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SUMMARY AND CONCLUSION

Herbal medicine has become an integral part of standard health care, based on a combination of time honored traditional usage and ongoing scientific research. Interest in medicinal herbs has increased scientific scrutiny of their therapeutic potential and safety. Some of the medicinal plants are believed to enhance the natural resistance of the body during infections. Extracts of plants have provoked interest as source of natural products and they have screened for their potential use as alternative medicines for the treatment of many infectious diseases.

Seeking remedies for human aliments from the environment has formed the basis for therapeutics. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefit and more affordable treatment. In modern days, the antioxidant and antimicrobial activities of plant extract have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy.

Some of the bioactive principles in plants are preferred for their therapeutic purposes either singly or in combination to inhibit the life processes of microbes. Besides, the rapid evolution of numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drugs underlies the urgency with which alternative chemotherapeutic agents with possible novel mechanisms of action to the microbes needs to be developed which formed the basis of our study.

India is one of the richest floristic regions of the world, well known for its famous heritage regarding medicinal plants and plant drugs. Since the time of Rig Veda medicinal plants are used pharmaceutically in various Indian medical systems in Ayurveda, Allopathy, Unani and Homeopathy. The significance of herbals and herbal products is gaining worldwide recognition. The concept of

complementary or alternative medicine is becoming much more widely accepted and there is an increasing belief in the efficacy of herbal remedies.

Tumors are mystifying diseases which remain completely incurable inspite of large number of current research works in this area. The major form of treatment of tumor is surgery and radiation. A chemotherapeutic agent can often provide temporary relief with painful side effects. A successful antitumor agent without causing any damage to normal cells are rare or practically nil. In this context, the use of plant extract in the treatment of tumor is more relevant. Eventhough India is enriched with a wide array of valuable medicinal plants, no comprehensive, systematic and controlled survey of antioxidative, antitumorigenic and antimicrobial activities have been undertaken so far.

The present research entitled, **"Antitumorigenic Effect in DLA Tumor Induced Mice and Antimicrobial Potential of *Ficus racemosa* and its Characterization by Spectral and *in silico* Studies"** was carried out and the results are summarized in this chapter.

The study was carried out in five phases.

The Phase I was designed to assess the *in vitro* antioxidative and antitumorigenic potential of methanolic extract of *Ficus racemosa* bark (MEFrB) fruits (MEFrF) and leaves (MEFrL). *In vitro* antioxidative role of different parts of *Ficus racemosa* was evaluated by their ability to scavenge the radicals such as ABTS, DPPH, Hydroxyl, Superoxide and non radicals such as Hydrogen peroxide and Nitric oxide. *In vitro* antitumorigenic potential was assessed by their cytotoxic effect to intraperitoneally propagated DLA tumor cells by MTT dye reduction and Trypan blue exclusion assays.

In Phase II the assessment of the *in vivo* antioxidative and antitumorigenic efficacy of MEFrB, MEFrF and MEFrL in DLA transplanted Swiss albino mice were carried out. The antioxidative role was assessed against the standard antioxidant silymarin. The antioxidative and antitumorigenic effect was assessed in DLA tumor transplanted mice.

In Phase III evaluation of the antimicrobial activity of MEFrB, MEFrF and MEFrL was carried out using the selected bacterial species *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri and Staphylococcus aureus.*

The Phase IV involved the characterization of the phytochemical constituents of MEFrB by chromatographic and spectral studies.

In the final Phase V *in silico* Glide docking studies were carried out to characterize the antitumorigenic efficacy against the tumor target proteins Histone deacetylase (1T69) and Tubulin (1Z2B) and antimicrobial efficacy against microbial target proteins Deacetylase C synthase (1W2N) and Pantothenate kinase (ISQ5) of phytochemical constituents of MEFrB.

All the three parts of *Ficus racemosa* scavenged the radicals such as ABTS, DPPH, hydroxyl, superoxide and non radicals such as hydrogen peroxide and nitric oxide in a concentration dependent manner. The ED_{50} of MEFrB was found to be in the order of OH (40µg) > ABTS (42µg) > H_2O_2 (43µg) > NO (44µg) > $O_2^{\bullet -}$ (47µg) > DPPH (48µg); the ED_{50} of MEFrF was found to be in the order of ABTS (46µg) >DPPH (49µg) > H_2O_2 (53µg) > OH (53µg) > NO (54µg) > $O_2^{\bullet -}$ (55µg) and the ED_{50} of MEFrL was found to be in the order of ABTS (43µg) > OH (45µg) > H_2O_2 (47µg) NO (48µg) > DPPH (48µg) > $O_2^{\bullet -}$ (52µg) and the ED_{50} of Standard ascorbic acid was found to be in the order of ABTS (49µg) >NO (55µg) > H_2O_2 (55µg) > $O_2^{\bullet -}$ (56µg) OH (59µg) >DPPH (60µg). The results indictaed the antioxidative role of all the extracts and was found to be more pronounced than that of the standand antioxidant ascorbic acid. The antioxidative role was found to be more pronounced in MEFrB.

The *in vitro* cytotoxicity study by MTT assay showed the cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA tumor cells in a dose dependent manner and ED_{50} was found to be 40µg, 42µg and 46µg and Trypan blue assay also revealed the concentration dependent cytotoxic effect of MEFrB, MEFrF and MEFrL to DLA

tumor cells with the fifty percent effective dose ED_{50} at 54µg, 58µg and 60µg respectively and indicated their antitumorigenic potential.

After establishing the *in vitro* antioxidant and antitumorigenic potential in phase I the *in vivo* antitumorigenic efficacy of MEFrB, MEFrF and MEFrL was evaluated in Phase II by the assessment of the liver marker enzymes, enzymic and non enzymic antioxidants and TBARS along with the histological analysis of the liver compared to the standard antioxidant silymarin after 20 days and 90 days of the experimental tenure in 11 groups (group 1 - PBS; group 2 - Paraffin oil; group 3 - DMSO; group 4 - Silymarin; group 5 – MEFrB ; group 6 -MEFrF; group 7 – MEFrL ; group 8 - MEFrB +DLA; group 9 - MEFrF +DLA; group 10 MEFrL +DLA and group 11 DLA) of mice with six Swiss albino mice in each. The experimental design was approved by the Ethical Committee (Reg no: 623/02/b/CPCSEA).

The liver marker enzymes namely AST, ALT and ALP were analysed in all the experimental animals to assess the normal functioning of the liver. The activities of all the three liver marker enzymes were found to be significantly increased in DLA tumor induced mice. The increase in the activities of these enzymes in serum may be due to hepatocellular damage which resulted in the leakage of cytosolic enzymes into the circulation. The activities of the liver test enzymes AST, ALT and ALP were found to be significantly decreased by the administration of MEFrB, MEFrF, MEFrL and silymarin. The coadministration of MEFrB, MEFrF and MEFrL to DLA tumor induced mice showed a significant decrease in the activities of the above enzymes. The results showed that the activity of all the three liver function test enzymes increased and induced the liver damage in DLA transplanted mice and this was reverted back towards the normal status by the coadministration of MEFrB, MEFrF and MEFrL to DLA induced mice. The above observations of the present study could be attributed to the significant protective effect of the MEFrB, MEFrF and MEFrL by establishing the normal functions of the liver.

Intraperitoneal transplantation of DLA tumor cells altered the antioxidant balance of the mice liver by significantly decreasing the activities of enzymic antioxidants CAT, SOD, GPx and also the levels of non enzymic antioxidants

Vitamin A, E, C and GSH. Administration of all the three extracts individually and to DLA tumor induced mice significantly enhanced the enzymic and non enzymic antioxidant status in all treatment periods and this was found to be more significant than that found in silymarin administration. These results indicated that all the three extracts significantly enhanced the enzymic and non enzymic antioxidant status and protected the cells from oxidative damage caused by DLA tumor cells. These results indicated the antioxidative potential of MEFrB, MEFrF and MEFrL.

Lipid peroxidation status was assessed by the level of TBARS and it was found to be significantly increased in the liver of mice transplanted with DLA tumor cells and decreased by the administration of MEFrB, MEFrF and MEFrL and silymarin and coadministration of MEFrB, MEFrF and MEFrL to DLA tumor cells treated group. Increase in the concentration of TBARS observed in tumor induced mice is the indication of membrane damage. Thus, it could be inferred that MEFrB, MEFrF and MEFrL would have strengthened the endogenous antioxidant defense and restored the optimal balance by neutralizing the ROS. Administration of MEFrB, MEFrF and MEFrL individually and to the DLA tumor induced mice showed significant decrease in the rate of lipid peroxidation and showed their antilipidperoxidative role.

The *in vivo* cytotoxic study in DLA tumor bearing mice showed an average life span of 20 days whereas administration of MEFrB, MEFrF and MEFrL to DLA transplanted mice increased the average life span to 90 days, 82 days and 81days respectively and indicated their antitumorigenic effect.

The results of histological examination of the liver of control groups, silymarin, MEFrB, MEFrF and MEFrL treated groups showed normal architecture in all the portal triads, sinusoids, kupffer cells and central veins. The histological observations in the DLA treated mice showed severe necrosis, with the peripheral rim of surviving liver cells with focal statosis and balloon degeneration. Liver of MEFrB + DLA, MEFrF + DLA and MEFrL + DLA treated mice also showed normal architecture. These observations indicated the reversal of DLA induced membrane

damage by the coadministration of MEFrB, MEFrF and MEFrL. The results of the histological studies also strongly supported the antioxidative and antitumor activity of MEFrB, MEFrF and MEFrL in DLA tumor induced Swiss albino mice.

The Phase III results showed that the exposure of MEFrB exhibited the maximum zone of inhibition against all the selected Gram negative bacterial pathogens namely *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri* and Gram positive pathogen *Staphylococcus aureus* which was followed by MEFrF and MEFrL. These observations clearly indicated the antibacterial activity of MEFrB, MEFrF and MEFrL.

In Phase IV, an attempt was made to determine the active principles present in all the three extracts. The results revealed the presence of fats, flavonoids, fixed oils, phenolics, saponins, steroids, tannins and terpenoids. To confirm the nature of the active principles GC-MS, HPTLC and FT-IR analysis were carried out. The GC-MS analysis showed the presence of 24 compounds in MEFrB and HPTLC analysis showed the presence of 12 flavonoids, 10 phenolic compounds and 5 terpenoids against the standards catechin, rutin and solanesol respectively. FT-IR analysis of MEFrB showed the peak at 3424.96 cm^{-1} revealed C-H, O-H and N-H stretching variations of alcohols and phenols. The peak at 1657.52 cm^{-1} revealed C=O stretching variations of aldehyde / ketones / carboxylic acids / esters and NH primary amines. The peak at 1392.35 cm^{-1}revealed C-H stretching variations of aromatic bonding and 667.25 cm^{-1} revealed C-H stretching variations of alkanes. The peak at 1108.87 cm^{-1} revealed C-O stretching variations of alcohols / ethers / carboxylic acids and esters, the absorbance bands known to be associated with the stretching vibrations for –C, C-CO, -C-C (aromatic ring), C-O (esters and ethers) and C-O (polyols). Thus, FT-IR analysis confirmed the presence of characteristic functional groups such as phenolic, aliphatic, aldehydes, cyanides and halogen compounds in MEFrB. Further studies have to be carried out to analyse the functional groups of each phytochemical constituents.

In Phase V the 24 compounds of MEFrB as identified in GC- MS analysis were subjected to Standard Precision (SP) docking and Extra Precision (XP) docking against the tumor target proteins histone deacetylase and tubulin with co-crystal interaction of Octanedioic acid hydroxyamide phenylamide (SHH) and 2alpha, 2'beta, 3beta, 4alpha, 5beta - vincaleukoblastine (VLB) and microbial target proteins deacetylase C synthase and pantothenate kinase with co-crystal interaction of 2s,6r)-6-{[(2r)-2-amino-2-phenylethanoyl]amino}-3,3-dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0]heptane-2-carboxy-licacid (PN1) and Pantothenoic acid (PAU) respectively. Out of 24 compounds, only seven compounds were found to be successful in Standard Precision docking and Extra Precision docking. Induced fit (IF) docking showed high score for two compounds namely compound 4 [(Z)-(2S, 3S)-2, 3 bis [(methoxy -methyl) oxy] -5-(4-methoxyphenyl) pent-4-enol] and compound 5 [(E)-(2S, 3S)-2, 3-bis [(methoxymethyl) oxy]-5-(4-methoxyphenyl) pent-4-enal] and confirmed their antitumorigenic and antimicrobial effect. ADME properties of these two compounds in QikProp program showed drugable property, solubility and permeability and also obeyed the Lipinski's rule.

The results of *in vitro* and *in vivo* studies showed the strong evidences for the antioxidative, antitumorigenic and antibacterial potential of MEFrB, MEFrF and MEFrL. The antioxidative, antitumorigenic and antimicrobial role of these extracts may be attributed to the presence of phytochemical constituents. Apart from the other minor and major components of MEFrB, the presence of two compounds namely [(Z)-(2S,3S)-2,3 bis [(methoxy -methyl)oxy]-5-(4-methoxyphenyl)pent-4-enol] and [(E)-(2S,3S)-2,3-bis [(methoxymethyl) oxy]-5-(4-methoxyphenyl) pent-4-enal] may be responsible for the *in vitro* and *in vivo* antioxidative and antitumorigenic potential against DLA tumor cells, tumor target proteins and antimicrobial activity against selected pathogen microbes and microbial target proteins. Thus, based on the above results, it can be suggested that all the three extracts and the two compounds of MEFrB can be recommended as antioxidative, antitumorigenic and antimicrobial agents to the individuals suffering from oxidative degenerative diseases and microbial infections.

SUGGESTIONS FOR FUTURE RESEARCH

The outcome of the present study has opened the way for addressing several other research problems in the current scenario. Some of the suggestions for the future research include:

- Isolation and purification of individual phytochemical constituents.
- The active component can be subjected to clinical trials to develop a novel drug to alleviate the side effects of chemotherapy and as antitumor drugs and natural as antibiotics.
- Before recommending as antitumor and antimicrobial agent the active components can further be docked against the other tumor markers and microbial target proteins.
- The lead compounds can also be screened for their efficacy against the other bacterial, fungal strains and other tumor cell lines.