

pathway. This miRNA could be a potent therapeutic target to inhibit the stellate cell activation.

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### MIRNA-181A OVER EXPRESSION ACTS AS POTENT ANTI-OXIDANT BY INCREASING SOD-1 AND NRF-2 IN HEPATIC CELLS

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**Background and Aims:** Oxidative stress plays an important role in inducing apoptosis and injury in hepatocytes. In response to the injury, the cells proliferate and replace the injured cells. There is a difference in the protein expression during liver injury, which is tightly regulated by several mechanisms. One of the mechanisms could be through miRNAs. In this study, we hypothesized that miRNA-181a could induce nrf-2, thereby acting as a potent anti-oxidant in decreasing the amount of ROS generated in hepatic cells.

**Methods:** Chang liver cell line was used for all the experiments. miRNA-181a was over expressed in these cells using siPORT NeoFX transfection reagent as per the manufacturer's instructions. The cells were collected after 72 h of transfection. The cells were collected for either protein or RNA isolation. Total RNA was isolated, cDNA was synthesized and the real time RT-PCR was performed for superoxide dismutase-1 (SOD-1), SOD-2 and glutathione peroxidase-1. Western Blots were performed for erk1/2, nrf2 and b-actin.

**Results:** miRNA-181a over expression resulted in a significant increase in the SOD-1 levels (5-fold increase compared to control). There was no significant change in SOD-2 and Gpx-1. Western Blot analysis revealed that over-expressing miRNA-181a also upregulated the protein levels of Nrf2 (2-fold as compared to control) and erk1/2.

**Conclusion:** The data from this study showed that the over expression of miRNA-181a in non-cancerous hepatic cells increased the SOD-1 levels and nrf-2 levels, which could play a role in decreasing ROS levels in hepatic injury.

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### ANTI-PROLIFERATIVE AND ANTICANCER PROPERTIES OF FULVIC ACID ON HEPATIC CANCER CELLS

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**Background and Aims:** Fulvic acid (FA) is considered as the most effective antioxidant present in the Nature and used for several body complaints. It is a member of humic substance found most abundantly in the Nature, and is a main active component of the mineral pitch. Although used for years, the anticancer properties and antiproliferative properties are not documented in detail. In this study, we, have analysed and antineoplastic and growth inhibitory properties of the Fulvic acid.

**Methods:** Different concentrations of fulvic acid (10–1000 µg/ml) was incubated with the Huh 7 cells for 24 h. To study the anticancer property, these cells were treated with fulvic acid and MTT assay, tunnel assay, DNA fragmentation assay were performed. The nitrous oxide production was determined using Griess reagent and the colonogenic assay was performed using crystal violet staining.

**Results:** MTT assay demonstrated the growth inhibition by FA with increasing concentrations. The 1000 µg/ml showed the highest inhibition (73.23%), while the 56.4% and 45.36% inhibition were measured by the 500 and 200 µg/ml concentrations. The production of nitric oxide was increased in the Huh-7 cells with increasing concentrations of FA. There was a 24.04%, 26.17%, and 45.59% increase in the nitric oxide levels when the cells were incubated with 100, 500, and 1000 µg/ml concentration respectively. There was a significant decrease in the colony formation, confirming the anti-proliferative property of the FA. The TUNEL assay and DNA fragmentation assay showed an increased DNA damage and apoptosis with increasing concentration of FA.

**Conclusion:** This study showed that FA has the anticancer properties by increasing apoptosis and via production of nitric acid and reactive oxygen species. Our study demonstrated that fulvic acid may be useful in the liver cancer and related disorders.

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