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Fermented Papaya Preparation (FPP) is a nutraceutical available online in the western countries by the name Immun'Age. It is a powerful antioxidant and is believed to cure several oxidative stress related disorders. Doxorubicin, a potent anticancer drug used to treat several kinds of tumors, has a limited applicability due to its severe toxic effects at higher dose. Therefore, the work presented herein deals with Fermented Papaya Preparation (FPP), its various beneficial functions and most importantly its protective and therapeutic property against chemotherapy induced toxicity. We conducted a systematic study on doxorubicin induced organ toxicity in rats. Organ toxicity was induced in the Wistar rats by injecting a chemotherapy drug, Doxorubicin 20mg/kgbw. FPP, an antioxidant, was given to the sets of rats in specific dose regime, to observe its preventive and therapeutic potency against Doxorubicin induced organ toxicity. FPP's efficacy was further confirmed by using cell lines H9c2 and BRL3a.

In, **the first experiment**, ameliorative effect of FPP on Doxorubicin induced multi organ toxicity model was studied. Dose of 100mg/kgbw and 250mg/kgbw of FPP were given to the rats of two different sets for 28 days. Toxicity was induced by giving Doxorubicin 20 mg/kg bw intraperitoneally on 29^{th} and 30^{th} day. Control and Dox alone sets were kept for comparison while FPP alone set to prove its non toxic nature. Twenty-four hours after the treatment period, animals were sacrificed and tissues and blood samples were collected. Serum was separated from each blood sample and was used for the biochemical analysis. Serum markers for cardiac, hepatic and renal function, lipid profile, lipid peroxidation, antioxidant status and histopathological changes in tissues were

assessed in control, doxorubicin treated, FPP alone treated and FPP+DOX treated (100mg/kg bw and 250 mg/kg bw) rats.

Doxorubicin treated rat resulted in significant ($p<0.001$) elevation in cardiac, hepatic and renal serum markers and cholesterol and triglyceride levels compared to control rats. However, FPP supplementation of dose 250mg/kg bw to rats resulted in significant decrement of cardiac function markers CK-MB and LDH ($p<0.001$), hepatic function marker SGPT ($p<0.001$), SGOT ($p<0.01$), and renal function markers Urea, Creatinine ($p<0.001$) compared to doxorubicin treated rats. Triglyceride ($p<0.05$) and Cholesterol ($p<0.01$) levels were also decreased significantly in the animals adminstered FPP 250mg/kg bw dose as compared to doxorubicin treated rats. MDA levels were found to be significantly higher in cardiac ($p<0.01$), hepatic and renal tissues ($p<0.001$) in doxorubicin treated rats as compared to control animals. In FPP treated (250mg/kg Bw) animals, the MDA levels were significantly decreased in heart ($p<0.05$), liver ($p<0.001$) and kidney ($p<0.01$) as compared to doxorubicin control animals. Pretreatment of FPP 250mg/kg bw counteracted doxorubicin induced organ damage by significant increase in GSH, GPx, SOD and Catalase activity levels. The histopathological examination of doxorubicin treated rat heart showed loss of striations and myofibrillar damage. These changes were less when hearts of 250mg/kg FPP treated rats were observed. The kidney sections of the doxorubicin treated animals showed thickening of the glomerular basement membrane and destructive changes in the renal tubules were seen, which were reduced to minimal in the animals pretreated with 250 mg/kg dose of FPP. In the liver sections, loss of tissue structural pattern and vacuolization seen in doxorubicin treated animals were reversed in pre-treatment of 250 mg/kg dose of FPP. Histological

examination of Pre treatment of FPP 100mg/kg dose was not much was not done as biochemical results revealed a better response in animals treated with 250mg/Kg dose of FPP.

Our second study was to simulate the human chemotherapy regime in rats. Here we evaluated multi organ toxicity induced by cumulative dose of Doxorubicin. The experiments were designed to assess the therapeutic potential of FPP 250mg/kgbw, co-administered with doxorubicin (20mg/Kg cumulative dose) along with, pre, and post administration in a 5 week regime. Serum markers of cardiac, hepatic and renal damage and lipid per oxidation, antioxidant status and histopathological changes in tissues were assessed in different groups.

Administration of cumulative dose of doxorubicin animals showed impaired function in all the organs, which was evident from the marked increase in hepatic markers (SGPT, SGOT), renal markers (Urea, Creatinine), and cardiac markers (CK MB, LDH, Triglycerides, Cholesterol) when compared to control animals ($p<0.001$).

FPP administration showed ameliorating effects in all groups of animals. However, the set of animals in which, the FPP treatment extended from 7 days prior to doxorubicin regime to 7 days post doxorubicin regime, showed the maximum amelioration. ($p<0.001$)

Cumulative administration of doxorubicin control group animals resulted in a significant decrease in the levels of cardiac, hepatic and renal enzymes viz. SOD and Catalase as well as decrease in levels of GSH and GPx as compared to the control group. Significant increase in cardiac, hepatic and renal levels of Lipid peroxidation was also observed, indicating induction of respective organ toxicity in these animals.

All groups treated with FPP showed much less toxic effect of doxorubicin treatment (cumulative dose) with minimum damage seen in the animals in which, FPP treatment extended from 7 days prior to doxorubicin regime to 7 days post doxorubicin regime. However, the group of animals that were treated with FPP along with Doxorubicin also showed significant amelioration, indicating therapeutic role of Fermented Papaya.

The histological architecture revealed that, the protective and therapeutic effect of FPP successfully overcomes the damage caused to organs by the cumulative dose of Doxorubicin.

It can therefore be concluded that FPP shows protective and curative effect by alleviating the effect of doxorubicin.

FPP, in both short term and long term study, did not reveal any toxic effect either to the animal as a whole or to the organs under study. However, to validate our claim that FPP was not only safe for consumption but also did not induce any adverse effect at cellular level, while performing its therapeutic role, we conducted *in vitro* studies. Cytoprotective role of FPP was assessed on Doxorubicin induced toxicity in H9c2 rat cardiac myoblast cell line and BRL-3A rat liver cell line. These cell lines were cultured and maintained in optimal conditions. Cells were plated at an appropriate density according to each experimental design. In the cells, ROS generation and Mitochondrial Membrane Potential (MMP) were assessed. Nuclear Staining and Bax and Bcl2 gene expression was studied to assess apoptosis.

ROS generation was assessed with DCFDA staining method. Cells treated with doxorubicin alone showed intense fluorescence due to high intracellular ROS activity, this could be correlated to mechanism of action of doxorubicin which generates Fe^{2+} and

O^{-}_{2} free radicals. In contrast FPP treated cells did not record any significant change in fluorescence with respect to control cells, suggesting thereby FPP did not cause any free radical generation in the system. In fact FPP prevented ROS generation in FPP + DOX group to significant levels in comparison to doxorubicin treated group.

Mitochondrial Membrane Potential (MMP) assessment was performed with Rhodamine 123. Treatment with 2ug/ml Dox showed a significant decrease in MMP as indicated by significant reduction in Rhodamine-123 fluorescence as compared to control group. Cells treated with *FPP* alone showed normal MMP. Pre and co-administration of *FPP with doxorubicin* showed a significant restoration of MMP as evident from the higher fluorescence in comparison to exclusively doxorubicin treated cells.

Nuclear Staining was done with DAPI .Counterstaining of cells with in fixed cells (fixed in 2% PFA) showed normal nuclear morphology in Control and FPP treatment, however in doxorubicin treated groups some of the nucleus showed condensation of chromatin and nuclear fragmentation. Treatment of FPP with doxorubicin showed reduced or no nuclear condensation per field in comparison to doxorubicin treatment. The results with DAPI staining were in correspondence with DCFDA and RHO123 staining performed.

Gene expression study was conducted to examine Bcl-2 and Bax protein expression in H9c2 & BRL3A cells during doxorubicin treatment. Our results show that Dox mitigated Bcl-2 expression and elevated Bax expression, while treatment with FPP preserved the Bcl-2 protein level and inhibited the elevation of Bax level.

The overall observation from the present study suggest that FPP is capable of reducing the toxic effect induced by doxorubicin in H9C2 and BRL 3A cell lines, which may be

due to the reduced drug metabolism, down regulation of pro-apoptotic molecules as well as reduction in oxidative stress by preventing ROS formation.

The extensive study carried out in Rat model as well as Cardiomyoblast and Hepatic cell line, provides evidence that doxorubicin induced organ toxicity can either be prevented or can be reversed to appreciable levels with FPP. Additional studies are warranted to obtain further insight into the clinical modalities through which FPP exerts its beneficial effects on patients who are at risk of side effects after receiving doxorubicin chemotherapy. Nevertheless, the consumption of FPP as a Nutraceutical may prove to be useful in patients to prevent doxorubicin-induced organ toxicity. FPP treatment is a strategy that may provide significant benefits to the cancer patients undergoing doxorubicin treatment. Patients taking FPP may show better tolerance to doxorubicin treatment or possibly even tolerate higher doses of doxorubicin. Such an improved response to doxorubicin treatment may ultimately increase cure rate, long term survival, and the quality of life for cancer survivors.

Therefore, although the results of our studies strongly suggest the possible use of FPP as nutraceutical against oxidative stress induced organ toxicities, the interference of FPP in the antitumor efficacy of Doxorubicin must be evaluated for its possible clinical application.