IMMUNOMODULATORY EFFECTS OF ETAHNOLIC EXTRACT OF *THYPHONIUM FLAGELLIFORME* (Lodd.) Blume ON CYCLOPHOSPHAMIDE−TREATED RATS

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Keladi tikus, a folklore name for *Thyphonium flagelliforme* (Lodd.) Blume, is a traditional medicine that used for cancer treatment in Indonesia and South East Asia. The present study aimed to examine the immunomodulatory effect of ethanolic extract of *Thyphonium flagelliforme* (ETF) in cyclophosphamide (CPA)-treated rats. To induce immunosuppression, 150 and 110 mg/kg of CPA were intraperitoneally injected at day 1 and day 4, respectively. Simultaneously, ETF were orally administered once daily for 7 days with doses 250, 500, and 1000 mg/kg. The immunomodulatory effect were determined lymphocytes proliferation and phagocitosis macrophages activity were determined. Changes in plasma cytokines of tumor necrosis factor (TNF)-α, interleukin (IL)-1α, and IL-10 production, and numbers of CD8+ were also monitored in CPA-treated rats. The results showed that administration of CPA with a dose of 150 and 110 mg/kg in rats significantly suppress lymphocyte proliferation, phagocytic activity, TNF-α and IL-1α, and CD8 + cells. Administration of ETF (250−1000 mg/kg) reduced immunosupessive effect on lymphocyte proliferation, increase the number and activity of macrophages phagocytic in CPA-treated rats. The results demonstrated that CPA significantly supressed CD8+. The administration of ETF (250−1000 mg/kg) significantly improve the immunosupressive effect of CD8-induced by CPA.The study of plasma cytokine levels, administration of ETF (250−1000 mg/kg) also significantly reduce the suppressive effect of CPA on cytokine levels. The results indicated that the optimum dose of immunomodulatory properties is 250 mg/kg in CPA-treated rats. All taken together, the results conclude that ETF have immunomodulatory properties in CPA treated rat. This results suggest that ETF can reduce the side effect of chemotherapy especially immunosupressive effect that can be used as co-chemotherapy for cancer treatment.

HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES INDUCED IN RABBITS BY PROLONGED ORAL CYANIDE INTOXICATION

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Cyanide is widely distributed in the ecosystem and has been associated with goiter, disturbances of thyroid and CNS pathology. However, little information is available in the literature concerning toxic effects of cyanide on liver and kidneys. Hence, the objective of the present study was to determine the deleteriouseffects of prolongedoral cyanide insult on liver and kidney tissues, and associated biochemical changes in rabbits. For this purpose,12 locally bred adult male rabbits were allocated into two groups of 6 viz. control and experimental. Rabbits incontrol group were offered feed only while the rabbits inexperimental group received feed plus potassium cyanide (KCN) at 3 mg/kg body weight orally for a period of 40 days. Results demonstrated significantly increased (<0.05) serum activities of alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase enzymes in experimental group rabbits compared to control group. Likewise, the serum concentrations of urea, uric acid and creatinin were also significantly increased (<0.05) in experimental group rabbits compared to control group. None of the rabbit in both the groups demonstrated any of the gross changes in any organ on postmortem examination. Liver was normal in size, shape, texture and color. Kidneys were also normal in size and color. Histopathological examination revealed severe hepatocyte vacuolation and degeneration in liver of rabbits in experimental group. There was also excessive congestion and bile duct hyperplasia in experimental group rabbits liver. Kidneys of rabbits in experimental group demonstrated severe glomerular and tubular necrosis and congestion. In the tubular epithelial cells, pyknotic nuclei were also present.In conclusion,prolonged oralcyanide administration could have harmful effects on liver and kidney functions.

DEVELOPMENT OF AH (DIOXIN) RECEPTOR-BASED APPROACHES FOR THE STUDY OF TOXICOLOGY AND HEALTH EFFECTS OF DIOXINS AND RELATED CHEMICALS

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Exposure to and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related compounds can produce a wide variety of species- and tissue-specific toxic and biological effects, including tumor promotion, lethality, birth defects, hepato-, neuro-, and immunotoxicity, endocrine disruption, dermal toxicity. Proper epidemiological, risk assessment and exposure analysis of dioxins requires accurate measurements of these chemicals both in the species of interest and in various exposure matrices. Currently, besides the high-resolution instrumental analysis method as the “gold standard”, the numerous bioanalytical methods have also been developed and used for the detection of these chemicals and also used for the related toxicological studies, the majority of which take advantage of the ability of these chemicals to activate the aromatic hydrocarbon receptor (AhR) and the AhR signal transduction pathway. The most sensitive and widely used bio-analytical approaches are cell-based reporter gene bioassay systems, including CALUX (Chemically Activated Luciferase Expression) and CAFLUX (Chemically Activated Fluorescent Expression) bioassays. These bioassays utilize recombinant cell lines containing stably transfected dioxin (AhR)- responsive firefly luciferase or enhanced green fluorescent protein (EGFP) reporter genes, respectively. The application of the bioassays has significantly promoted the basic research on dioxin induced toxic and health effects. While the current bioassays are very sensitive, increasing their lower limit of sensitivity, magnitude of response and dynamic range for chemical detection would significantly increase their utility, particularly for the exposure situation containing low levels of dioxins.

A COMPARISON STUDY OF SINGLE AND COMBINED CYTOTOXIC EFFECTS OF FUMONISIN B1, AFLATOXIN B1 AND OCHRATOXIN A ON HUMAN MONONUCLEAR BLOOD CELLS USING METHYL TETRAZOLIUM ASSAY, COMET ASSAY AND FLOW CYTOMETRY

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Simultaneous exposure of human to different mycotoxins at low and/or high doses might have additive or synergistic effects on consumers' health. This study investigated the single and combined effects of fumonisin B1 (FB1) and ochratoxin A (OTA), and aflatoxin B1 on mononuclear cells obtained from healthy human. The MTT assay test showed an inhibition of cell viability over time and concentration. While the assessment of the cell DNA damage by Comet assay technique and Flow Cytometryfor apoptosis and DNA cleavage induction confirmed the results previously obtained on MTT assay that the effects of the studied mycotoxins in combination showed synergistic cytotoxic effects as compared to the effect of single mycotoxin and this increased over time and concentration of exposure. Additive to synergistic effects were observed at high dose (40) suggesting that high doses or chronic exposure for low doses were able to induce synergism. In addition, it was also shown that FB1 induced slowly its cytotoxicity, toxic and/ or inhibitive effect, as compared to AFB1 and OTA when exposed singularly to mononuclear cells. Finally a correlation study done on the results obtained from the three techniques revealed that all of technique positively correlated and in addition it showed that from one study, prediction of results for another technique could have been done. in conclusion, the exposure to to several mycotoxins simultaneously can induce different symptom and effect on the immune system.

RISK EVALUATION OF EXPOSURE TO LOW CONCENTRATION OF CARCINOGENIC MYCOTOXINS – MOLECULAR EVIDENCE OF SYNERGYSTIC EFFECTS

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Crops are susceptible to fungal attack in field or during storage. These fungi may produce mycotoxins which are very stable. We have analysed several cereals (rice, wheat, maize) but also olives; coffee; spice, from different origin (Vietnam, Thailand, France, Moldavia, Czech Republic, Morocco). The contamination of rice and spice was at alarming rate; especially AFB1. OTA, AFB, CIT and FB were also detected in maize and wheat. All samples of ground coffee contain OTA ranging from trace (< LOQ, 5 samples) to 11.9 µg/kg. The amount of OTA passing in the beverage ranged between 20-140%. Based on a typical menu including some of these ingredients and using the average mycotoxin's amount for calculation, we observed that the tolerable daily intake (TDI) was respectively 39-fold; 7-fold and 3 fold higher than the virtual safety dose (VSD) established for AFB1, OTA and FB. The simultaneous presence of OTA with either CIT or FB or ZEA, modify human kidney cells (HK2) cell viability. The main covalent OTA DNA-adduct, found in human tumours, identified as C8 dG-OTA was increased by simultaneous presence of OTA with CIT/ZEA/FB. The synergistic effects are due to increase of ERKs and COX_2; and modulation of biotransforming enzymes. In the same way, in *in vivo* studies on rat and pig fed simultaneously by OTA and FB or CIT in feed, the formation of OTA specific DNA adducts including C-C8dG OTA adduct and the both OTHQ related adduct increased. The data indicate clearly that exposure to low concentration of mycotoxin which is considered as safe when they are present together can lead to dramatic effect. Until now, regulation does not take into account co-contamination and it is urgent to develop decontaminating process.

Keywords*:* Mycotoxin*,* risk evaluation, genotoxicity, OTA DNA adduct