. 2017.Treatment with *Nigella sativa* oil ameliorates hepato-renal toxicities induced by cyclophosphamide in splenectomised mice Egypt. *The Egyptian Journal of Experimental Biology (Zoology)*, 13:291–299.

El-Naggar SA, El-Said KS, Albagoury AA, Awad VS, and Attia LH. 2020.Cancer-fighting Phytochemicals of *Prunus armeniaca* and *Prunus domestica* Seeds Extracts. *International Journal of Cancer Research*, 16: 10-17.

El-Naggar SA, El-Said, K. S, Mobasher M., Elbakry M. 2019.Enhancing antitumor efficacy of cisplatin low Dose by EDTA in Ehrlich Ascetic Carcinoma bearing mice. *Brazilian Archives of Biology and Technology*,62. e19180716

El-Sawalhi MM, Ahmed LA. 2014.Exploring the protective role of apocynin a specific NADPH oxidase inhibitor in cisplatin-induced cardiotoxicity in rats. *Chemico-biological Interaction*, 207:58-66.

Gahr, S. T. Wissniowski, S. Zopf, D. Strobel, A. Pustowka, and M. Ocker. 2012. Combination of the deacetylase inhibitor panobinostat and the multi-kinase inhibitor sorafenib for the treatment of metastatic hepatocellular carcinoma-review of the underlying molecular mechanisms and first case report. *Journal of Cancer*, 3:158- 165

Hassan A, Mohammed L, Abd ElMoneim M, and Abd ElBaky A. 2019. Hepatic and Renal Protective Effects of Annona muricata Leaf and Fruit Extracts on Ehrlich Ascites Carcinoma in Mice. *Zagazig Veterinary Journal*, 47(3): 234–247.

Hwang HJ, Kim P, Kim CJ, Lee HJ, Shim I, Yin CS, Yang Y, Hahm DH. 2008. Antinociceptive effect of amygdalin isolated from Prunus armeniaca on formalin-induced pain in rats. *Biological & pharmaceutical bulletin*, 31(8):1559-64.

Jasar D, Filipovski V, Kubelka-Sabit K, and Curcic-Trajkovska B. 2016. Potential benefits and controversies related to use of amygdalin (vitamin b17). *Journal of Hygienic Engineering and Design*, 615:164-577.

Keith VM, Kronenfeld JJ, Rivers PA, and Liang SY. 2005.Assessing the effects of race and ethnicity on use of complementary and alternative therapies in the USA. *Ethnicity and Health*,10(1):19-32.

Kumar RS, Rajkapoor B, and Perumal P. 2011.*In vitro* and *in vivo* anticancer activity of Indigofera cassioides Rottl. Ex. DC. *Asian Pacific Journal of Tropical Medicine*, 4(5): 379–385.

Liao CY, Lee CC, Tsai CC, Hsueh CW, Wang CC, Chen IH, Tsai MK, Liu MY, Hsieh AT, Su KJ, Wu HM, Huang SC, Wang YC, Wang CY, Huang SF, Yeh YC, Ben RJ, Chien ST, Hsu CW and Kuo WH: 2015.Novel investigations of flavonoids as chemo preventive agents for hepatocellular carcinoma.

Biomedical Research International,840542.

Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, Wilhelm S, Lynch M and Carter C. 2006.Sorafenib blocks the RAF/MEK/ERK pathway, inhibits tumor angiogenesis, and induces tumor cell apoptosis in hepatocellular carcinoma model PLC/PRF/5. *Cancer Research*,66: 11851-11858.

Mansour MA, Salama AF, Ibrahim WM, and Shalaan ES. 2019.Assessment of autophagy as possible mechanism of the antitumor effects of arsenic trioxide and/or cisplatin on ehrlich ascites carcinoma model. *Alexandria Journal for Veterinary Sciences*, 61:159–167.

Milazzo S, Ernst E, Lejeune S, and Boehm K. 2006.Laetrile treatment for cancer. *Cochrane Database of Systematic Reviews*, 2.

Nasser HM, El-Naggar SA, Rizk ME, Elmetwalli A, Salama AF. 2021.Effect of sorafenib on liver biochemistry prior to vitamin b17 coadministration in ehrlich ascites carcinoma mice model: preliminary phase study. *Biochemistry Letters*, 17: 40-49

Nurgali K, Jagoe RT, Abalo R. 2018. Editorial article: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae? *Frontiers in Pharmacology;* 9:245.

Olga N, Claudio C, Ilaria P. 2021.Molecularly targeted therapy for advanced gastrointestinal noncolorectal cancer treatment: how to choose? Past, present, future. *Anti-Cancer Drugs*, 32(6):593-601.

Pal HC, Baxter RD, Hunt KM, Agarwal J, Elmets CA, Athar M and Afaq F. Fisetin, 2015. A phytochemical, potentiates sorafenib-induced apoptosis and abrogates tumor growth in athymic nude mice implanted with BRAF-mutated melanoma cells. *Oncotarget*, 6: 28296-28311.

Patil AS. Plant Secondary Metabolites: Isolation, Characterization and Biological Properties; Studera Press. 2020.

Pitoia F., E. Abelleira, F. Jerkovich, C. Urciuoli, and G. Cross, 2015. "Partial response to sorafenib treatment associated with transient grade 3 thrombocytopenia in a patient with locally advanced thyroid cancer. *Archives of Endocrinology and Metabolism*, 59, 4,47–350.

Rong LW, Wang RX, Zheng XL, Feng XQ, Zhang L, Zhang L, Lin Y, Li ZP and Wang X. 2017.Combination of wogonin and sorafenib effectively kills human hepatocellular carcinoma cells through apoptosis potentiation and autophagy inhibition. *Oncology Letters*, 13:5028-5034.

Russo GL. 2007.Ins and outs of dietary phytochemicals in cancer chemoprevention. *Biochemical Pharmacology*, 74(4): 533–544.

Sannigrahi S., U. K. Mazumder, D. Pal, and S. L. Mishra, 2012.Terpenoids of methanol extract of Clerodendrum infortunatum exhibit anticancer activity against Ehrlich's ascites carcinoma (EAC) in mice. *Pharmaceutical Biology*, 50, 3: 304–309.

Santos Pimenta LP, Schilthuizen M, Verpoorte R, and Choi YH. 2014.Quantitative analysis of amygdalin and prunasin in Prunus serotina Ehrh. using 1H-NMR spectroscopy. *Phytochemical Analysis*, 25(2): 122–126.

Sharma S, and Naura AS. 2020.Potential of phytochemicals as immune-regulatory compounds in atopic diseases: A review. *Biochemical Pharmacology,* 173: 113790.

Steensma DP. 2008.Is anemia of cancer different from chemotherapy-induced anemia? *Journal of Clinical Oncology,* 26(7): 1022–1024.

Suchard JR, Wallace KL, Gerkin RD. 1998.Acute cyanide toxicity caused by apricot kernel ingestion. *Annals of Emergency Medicine,*32(6):742–744