SUMMARY AND CONCLUSIONS

Skin is the outermost important organ that forms the major organic barrier to external xenobiotics and stimuli such as ultraviolet radiation. The intensity levels of ultraviolet (UV) radiation, predominantly UVB, can act as a complete carcinogen by multiple numbers of intracellular signaling cascades. Recently, increasing effort has been put into finding novel approaches to manage risk factors for skin cancers, including the damage from frequent exposure to solar UV-radiation, in particular from its UVB component. Polyphenols, from natural and herbal extract are raising great interest as powerful and safe anticancer strategy for their broad range targeting capability and low side effects. In this study, we investigated the preventive effect of ferulic acid, a naturally occurring phenolic compound, on UVB-induced cellular and molecular changes in human dermal fibroblasts (HDFa) and in chronic UVB-exposed tumor bearing mice skin. The summary of the present findings are given below.

**Sun protection factor of FA**

In this study, FA exhibits maximum absorbance at 258 nm with an additional absorbance at 335 nm. FA showed SPF value of 9.5 and this was a significant value when compared with standard commercially available sunscreens.

**FA modulates acute UVB-effects in HDFa**

**Protective effect of FA against UVB-induced cytotoxicity in HDFa cells**

In this study, one time UVB-exposure significantly decreased HDFa viability. Conversely, FA pretreatment (10, 20, 40 µg/ml) significantly prevented UVB-induced cell death and restored cell viability in a concentration dependent manner.
FA prevents UVB-induced ROS generation

The intracellular ROS production was significantly higher in acute UVB-irradiated HDFa cells compared to the control. FA pretreatment (10, 20, 30 µg/ml) significantly decreased the intracellular ROS production in UVB-irradiated HDFa cells in a dose dependent manner. Among all the concentrations studied, 40 µg/ml of FA treatment almost restored ROS accumulation to the basal level.

FA prevents UVB-induced lipid peroxidation and restores cellular antioxidant status

Levels of TBARS were increased significantly in UVB-irradiated HDFa cells. FA pretreatment shows progressively decreased levels of TBARS when compared with UVB-irradiated HDFa cells. Our study shows that UVB-irradiation caused significant decrease in the activities of enzymatic antioxidants such as SOD, CAT and GPX in HDFa cells. Significantly increased activities of SOD, CAT, GPX and GSH were observed in FA pretreated HDFa cells.

FA prevents UVB-induced mutagenesis and DNA-damage

Marked increase in the number of revertant colonies was observed in the UVB-exposed cells compared with control group. No mutagenicity was observed in FA alone treated group. Whereas, FA pretreated plus UVB-irradiated cells showed significantly decreased number of revertant colonies.

UVB-irradiation significantly increased % head DNA, tail length, tail moment and OTM in HDFa cells. FA (10, 20, 40 µg/ml) pretreatment significantly decreased the levels of DNA damage in a concentration dependent manner.
**FA inhibits UVB-induced activation of inflammatory markers in HDFa**

FA (40 µg/ml) treatment downregulated TNF-α and COX-2 expression compared with control group. UVB-treated cells showed increased TNF-α and COX-2 expression 4 h after irradiation. Treatment with FA (40 µg/ml) before UVB-irradiation markedly decreased the TNF-α and COX-2 expression.

**Molecular docking of FA with PPARα/γ**

FA was docked against PPARα (PDB code: 1K7L). The energy score of PPARα with cocrystralized ligand was -86.02 (kcal/mol) and with FA was -39.31 (kcal/mol). PPARα has a common hydrogen bond interaction (Tyr 464 and Ser 280) with cocrystralized ligand and FA. The amino acid residues such as Phe 273, Cys 276 and Ile 354 showed hydrophobic bond interactions with FA and the cocrystralized ligand.

The energy score of PPARγ with ligand retinoic acid was -49.18 (kcal/mol) and with the FA was -40.44 kcal/mol. PPARγ possess a common hydrogen bond interaction (Arg 316) with retinoic acid and FA. The amino acid residues such as Ala 272, Ile 268, Leu 326, Leu 309, Phe 313 and Ile 310 showed hydrophobic interactions with FA and retinoic acid. The amino acid Ala 271 was interacting with FA through hydrogen bonding and interacting with retinoic acid through hydrophobic interaction.

**Molecular docking of FA with COX-2**

The energy score of COX-2 with the cocrystralized ligand was -62.09 kcal/mol and with FA the energy score was found to be -37.82 kcal/mol. COX-2 posses a common hydrogen bond interaction (His 90) with cocrystralized ligand and FA. The amino acid Leu 352 was interacting with FA through hydrogen bonding and interacting with cocrystralized ligand through hydrophobic interaction.
The amino acids such as Ser 353 and Val 523 shows hydrophobic bond interactions with FA and the cocrystallized ligand.

**FA activates PPARα/γ mRNA expression in HDFa**

The mRNA levels of *PPARα/γ* were downregulated in UVB-exposed HDFa. Whereas, FA treatment prevented the UVB induced loss of *PPARα/γ* and up-regulated these proteins expression in HDFa cells.

**Effect of FA on mRNA expressions in UVB irradiated HDFa**

The mRNA levels of *XRCC1, GADD45a, ATM* and *hOGG1* were overexpressed in UVB-exposed HDFa than non-UVB-exposed control HDFa. FA pretreatment (40 μg/ml) downregulated this expression pattern of these gene expressions in HDFa.

**FA on UVB-induced apoptotic incidence in HDFa**

UVB treatment significantly decreased the mitochondrial membrane potential levels. FA treatment before UVB exposure significantly prevented the loss of mitochondrial membrane potential in a dose-dependent manner. Further, The amount of nuclear fragmentation and apoptotic incidence were dramatically reduced when the cells were pretreated with FA.

**Preventive role of FA on UVB-induced photocarcinogenesis**

**FA inhibits UVB-induced skin carcinogenesis**

The tumor incidence was decreased in the groups of mice treated with FAT, FAIP (50 mg/kg b.wt.) before UVB-exposure. A total of 35 tumors/8 mice and 400 mm³ tumor volumes were recorded in the chronic UVB-irradiated mice skin. FAIP plus UVB irradiated group of animals showed only 12 tumors/8 mice; FAT – UVB group of animals showed only 11 tumors/8 mice.
FA attenuates UVB-induced histopathological changes in mouse skin

UVB-irradiation significantly increased hyperplasia, dysplastic feature and microinvasive squamous-cell carcinomas (SCC) in the epidermis and dermis part of the mice skin. FA treatments attenuated the UVB-induced hyperplasia, dysplastic feature and microinvasive squamous-cell carcinomas and degradation of collagen fibers in the histological section.

FA modulates UVB-induced oxidative stress

We found that exposure of UVB showed increased levels of TBARS and LPH. Topical treatment of FA reduced lipid peroxidative markers in mice skin homogenate. It has been also noticed that treatment of FA prevented UVB-induced depletion of enzymatic activities (SOD, CAT and GPX) and non-enzymatic antioxidants (GSH, vitamin-C and vitamin-E) levels in UVB-exposed tumor bearing mice skin tissue.

FA inhibits UVB-induced VEGF and iNOS expression

UVB-exposure caused enhanced expression of VEGF and iNOS in epidermal and dermal cells as evidenced by the intensive dark brown staining (positive cells) than non-UVB-exposed control mice skin. The treatment with FA (1 mg/mouse) suppressed UVB-induced expression of tumor angiogenic growth factors in epidermal and dermal portions.

FA inhibits TNF-α and IL-6 expressions

Exposure of the skin to UVB-radiation resulted in the induction of inflammatory responses. Chronic UVB-irradiation induced increased expression of TNF-α and IL-6 in the tumor bearing mice skin. Topical application of FAIP, FAT (50 mg/kg b.wt.) before each irradiation decreased the expressions of these inflammatory markers.
**FA modulates apoptotic signaling in Chronic UVB-exposed mice skin**

Immunohistochemical analysis revealed that chronic UVB-exposure resulted in enhanced expressions of mutated p53 and Bcl-2 expressions and decreased expression of Bax when compared to the non-UVB-exposed mice skin tumor. FAIP and FAT application before each UVB-exposure enhanced the expression of Bax and reduced mutated p53 and Bcl-2 expressions in the tumor of the mice skin.

**Conclusion**

We investigated the preventive effect of FA on UVB-radiation induced cytotoxicity, DNA damage and repair signaling, mutagenesis, lipid peroxidation and antioxidants status in cultured skin fibroblast cells. Chronic UVB-irradiation increased the skin tumor incidence, oxidative imbalance angiogenic and inflammatory signaling events. It has also been observed that decreased apoptotic events with concomitant increases of mutated p53 in the chronic UVB-irradiated mice skin. FA administration before each UVB-irradiation significantly decreased the skin tumor incidence, oxidative imbalance, angiogenic and inflammatory signaling with increased apoptotic signaling. Thus, FA modulates UVB-induced cellular and molecular events in human dermal fibroblasts and Swiss albino mice. In this study both intraperitonial (FAIP) and topical (FAT) treatments of ferulic acid offers similar photoprotection efficacy in Swiss albino mice.

**Recommendation of the study**

The use of FA in skin care lotions may be an effective strategy for mitigating the biological effects of solar UVB-radiation that will lead to the protection of the skin from various skin diseases caused by excessive sun exposure.