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Antioxidant and Cytotoxicity Potential of Piperine and Sorafenib combination in human MDA-MB-231 Breast Cancer cells

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ABSTRACT

Background: Breast cancer is the most common cancer diagnosed in women. Worldwide, it is the second most common cause of cancer-related mortality among women. Plants have been the primary sources of natural product drug discovery, over 60% of the current anticancer drugs were derived from natural sources. Piperine (PIP) is a major bioactive constituent of the black pepper (*Piper nigrum*). **Aim**: We have assessed the cytotoxicity of PIP and the anti-hepatocellular carcinoma drug Sorafenib (SOR) against human triple negative breast cancer MDA-MB-321 cell line. Furthermore, we have investigated the potential effect of single and their combined treatment on antioxidant enzyme activity and lipid peroxidation status. **Methods**: We measured cytotoxicity of PIP and SOR in MDA-MB-321 cells by MTT assay after 48 hours treatment. Activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and the lipid peroxidation marker Malondialdehyde (MDA) were measured colorimetrically. **Results**: Both PIP and SOR caused a significant ($P<0.001$) dose dependent cell death in MDA-MB-321 cells. However, SOR showed higher cytotoxicity (IC_{90}= 144.8 ± 5.1µg/ml) compared to PIP (IC_{90}= 252.4 ± 6.7µg/ml). Consistent with these data, SOR individual treatment caused the highest significant SOD and CAT activities while their combined treatment caused the lowest MDA levels (4.2 ± 0.2 nmol/ml) compared with the control untreated (7.7 ± 0.2 nmol/ml) or DMSO treated cells (7.76 ± 0.25 nmol/ml).
Conclusion: This study points out a potential mode of action for PIP and SOR as anticancer agents in triple negative breast cancer cells through the regulation of their antioxidant and lipid peroxidation status.

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1. Introduction

Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy in every country of the world (1). Female breast cancer is the most commonly occurring cancer worldwide (11.7 % of the total new cases) with approximately 6.9 % of new deaths.

1) Chemotherapy uses powerful chemicals to kill fast-growing cancer cells, however, drugs used for chemotherapy causes damage to healthy cells. The most common side effects of chemotherapy include; fatigue, pain, diarrhea or constipation, blood disorders, nausea, nervous system effects, appetite and hair loss. Current chemotherapeutic options for liver and breast cancer patients include; Sorafenib (SOR), Doxorubicin and Cisplatin. Sorafenib is an oral multikinase inhibitor (2) that blocks tumor cell proliferation and angiogenesis by inhibiting a Raf serine/threonine kinase and vascular endothelial growth factor (VEGF) receptors. Sorafenib is currently used in clinics to treat patients with advanced renal cell carcinoma and hepatocellular carcinoma (3).

Natural products have been regarded as one of the richest sources of chemotherapeutic drug development, especially for cancer and infectious diseases (4), but also in other therapeutic areas, including cardiovascular diseases and multiple sclerosis (5, 6). Natural compounds are excellent candidates in cancer prevention and therapy because scientific evidence has shown that these compounds can directly or indirectly target and regulate genetic expression by interfering with genetic and/or epigenetic machineries (7).

Piperine (PIP) is one of the most widespread dietary alkaloid principally found in the fruits and roots of black pepper (*Piper nigrum)* (8). This compound is well known for its anti-inflammatory, immunosuppressive and anti-cancer actions (9).

In this study, we have assessed the cytotoxicity of PIP and SOR in MDA-MB-321 cells. We have also investigated the potential effect of their single and this novel combination treatment on antioxidant enzyme activity and lipid peroxidation status as a potential anticancer mechanism of action.

2. Materials and Methods

2.1. Chemicals and drugs

PIP was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), SOR was purchased from Cipla Ltd, India. Media, fetal bovine serum and other cell culture materials were purchased from Gibco, Thermo Fisher Scientific (Grand Island, NY, USA).

2.2. Cell line and cell culture conditions

Human breast cancer cell line MDA-MB-321 was supplied from American Type Culture Collection (ATCC). Cells were cultured in a complete DMEM medium and incubated at 37^{o}C in an atmosphere containing 5% CO_2.

2.3. Cytotoxicity assay

MTT assay was performed as described by (10). In brief, MDA-MB-321 cells were seeded at 15×10^3 per well in 96-well plate with 100 µl of fresh complete medium for 24 h before treatment. Cells were then treated and incubated with different concentrations of PIP (12.5–200 µM) and SOR (6.25-100 µM) for 48 h. The "IC_{90}" which is the compound concentration the causes 90% cell death for each compound was calculated by nonlinear regression analysis of the dose response curve in each cell line.

2.4. Cell line treatment

For all biochemical analysis, MDA-MB-321 cells were treated with single or combined 53 µM of PIP and 14

µM of SOR. DMSO treated cells were treated with a final concentration of 0.1% DMSO. All treatments were performed for 48 hours followed by respective analysis. All experiments were performed in triplicate and were independently repeated for at least 3 times.

2.5. Biochemical analysis for antioxidant activity and lipid peroxidation

2.5.1. Superoxide dismutase assay

Superoxide dismutase (SOD) activity was measured using a colorimetric assay kit (Cat. no: SD 25 21) from Bio-diagnostic Co.,Ltd, following the manufacture instructions. In brief, cells were incubated and treated for 48 h prior to analysis. The activity was measured in cell lysate and the absorbance was assessed at 560 nm.

2.5.2. Catalase assay

Catalase (CAT) activity was measured using assay kit (Cat. no: CA 25 17) from Bio-diagnostic Co.,Ltd (11), following the manufacture instructions. In brief, cells were incubated and treated for 48 h prior to analysis. The activity was measured in cell lysate and the absorbance was assessed at 510 nm.

2.5.3. Lipid peroxidation analysis

Considered as a biomarker of lipid peroxidation, malondialdehyde (MDA) was quantified using the MDA kit (Cat. no: MD 25 29) from Bio-diagnostic Co.,Ltd (12) following the manufacture instructions. The activity was measured in cell lysate and the absorbance was assessed at 534 nm.

2.6. Statistical analysis

All quantitative data were presented as the mean ± standard deviation. Repeated measure analysis of variance with post hoc test was performed to determine the statistical significance among different groups using the SPSS software package 20.0 IBM modeler (2009). $P<0.05$ was regarded as significant.

3. Results

3.1. Cytotoxicity of PIP and SOR in MDA-MB-321 breast cancer cell line

The cytotoxic effect of PIP and SOR was investigated in MDA-MB-321 cells after 48 h treatments using the MTT viability assay. Both PIP (Figure 1A) and SOR (Figure 1B) caused a significant ($p < 0.001$*) dose-dependent decrease in cell viability compared to control untreated cells.

The MTT assay showed a gradual reduction in cell viability at high concentrations of PIP and SOR, the highest cytotoxic effect was observed for SOR treatment compared to PIP treated cells. Of note, low doses of PIP did not show significant ($p \geq 0.05$) reduction in MDA-MB-321 cell viability compared to control cells since first significant ($p < 0.001$) reduction in viability compared to control cells was observed only at a concentration of 100 µg/ml. On the other hand, SOR caused a significant ($p < 0.001$) reduction in cell viability using a concentration as low as 6.25 µg/ml.

The results also showed that the IC_{90} values for PIP and SOR which is reflective of cells fully affected by the compound (90% mortality) were 252.4±6.7 µg/ml and 144.8±5.1 µg/ml for PIP and SOR, respectively.

3.2. Effect of PIP and SOR on oxidative stress-related biochemical markers

Sorafenib treatment caused the highest significant ($P< 0.001$) increase in SOD activity (49.1 ± 0.9 U/ml) then combined PIP+SOR treatment (47.2 ± 0.6 U/ml) compared to control untreated (38.7 ± 1.7 U/ml) and DMSO treated cells (35.7 ± 1.5 U/ml), (Figure 2).

Similarly, Sorafenib caused the highest significant ($P< 0.001$) increase in CAT activity (56.0 ± 1.0 U/L) followed by PIP alone treatment (51.0 ± 2.0 U/L) compared to control untreated (29.0 ± 1.0

U/L) and DMSO treated cells (29.8 ± 2.0 U/L), (Figure 3).

On the other hand, the lowest significant (P< 0.001) MDA levels were detected after combined treatment of cells with PIP + SOR (5.8 ± 0.1 nmol/ml) and SOR treatment (4.9 ± 0.2 nmol/ml) compared to control untreated (7.56 ± 0.15 nmol/ml) and DMSO treated cells (7.36 ± 0.15 nmol/ml), (Figure 4).

Collectively, the antioxidant SOD and CAT enzyme activity showed a significant increase while MDA lipid peroxidation biomarker levels showed a significant decrease in PIP and SOR treated MDA-MB-321 cells depending on the treatment.

4. Discussion

Cancer is a multi-factorial disease characterized by uncontrolled proliferation of cells, it usually arises from alterations in several signaling pathways and multiple DNA affecting the survival and development of cells (13, 14). Treatment options for most types of cancers include chemotherapy, surgery and radiotherapy (15). Limitation of chemotherapy are mainly due to its severe life-threatening side effects and chemoresistance (16).

Sorafenib (Nexavar®) is a multi-kinase inhibitor approved by FDA for the treatment of hepatocellular carcinoma (17), renal (18) and thyroid cancer (19). Ongoing clinical trials are also testing the effectiveness of Sorafenib in the treatment of breast cancer patients (20). Overtime SOR resistance and toxicity was reported in hepatocellular carcinoma (21). Combination of natural products with chemotherapies have become the focus of many studies aiming at improving the effect of chemotherapeutic drug and minimize its toxicity (22, 23). Piperine is commonly used for several medicinal purposes (24, 25). To the best of our knowledge, the potential anticancer combinations of PIP with SOR was not investigated before against any type of cancer.

In the current study, we investigated the potential cytotoxicity and mechanisms leading to PIP and SOR anti-tumor actions including; cellular antioxidant and lipid peroxidation status in MDA-MB-231 triple negative breast cancer cell line.

In the current study, we showed that PIP and SOR caused significant cytotoxicity to a variable potency in a concentration-dependent manner. PIP alone treatments showed growth inhibitory effects which agrees with Greenshields et al. who observed that PIP inhibited the growth and motility of MDA-MB-231 cells (26). Piperine was found to target different drug resistance mechanisms in human ovarian cancer cell lines leading to increased sensitivity to cytotoxic drugs such as paclitaxel and topotecan (27). Moreover, PIP was found to suppress the Wnt/β-catenin pathway and has anti-cancer effects on colorectal cancer cells (28). Piperine also was shown to depress the migration progression of prostate cancer cells via downregulating the Akt/mTOR/MMP-9 signaling pathway (29). This wide range anticancer effects of PIP suggests that PIP has broad cytotoxicity actions on different types of cancer cells. Many recent reports including the current study showed promising chemo sensitization effects of PIP when used in combination with several chemotherapeutic drugs used against different types of drug-resistant cancer cells (30, 31). PIP was also reported to exert a synergistic chemomodulatory effects to chemotherapeutic drugs in different types of cancers (32). Piperine has also enhanced docetaxel efficiency in vitro and in vivo in prostate cancer model (33).

Our data also showed that PIP and SOR increased the activity of SOD and CAT, the two enzymes which aid to scavenge superoxide ions and hydroxyl ions, respectively, and caused a

significant reduction in the MDA lipid peroxidation marker. This result agrees with a previous study in colon cancer that suggested piperine to trigger the antioxidant response machinery including CAT and SOD, scavenging ROS, and decreasing lipid peroxidation (34). Similarly, a previous study with PIP indicated has a free radical scavenging capacity (35).

Conclusion

Piperine and SOR have shown a significant cytotoxic action in human triple negative breast cancer cell lines with variable potency. We have also shown that one of the major modes of action of these compounds is by regulating the activity of cellular antioxidant enzymes and lipid peroxidation status.

References

1. Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. 2021.
2. Iyer R, Fetterly G, Lugade A, Thanavala Y. Sorafenib: a clinical and pharmacologic review. Expert opinion on pharmacotherapy. 2010;11(11):1943-55.
3. Siegel AB, Olsen SK, Magun A, Brown Jr RS. Sorafenib: where do we go from here? Hepatology. 2010;52(1):360-9.
4. Harvey AL, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. Nature reviews drug discovery. 2015;14(2):111-29.
5. Tintore M, Vidal-Jordana A, Sastre-Garriga J. Treatment of multiple sclerosis—success from bench to bedside. Nature Reviews Neurology. 2019;15(1):53-8.
6. Waltenberger B, Mocan A, Šmejkal K, Heiss EH, Atanasov AG. Natural products to counteract the epidemic of cardiovascular and metabolic disorders. Molecules. 2016;21(6):807.
7. Kar S, Parbin S, Deb M, Shilpi A, Sengupta D, Rath SK, et al. Epigenetic choreography of stem cells: the DNA demethylation episode of development. Cellular and molecular life sciences. 2014;71(6):1017-32.
8. Kumar S, Kamboj J, Sharma S. Overview for various aspects of the health benefits of Piper longum linn. fruit. Journal of acupuncture and meridian studies. 2011;4(2):134-40.
9. Zadorozhna M, Tataranni T, Mangieri D. Piperine: role in prevention and progression of cancer. Molecular biology reports. 2019;46(5):5617-29.
10. Ismail N, Abdel–Mottaleb Y, Ahmed AAE, El-Maraghy NN. Novel combination of thymoquinone and resveratrol enhances anticancer effect on hepatocellular carcinoma cell line. Future Journal of Pharmaceutical Sciences. 2018;4(1):41-6.
11. Aebi H. [13] Catalase in vitro. Methods in enzymology. 1984;105:121-6.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979;95(2):351-8.
13. Kamb A, Wee S, Lengauer C. Why is cancer drug discovery so difficult? Nature reviews Drug discovery. 2007;6(2):115-20.
14. Ponder BA. Cancer genetics. Nature. 2001;411(6835):336-41.
15. Tappenden P, Chilcott J, Ward S, Eggington S, Hind D, Hummel S. Methodological issues in the economic analysis of cancer treatments. European journal of cancer. 2006;42(17):2867-75.
16. Schirrmacher V. From chemotherapy to biological therapy: A review of novel concepts to reduce the side effects of systemic cancer treatment. International journal of oncology. 2019;54(2):407-19.
17. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J-F, et al. Sorafenib in advanced hepatocellular

carcinoma. New England journal of medicine. 2008;359(4):378-90.
18. Guevremont C, Jeldres C, Perrotte P, Karakiewicz P. Sorafenib in the management of metastatic renal cell carcinoma. Current Oncology. 2009;16(s1):27-32.
19. White PT, Cohen MS. The discovery and development of sorafenib for the treatment of thyroid cancer. Expert opinion on drug discovery. 2015;10(4):427-39.
20. Zafrakas M, Papasozomenou P, Emmanouilides C. Sorafenib in breast cancer treatment: A systematic review and overview of clinical trials. World journal of clinical oncology. 2016;7(4):331.
21. Blanchet B, Billemont B, Barete S, Garrigue H, Cabanes L, Coriat R, et al. Toxicity of sorafenib: clinical and molecular aspects. Expert opinion on drug safety. 2010;9(2):275-87.
22. Garcia-Oliveira P, Otero P, Pereira AG, Chamorro F, Carpena M, Echave J, et al. Status and challenges of plant-anticancer compounds in cancer treatment. Pharmaceuticals. 2021;14(2):157.
23. Nair HH, Alex VV, Anto RJ. Significance of nutraceuticals in cancer therapy. Evolutionary Diversity as a Source for Anticancer Molecules: Elsevier; 2021. p. 309-21.
24. Khader M, Eckl PM. Thymoquinone: an emerging natural drug with a wide range of medical applications. Iranian journal of basic medical sciences. 2014;17(12):950.
25. Shityakov S, Bigdelian E, Hussein AA, Hussain MB, Tripathi YC, Khan MU, et al. Phytochemical and pharmacological attributes of piperine: A bioactive ingredient of black pepper. European journal of medicinal chemistry. 2019;176:149-61.
26. Greenshields AL, Doucette CD, Sutton KM, Madera L, Annan H, Yaffe PB, et al. Piperine inhibits the growth and motility of triple-negative breast cancer cells. Cancer letters. 2015;357(1):129-40.
27. Wojtowicz K, Sterzyńska K, Świerczewska M, Nowicki M, Zabel M, Januchowski R. Piperine Targets Different Drug Resistance Mechanisms in Human Ovarian Cancer Cell Lines Leading to Increased Sensitivity to Cytotoxic Drugs. International Journal of Molecular Sciences. 2021;22(8):4243.
28. de Almeida GC, Oliveira LF, Predes D, Fokoue HH, Kuster RM, Oliveira FL, et al. Piperine suppresses the Wnt/β-catenin pathway and has anti-cancer effects on colorectal cancer cells. Scientific reports. 2020;10(1):1-12.
29. Zeng Y, Yang Y. Piperine depresses the migration progression via downregulating the Akt/mTOR/MMP‐9 signaling pathway in DU145 cells. Molecular medicine reports. 2018;17(5):6363-70.
30. Jeong S, Jung S, Park G-S, Shin J, Oh J-W. Piperine synergistically enhances the effect of temozolomide against temozolomide-resistant human glioma cell lines. Bioengineered. 2020;11(1):791-800.
31. Li H, Krstin S, Wang S, Wink M. Capsaicin and piperine can overcome multidrug resistance in cancer cells to doxorubicin. Molecules. 2018;23(3):557.
32. Fattah A, Morovati A, Niknam Z, Mashouri L, Asadi A, Rizi ST, et al. The Synergistic Combination of Cisplatin and Piperine Induces Apoptosis in MCF-7 Cell Line. Iranian Journal of Public Health. 2021;50(5):1037-47.
33. Li C, Wang Z, Wang Q, Ho RLKY, Huang Y, Chow MS, et al. Enhanced anti-tumor efficacy and mechanisms associated with docetaxel-piperine combination-in vitro and in vivo investigation using a taxane-resistant prostate cancer model. Oncotarget. 2018;9(3):3338.
34. Rehman MU, Rashid S, Arafah A, Qamar W, Alsaffar RM, Ahmad A, et al. Piperine Regulates Nrf-2/Keap-1 Signalling and Exhibits Anticancer Effect

in Experimental Colon Carcinogenesis in Wistar Rats. Biology. 2020;9(9):302.
35. Mittal R, Gupta R. In vitro antioxidant activity of piperine. Methods and findings in experimental and clinical pharmacology. 2000;22(5):271-4.

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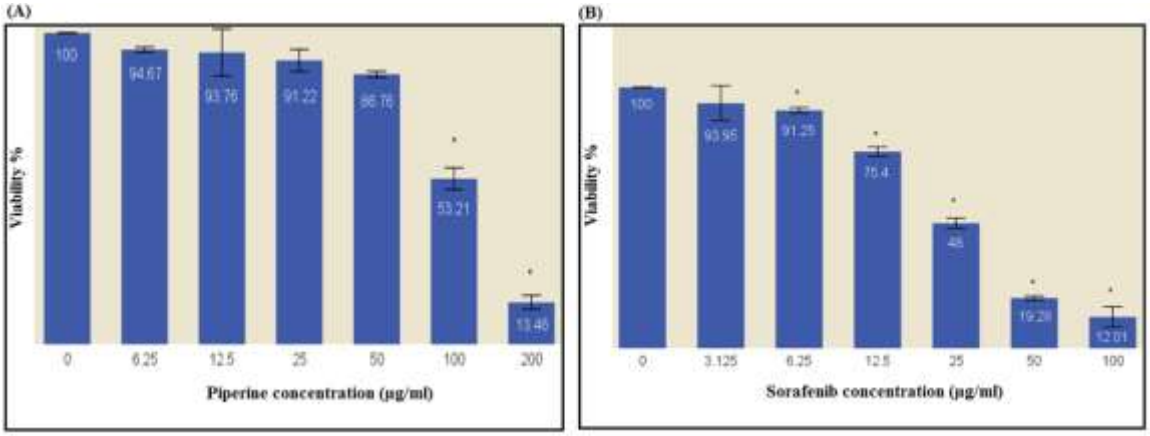

Figure 1. Dose–response bar graph showing the cytotoxic effects of: (A) PIP and (B) SOR, on MDA-MB-231 cells. Cells were exposed to different concentrations of each compound for 48 h and cell viability was determined by MTT assay. Data are expressed as mean ± SD (n = 3). * P<0.05 was regarded as significant compared to control group.

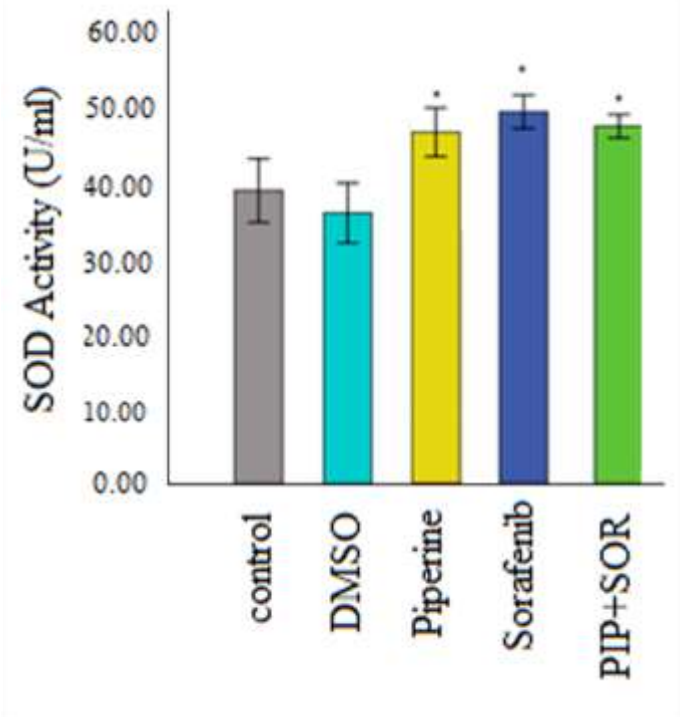

Figure 2. Effect of PIP and SOR treatments on SOD activity: MDA-MB-231 cells were treated individually or in combination with PIP and SOR for 48 hours and enzymatic activity was measured colorimetrically. The experiment was performed in triplicate and was independently repeated for 3 times. Data are expressed as mean ± SD, * P<0.05 was regarded as significant compared to control group.

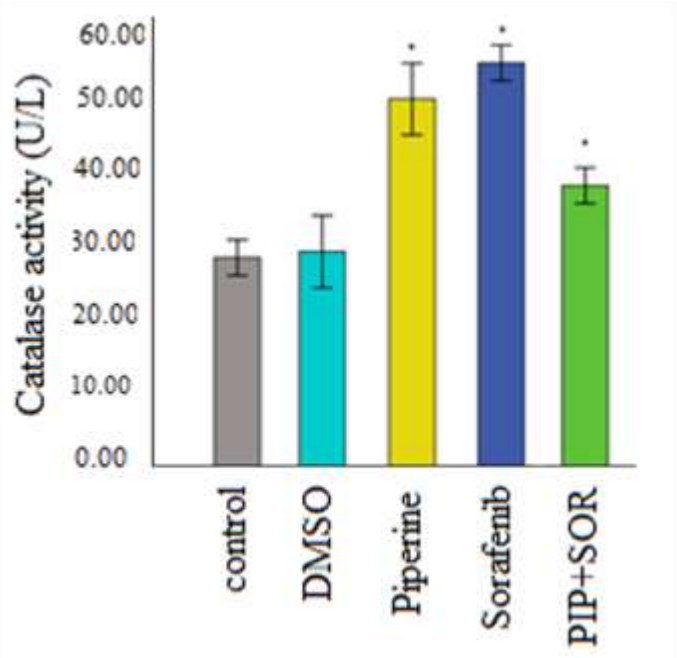

Figure 3. Effect of PIP and SOR treatments on CAT activity: MDA-MB-231 cells were treated individually or in combination with PIP and SOR for 48 hours and enzymatic activity was measured colorimetrically. The experiment was performed in triplicate and was independently repeated for 3 times. Data are expressed as mean ± SD, * P<0.05 was regarded as significant compared to control group.

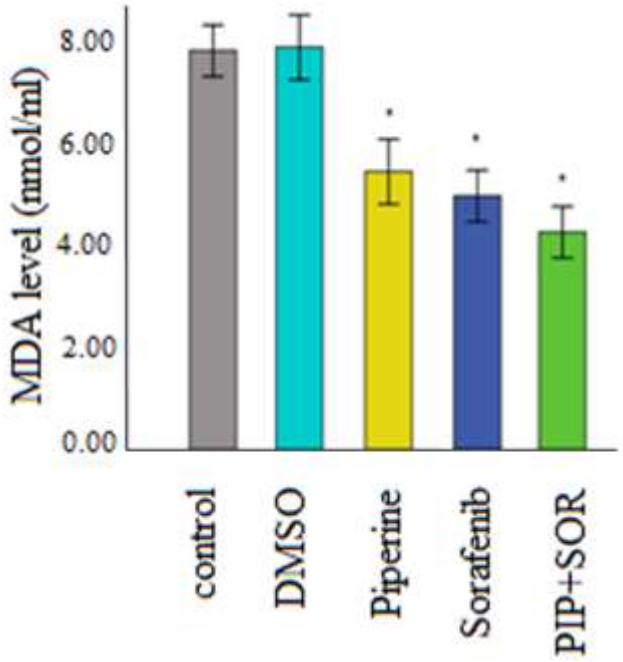

Figure 4. Effect of PIP and SOR treatment on lipid peroxidation MDA marker: MDA-MB-231 cells were treated individually or in combination for 48 hours and MDA content was measured colorimetrically. The experiment was performed in triplicate and was repeated for 3 times. Data are expressed as mean ± SD, * P<0.05 was regarded as significant compared to control group.