Protective Effect of Black Tea Infusion on Aflatoxin-Induced Hepatotoxicity in Mice

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Background: **Aflatoxins are a group of mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* and are potent inducers of hepatotoxicity.** ***Objective:*** **The present study was carried out to investigate the effect of black tea infusion on aflatoxin—induced hepatotoxicity in male mice.** ***Methods:*** **A 2% black tea infusion in drinking water was prepared and orally administered along with aflatoxin (750 and 1500 μg/kg body weight) for 30 days. Morphological investigation, body weight and organ weight calculations and histopathological analysis were carried out. Serum hepatic marker enzymes namely alanine aminotransferase and aspartate aminotransferase were estimated.** ***Results:*** **The results clearly indicated that aflatoxin treatment for 30 days caused significant dose-dependent reduction in body weight and increase in liver weight. The activities of ALT and AST were found to be elevated while protein content was found to be decreased in aflatoxin-treated mice as compared to vehicle control. Histopathological analysis showed hepatocellular necrosis and cytoplasmic vacuolization along with fatty infiltration in toxin-treated animals. Results revealed significant ($p < 0.05$) restoration of aflatoxin-induced damages in body weight, organ weight, serum chemistry and histopathological features in aflatoxin plus black tea infusion administered mice in a dose dependant manner.** ***Conclusion:*** **It is concluded from the present study that supplementation of black tea infusion can be beneficial in positively modulating aflatoxin-induced alterations in liver.** **(J Clin Exp Hepatol 2013;3:29–36)**

Hepatotoxicity

Aflatoxins are a group of well-known mycotoxins (secondary toxic fungal metabolites) produced by *Aspergillus flavus* and *Aspergillus parasiticus* which are known to contaminate a wide variety of food stuffs. The term Aflatoxins is used to collectively represent the four major naturally occurring secondary compounds namely B1, B2, G1 and G2. Aflatoxins B1 (AFB1) is considered as the most potent of these toxins and is attributed with hepatotoxic and hepatocarcinogenic properties.[1] The International Agency for Research on Cancer (IARC) has classified AFB1 and the mixtures of aflatoxins as Group 1 carcinogens. The liver is known to be the main target organ for aflatoxin and chronic exposure to low levels of these through intake of contaminated food stuffs can cause liver fibrosis and also primary liver cancer.[2]

Aflatoxins enter the dietary systems of human or animals through direct or indirect routes and once inside they are acted upon by microsomal mixed function oxidase (MFO) primarily in the liver and to some extent in the lungs, kidneys and other organs. AFB1 is first metabolized (Phase 1) by the Cytochrome P450 enzyme (CYP450) system found in the microsomes, producing a variety of intermediary metabolites such as AFB1 epoxide and other hydroxylated metabolites like AFM1, AFP1, AFQ1 and aflatoxicol. AFB1 epoxide is highly reactive and relatively unstable with inbuilt capacity to bind to cellular macromolecules like DNA, RNA, lipids and proteins, initiating the vicious cycle of lipid peroxidation and culminating in cellular injury.[3] Aflatoxins are thus responsible for a wide range of pathological abnormalities in humans and animals. Aflatoxin-albumin adduct, a biomarker for aflatoxin exposure was found to be higher in the serum of individuals at risk for hepatocellular carcinoma thus indicating its importance in human health and diseases.[4]

Tea intake either as an herbal medicine or as a rejuvenating drink is a common practice among people belonging to different parts of the world. Tea infusions are estimated to be consumed by two thirds of the world's population. Black tea represents approximately 72% of total consumed tea in the world, whereas green tea accounts for approximately 26%.[5] Tea infusions are used as therapeutic agent keeping in mind its ready availability and cost effectiveness. Tea is known to contain very high levels of total

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Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AFB1: aflatoxins B1; IARC: International Agency for Research on Cancer; MFO: mixed function oxidase; TLC: thin-layer chromatography; LD: low dose; HD: high dose; ANOVA: Analysis of Variance; WHO-ART: World Health Organization for Adverse Reaction Terminology
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flavonoids and polyphenols.[6] Tea polyphenols have been reported to inhibit DNA synthesis of leukemia and lung carcinoma cells.[7] Tea flavonoids have been attributed with enormous antioxidative activity by a number of previous studies.[8,9]

It is widely believed that green tea consumption gives more beneficial health effects as compared to black tea due to the presence of catechins which are converted to theaflavins and thearubingins by fermentation during the transformation process.[10] However many study reports have confirmed positive modulatory effect of black tea on a number of carcinogens and toxicants. Black tea has been reported to significantly protect against oxidative damage induced by a number of agents like hydrogen peroxide, primaquine and phenylhydrazine in red blood cells.[11] Administration of 2% black tea extract along with sodium fluoride was found to produce a profound neuroprotective effect on fluorotic rats with improved motor functions and coordination performance.[10] In one another study 2% black tea infusion was used to effectively combat sodium fluoride-induced behavioral and reproductive toxicity.[12] Chung et al[13] have reported the advantage of using 2% black tea over a 1% or 0.5% infusion on lung tumorigenesis induced by the nicotine-derived carcinogen 4-(methylnitrosamino)-L-(3-pyridyl)-L-butanone (NNK) in rats. Similarly 2% black tea preparation was found to prevent cigarette smoke-induced apoptosis and lung damage in guinea pigs.[14] The protective effect of black tea against many potent toxins has been extensively studied in our lab and 2% infusion was found to be the most effective and further increase in tea concentration produced insignificant results.[15,16] This prompted us to study the effect of 2% black tea infusion against aflatoxin which is one of the most potent hepatotoxin known thus far.

MATERIALS AND METHODS

Chemicals

Black tea (Lipton Yellow Label) was purchased from Hindustan lever Ltd., Mumbai, India. Olive oil was procured from Figaro, Madrid, Spain. Chemicals used in the present study were purchased from standard agencies and were of analytical grade. Aflatoxin standard was obtained as a gift from the International Agency for Research on Cancer, Lyon, France.

Aflatoxin Production

A. parasiticus var. globusus strain (MTCC 411) was procured from Institute of Microbial Technology, Chandigarh, India and was grown on sucrose-magnesium sulfate-potassium nitrate-yeast extract (SMKY) liquid medium at 28 ± 2 °C for 10 days. Briefly 50 ml of SMKY liquid medium was taken in a 500 mL Erlenmeyer flask and sterilized at 15 lb pressure for 20 min. The sterilized medium was inoculated with 0.5 ml spore suspension of *A. parasiticus* having 10^8 conidia/mL under strict aseptic condition. On the 11th day the contents were autoclaved and filtered and the pooled filtrate was extracted in chloroform and passed through anhydrous sodium sulfate, evaporated and was re-suspended in chloroform and qualitatively analyzed using thin-layer chromatography (TLC) technique. Briefly 100 μL of extracted aflatoxin was spotted along with standard aflatoxin on silica gel G coated activated TLC plates, developed using toluene: iso-amyl alcohol: methanol (90:32:2, v/v) and observed at 360 nm under UV light.[11] Aflatoxin B_1 and B_2 were seen as blue colored fluorescent spots, while aflatoxin G_1 and G_2 were identified by their characteristic bluish-green fluorescent color. The spots were also chemically analyzed by spraying trifluoroacetic acid or 25% acid. Subsequently the individual spots were eluted, dissolved in chilled methanol and quantified and stored until use.

Preparation of Black Tea Infusion

The effective dose of black tea infusion was based on our previous study report.[11] Briefly 2% black tea infusion was made using 2 g of black tea solids in 100 ml of deionized water and used.

Experimental Animals

Healthy male mice of Swiss strain weighing between 37–40 g of equivalent age groups were acclimatized for 15 days in polypropylene cages in the Animal House of Zoology Department, Gujarat University, Ahmedabad, India under controlled conditions of temperature (25 ± 2 °C) and relative humidity (50–55%) and12 h light/dark cycle. Animals were maintained on certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Limited, Pune, India and given water *ad libitum*. The experimental procedures were approved by "The Committee for the Purpose of Control and Supervision of Experiment on Animals" (Reg—167/1999/CPCSEA), New Delhi, India.

Experimental Design and Treatment Schedule

Seventy animals were randomized into seven groups and caged separately. Group 1 animals were marked as untreated control group and maintained without any treatment. Animals of Group 2 received olive oil as vehicle (0.2 mL/animal/day) and group 3 animals were administered with black tea infusion. Animals of Group 4 and 5 were orally administered with aflatoxin in olive oil (containing B_1, B_2, G_1 and G_2 in the ratio of 8:3:2:1 respectively) using a feeding needle attached to a hypodermic syringe with a dosage regimen of 750 μg/kg body weight (low dose i.e. LD) and 1500 μg/kg body weight (high dose i.e. HD) in 0.2 mL olive oil/animal/day respectively.[17] The selected dose of aflatoxin was based on its LD_{50} value in male mice (9 mg/kg body wt).[18] The experiments were