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Article

Effect of Kombucha Tea on Aflatoxin B_1 Induced Acute Hepatotoxicity in Albino Rats-prophylactic and Curative Studies

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Kombucha tea is sugared black tea fermented with a consortium of bacteria and yeasts which forms tea fungus (*Medusomyces gisevii*) for 14 days. Kombucha tea is claimed to have various beneficial effects on human health but very less scientific evidences are available in the literature. In the present study, the prophylactic and curative effect of black tea (unfermented black tea) and kombucha black tea (KBT) on aflatoxin B_1 induced liver damage was studied in male albino rats by analyzing hepatotoxicity markers (aspartate transaminase, alanine transaminase, alkaline phosphatase, gammaglutamyl transpeptidase), lipid peroxidation, reduced glutathione and antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase). Histopathological analysis of liver tissue was also carried out. Results revealed that kombucha tea is more potent in preventing hepatotoxicity than unfermented black tea. The mechanism of hepatoprotection offered by KBT treatment may involve the facilitation of both antioxidant and detoxification processes in the liver.

Key words: aflatoxinB_1, Kombucha, liver, tea fungus

One of the most serious problems to deal with the quality of food products is the presence of mycotoxins which has become a worldwide concern. Among mycotoxins, aflatoxins are of greatest concern as they are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extra hepatic carcinogenesis. The positive correlation between the consumption of aflatoxin B_1 (AFB_1) contaminated foods and the increased incidence of liver cancer in several Asian and African populations has led to the classification of AFB_1 as a group IA carcinogen by the International Agency for Research on Cancer (IARC) of World Health Organization (WHO) in 1993 [Hussein and Brasel, 2001]. It has been shown that AFB_1 is activated by hepatic cytochrome P450 enzyme system to produce a highly reactive intermediate, AFB_1-8,9-epoxide, which subsequently binds to nucleophilic sites in DNA, and the major adduct 8,9-dihydro-8-(N7guanyl)-9-hydroxy-AFB_1 (AFB_1 N7-Gua) is formed. The formation of AFB_1-DNA adducts is regarded as a critical step in the initiation of AFB_1-induced hepatocarcinogenesis [Preston and Williams, 2005].

Opportunities for primary prevention against AFB_1 toxicity and carcinogenicity include drugs that interfere with carcinogenic process through pharmacologic interventions. Since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, there is a worldwide trend to go back to natural resources which are therapeutically effective, culturally acceptable and economically within the reach of even the poor people. WHO has called the attention of many countries to the ever increasing interest of the public in the use of herbal medicines and

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encourages countries to identify and exploit those aspects of traditional medicine that provide safe and effective remedies.

Kombucha tea is sugared black tea fermented with a symbiotic association of bacteria and yeasts which forms tea fungus, *Medusomyces gisevii*. Kombucha tea is composed of two portions, a floating cellulosic pellicle layer and sour liquid. The liquid portion of kombucha tea is claimed to have various medicinal effects on human health. Recent studies have suggested that kombucha tea prevents paracetamol induced hepatotoxicity [Pauline *et al*., 2001] and chromate (VI) induced oxidative stress in albino rats [Sai Ram *et al*., 2000]. As kombucha tea is rich in compounds known to be strong antioxidants, it is expected to ameliorate liver damage induced by AFB_1.

In the present study, the prophylactic and curative effect of plain black tea (BT, unfermented black tea) and kombucha black tea (KBT) on AFB_1 induced liver damage was studied in male albino rats by analyzing hepatotoxicity markers (aspartate transaminase (SGOT), alanine transaminase (SGPT), alkaline phosphatase (ALP), gammaglutamyl transpeptidase (GGT)), lipid peroxidation, reduced glutathione and antioxidant enzymes (glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GRx), catalase and superoxide dismutase (SOD)). Histopathological analysis of liver tissue was also carried out.

Materials and Methods

Materials. "Institute Ethical Committee" clearance was obtained prior to our study to use rats as experimental models (588/02/A/CPCSEA; CPCSEA, 2003). AFB_1 was purchased from Sigma Chemical Co. (St. Louis, MO). Liv52 (supplied by The Himalaya Drug Company, Bangalore, India), an herbal formulation based on Ayurvedic principles, contains a number of hepatoprotective ingredients and is widely prescribed in Indian and European countries for various liver disorder.

Starter culture. Starter culture or tea fungal mat of *Medusomyces gisevii* was obtained from the tribal people of Kolli hills, Tamil Nadu, India and was maintained in sugared black tea. Bacterial component was identified as *Acetobacter aceti* MTCC 2945, and the yeast components were identified as *Zygosaccharomyces bailii* MTCC 8177 and *Brettanomyces claussenii* MTCC 7801 at Institute of Microbial Technology (IMTECH), Chandigarh, India. Identified cultures were deposited in Microbial Type Culture Collection, IMTECH, India [Jayabalan *et al*., 2008b].

Preparation of Kombucha tea. Brooke Bond Red Label Tea (Hindustan Lever Limited, Mumbai, India) was used for the preparation of kombucha tea. Black tea (1.2%, w/v) was added to boiling water and allowed to infuse for about 5 min after which the infusions were filtered through sterile sieve (black tea, BT). Sucrose (10%, w/v) was dissolved in hot tea and the preparation was left to cool. The cooled tea (200 mL) was poured into 500 mL glass jars that had been previously sterilized at 121°C for 20 min and inoculated with 3%(w/v) of freshly grown tea fungus that had been cultured in the same medium for 14 days and 10%(v/v) of previously fermented liquid tea broth aseptically. The jar was covered with a clean cloth and fastened properly. The fermentation was carried out in dark at 24±3°C for 14 days. After 14 days of fermentation, it was centrifuged at 10,000 rpm and the supernatant was collected (Kombucha black tea, KBT) [Jayabalan *et al*., 2007; 2008a]. Both BT and KBT were used as hepatoprotective agents. Liv52 was used as positive control.

Ethical guidelines. The experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India. Ethical care and treatment of animals were undertaken following the guidelines of the Animal Care and Ethics Committee of I.R.T. Perundurai Medical College and Research Center, Perundurai, Tamil Nadu, India (588/02/A/CPCSEA) [CPCSEA, 2003].

Hepatoprotective effect (Pre-Treatment). The prophylactic effect of BT and KBT on AFB_1 induced liver damage was studied in male albino rats weighing 160 to 220 g. Rats were randomly separated into 9 groups; each group consisted of 6 rats. Rats were maintained in polypropylene cages according to CPCSEA guidelines [CPCSEA, 2003]. Rats were fed with rat feed (Sai Durga Feed and Foods, Bangalore, India) *ad libitum*. Rats in group 1 (Normal), 2 (DM-Dimethyl sulfoxide) and 3 (AFB_1) were provided with tap water. Rats in group 4 (BT) and 7 (BTAF) were provided with BT. Rats in group 5 (KBT) and 8 (KBTAF) were provided with KBT. BT and KBT (300 mL/cage) were provided three times a day (usually kombucha tea is taken by the drinkers three times in a day) and tap water was provided in rest of the time period. Rats in group 6 (Liv52) and 9 (Liv52AF) were given Liv52 (3 mL/rat/day) by intragastric intubation and were provided with tap water *ad libitum*. The experiments were carried out for a period of 30 days. After the experimental period, rats in group 3, 7, 8 and 9 were injected with AFB_1 (3 mg/kg body weight) dissolved in 30% dimethyl sulphoxide solution in saline (0.2 mL/rat) and rats in group 2 were injected with 30% dimethyl sulfoxide solution in saline (0.2 mL/rat) intraperitoneally.

Hepatocurative effect (Post-Treatment). Rats in group 3 (AFB_1), group 7 (BTAF), group 8 (KBTAF) and group 9 (Liv52AF) were injected with AFB_1 (3 mg/kg body weight) dissolved in 30% dimethyl sulphoxide solution in saline (0.2 mL/rat). Rats in group 4 (BT) and 7 (BTAF), group 5 (KBT) and 8 (KBTAF) were provided respectively with plain black tea and kombucha black tea (300 mL/cage) three times a day. Rats in group 6 and group 9 were given with Liv 52 (3 mL/rat/day) by intragastric intubation. The rats were allowed to take tap water *ad libitum* and the animals were maintained for 48 h.

Rats in all groups were sacrificed after an over night fast by decapitation after 48 h of AFB_1 injection and blood samples were collected from the stump. EDTA was used as anticoagulant. Blood was centrifuged at 3,000 rpm for 15 min at 4°C. Plasma layer (top yellow) was removed by pipette without disturbing the white buffy layer (leukocytes). Plasma was stored on ice. If not assayed on the same day, the plasma was freezed at −80°C. Red blood cells were used for the analysis of SOD.

Histopathological studies. The liver was removed after perfusion *in situ* with phosphate buffered solution, pH 7.4, containing 0.16 mg/mL heparin to remove any red blood cells and clots. A portion of the liver was fixed in 10% formalin. The washed tissue was dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissue was then embedded in molten paraffin wax. Sections were cut at 5 μm thickness using microtome, stained with haematoxylin and eosin. The sections were then viewed under high power objective (100X) of light microscope for histopathological observations.

Biochemical analysis of blood. Activities of aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) in blood plasma were assayed by UV Kinetic (International Federation of Clinical Chemistry) method using Bayer Diagnostics Kits (Bayer Diagnostics India Limited, Baroda, India) in semiauto analyzer (Technicon Ames RA 50). Alkaline phosphatase (ALP) activity in blood plasma was assayed by ρ-nitrophenyl phosphate method using Bayer Diagnostic Kits (Bayer Diagnostics India Limited, Baroda, India) in semiauto analyzer (Technicon Ames RA 50). Haemoglobin (Hb) content of red blood cells was estimated by the method described by Van Kampen and Zijlstra [1965]. The conversion of Hb to cyanmethemoglobin by Drabkin reagent was measured at 540 nm after 10 min of the addition of the reagent. Hb content was estimated from a standard curve of Hb. The protein content of blood plasma was determined by the method of Lowry *et al*. [1951] using bovine serum albumin as the standard. Gamma glutamyl transpeptidase (GGT) in blood plasma and SOD in red blood cells were assayed by using kit developed by Randox Laboratories, UK. SOD kit employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity of red blood cells was then measured by the degree of inhibition of this reaction by means of the decrease in absorbance at 505 nm at 37°C.

Biochemical analysis of liver. One portion of the liver was homogenized in 5-10 mL of cold buffer (50 mM Tris-HCl, pH 7.5; 5 mM EDTA; 1 mM DTT) per gram tissue. Homogenized tissue was centrifuged at 10,000 rpm for 15 min at 4°C. The supernatant (liver tissue homogenate) was removed and stored on ice. If not assayed on the same day, the supernatant was freezed at −80°C. The protein content of liver homogenate was determined by Lowry *et al.* [1951]. Reduced glutathione was estimated by the method of Moron *et al*. [1979]. Reduced glutathione (0.01%) was used as standard and the results were expressed as mg/g protein. The kits for the analysis of GST, GPx and GRx were purchased from Cayman Chemical Kits (Cayman Chemical Company, Ann Arbor, MI). GST kit measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample [Habig *et al*., 1974]. One unit of enzyme will conjugate 1.0 n mol of CDNB with reduced glutathione per minute at 25°C. GPx kit measures GPx activity indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx, recycled to its reduced state by glutathione reductase and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. The rate of decrease in the A_{340} is directly proportional to the GPx activity in the sample [Paglia and Valentine, 1967]. One unit of enzyme is defined as the amount of enzyme that will cause the oxidation of 1.0 n mol of NADPH to $NADP^+$ per minute at 25°C. GRx kit measures the rate of NADPH oxidation. The oxidation of NADPH to $NADP^+$ is accompanied by decrease in absorbance at 340 nm and is directly proportional to the GRx activity in the sample. One unit is defined as the amount of enzyme that will cause the oxidation of 1.0 n mol of NADPH to $NADP^+$ per minute at 25°C. Catalase activity was assayed by the method proposed by Sinha [1972] using dichromate-acetic acid reagent. Catalase activity was expressed in μmoles of hydrogen peroxide consumed/min/mg protein. Thiobarbituric acid reactive substances (TBARS) released from endogenous lipid

peroxides reflect lipid peroxidation and this was assayed by the method described by Ohkawa *et al*. [1979]. Tetramethoxy propane (standard) was treated similarly and was read against distilled water blank. The lipid peroxidation was expressed in terms of nmoles of malondialdehyde (MDA) formed/mg protein.

Statistical analysis. The data were analyzed by the Statistical Package for the Social Science (SPSS Inc. Chicago, IL) program. The results were expressed as mean±standard deviation. Statistical comparisons were made by means of one-way analysis of variance (ANOVA) followed by a Duncan multiple range comparison test (DMRT). Differences were considered significant when the *p*-values were <0.05.

Results

Preliminary studies indicated that rats can tolerate BT and KBT upto 100% without any adverse effect on their body weight and food intake. Therefore, in the present study, BT and KBT are fed to rats without any dilution. There were no significant changes observed in body weight of rats and food intake due to tea treatments (data not shown).

Effect on hepatotoxicity markers. In pretreatment (hepatoprotective) groups, the severe liver injury by AFB1 was evidenced by increase in serum activity of alanine and aspartate aminotransferases (SGOT and SGPT) by 2.5 fold. BT (group 7) treatment reduced SGOT and SGPT activity by 50% and KBT (group 8) treatment also reduced these enzyme activities by 53% and 55% respectively. ALP and GGT were also observed to be elevated by 4 and 6 fold respectively in AFB1 treated rats whereas, rats treated with BT (group 7) showed decrease in ALP and GGT by 50% and 46.3% respectively. Similarly rats treated with KBT (group 8) showed decrease in ALP and GGT by 52% and 53% respectively. The level of lipid peroxidation was increased in rats treated with AFB1 by 18 fold as compared to control group. Pretreatment with BT (group 7) and KBT (group 8) reduced the thiobarbituric acid reactive substances (TBARS) level by 9 fold and 7 fold respectively. Similar reduction of lipid peroxidation by 5 fold was observed in rats administered with Liv52 (group 9). Reduced glutathione in the liver of rats which were administered AFB1 was markedly depleted by 79% in comparison with normal rats. Liver glutathione levels were found to be increased by 13%, 23.1% and 19% in rats fed with BT, KBT and Liv52 (group 4, 5 and 6, respectively) alone as compared to control groups (Table 1).

In post treatment (hepatocurative) groups, rats administered with AFB_1 showed an elevated level of serum SGOT, SGPT, ALP and GGT by 61, 60, 73, and 83% respectively. Treatment with BT, KBT and Liv52 (group 7, 8 and 9, respectively) did not show protection against AFB_1 induced increase of hepatotoxic markers. A significant increase (94%) in tissue malaondialdehyde (MDA) level was observed in AFB_1 alone treated rats. However, AFB_1 induced elevation of tissue MDA concentration were lowered by 14% and 40% in rats treated with BT (group 7) and KBT (group 8). Liv52 (group 9) also prevented the elevation of plasma MDA levels by 53%. Rats administered with AFB_1 showed lowered hepatic glutathione level (4.25 fold). Treatment with BT (group 7) and KBT (group 8) exhibited significant increase i.e., 50 and 67%, respectively, in hepatic glutathione levels. Liv52 treatment (group 9) also

Table 1. Hepatoprotective effect of kombucha black tea on hepatotoxicity markers changed by AFB_1 administration

Groups	SGOT (U/L)	SGPT (U/L)	Alkaline phosphatase (U/L)	Gamma GT (U/L)	Lipid peroxidation nmoles of MDA/mg protein	Glutathione mg/g protein
Normal (Group 1)	92.33±7.94	71.50±15.79	43.50±2.73	1.54±0.59	8.03±1.41	5.40±0.20
DM (Group 2)	83.55±7.50	64.66±3.72	38.16±8.18	2.04±0.97	9.20±1.43	5.15±0.15
AF (Group 3)	236.66±34.98	183.83±52.51	163.66±30.31	9.23±1.37	142.90±13.50	1.15±0.15
BT (Group 4)	84.66±9.26	70.16±8.30	44.50±6.02	1.73±0.63	9.43±1.38	6.10±0.20
KBT (Group 5)	97.16±7.65	75.50±8.04	41.16±4.99	1.28±0.20	9.23±1.12	6.65±0.24
Liv52 (Group 6)	91.95±6.04	70.15±7.14	42.30±1.05	1.65±0.24	8.67±0.25	6.40±0.21
BTAF (Group 7)	117.16±25.96[c]	90.50±16.97[c]	82.33±26.54[c]	4.95±1.18[c]	70.16±6.62[c]	3.40±0.20[c]
KBTAF (Group 8)	110.83±27.57[b]	81.66±11.21[b]	79.00±17.79[b]	4.31±1.07[b]	56.83±3.71[b]	4.30±0.17[b]
Liv52AF (Group 9)	101.80±20.26[a]	88.90±10.33[a]	66.80±9.46[a]	3.63±0.14[a]	37.55±1.94[a]	4.90±0.22[a]

SGOT, Serum glutamate oxaloacetate transaminase; SGPT, Serum glutamate pyruvate transaminase; Gamma GT, Gamma glutamyl transpeptidase.

Values are mean±S.D., n=6.

The letters mean the statistically significant difference compared with the group 3 (p<0.05).

Table 2. Hepatocurative effect of kombucha black tea on hepatotoxicity markers changed by AFB_1 administration

Groups	SGOT (U/L)	SGPT (U/L)	Alkaline phosphatase (U/L)	Gamma GT (U/L)	Lipid peroxidation nmoles of MDA/mg protein	Glutathione mg/g protein
Normal (Group 1)	92.33±7.94	71.50±15.79	43.50±2.73	1.54±0.59	8.03±1.41	5.40±0.20
DM (Group 2)	83.55±7.50	64.66±3.72	38.16±8.18	2.04±0.97	9.20±1.43	5.15±0.15
AF (Group 3)	236.66±34.98	183.83±52.51	163.66±30.31	9.23±1.37	142.90±13.50	1.15±0.15
BT (Group 4)	91.11±0.88	71.71±2.52	42.00±1.13	1.33±0.14	8.45±0.11	5.67±0.14
KBT (Group 5)	90.86±0.95	71.53±1.27	41.83±2.04	1.35±0.10	8.32±0.17	5.53±0.18
Liv52 (Group 6)	91.35±1.37	72.63±1.05	44.40±0.92	1.51±0.18	8.65±0.16	6.31±0.25
BTAF (Group 7)	214.35±1.29[c]	180.32±1.70[c]	162.35±1.33[c]	7.97±1.09[c]	125.55±1.67[c]	2.23±0.17[c]
KBTAF (Group 8)	209.00±1.70[b]	175.60±1.67[b]	159.40±3.04[b]	8.52±1.12[b]	101.80±1.83[b]	3.53±0.15[b]
Liv52AF (Group 9)	197.80±3.12[a]	160.25±1.25[a]	154.30±1.44[a]	8.15±0.39[a]	93.35±1.63[a]	4.27±0.22[a]

SGOT, Serum glutamate oxaloacetate transaminase; SGPT, Serum glutamate pyruvate transaminase; Gamma GT, Gamma glutamyl transpeptidase.
Values are mean±S.D., n=6.
The letters mean the statistically significant difference compared with the group 3 ($p<0.05$).

prevented the lowering of hepatic glutathione by 73% (Table 2).

Effect on antioxidant enzymes. As shown in Table 3, the activity of GST was increased by 45% in rats treated with AFB1 whereas, the activity of catalase, SOD, GPx and GRx were found to be reduced by 30, 38.2, 17, and 21% respectively. The remarkable change was observed in the GST activity. AFB1 alone caused 45% increase in the activity of the enzyme, while the pretreatment with BT and KBT (group 7 and 8) reduced this activity by 93 and 80% respectively. Rats treated with KBT alone (group 5) caused the increase in the activity of GPx, GRx and GST and catalase by 1.2, 48, 10.4, and 12.2% respectively and the decrease in the activity of SOD by 23%. BT (group 4) was less active and increased only the activity of GRx and catalase by 29.1 and 10.3% respectively.

In post-treatment groups (Table 4), rats administered with AFB_1 alone were found significantly lowered the catalase and superoxide dismutase levels by 41 and 62%, respectively. Treatment with KBT and Liv52 (group 8 and 9) prevented the decrease in catalase by 13 and 33%, respectively. Treatment with BT (group 7) did not show protection for lowering of catalase level. Liv52 (group 9) showed better protection towards SOD by increasing its level when compared to AFB_1 group. Post treatment of AFB_1 found to decrease the levels of GPx and GRx by 21 and 26%, respectively and significantly increased the glutathione-S-transferase level by 31%. Treatment with BT, KBT and Liv52 (group 7, 8 and 9, respectively) did not show protection towards GPx and GRx. But, BT (group 7) and KBT (group 8) showed protection on glutathione-S-transferase level by decreasing the elevation caused due to AFB_1 treatment by 4 and 6% respectively.

Histopathological studies. In rats treated with AFB_1 (Fig. 1B), the normal architecture of liver (Fig. 1A) was completely lost with the appearance of degenerative changes in hepatocytes with focal necrosis and bridging hepatic necrosis. In pretreatment, animals administered with BT, KBT and Liv52 (Fig. 1C, 1D and 1E, respectively) showed protection from AFB_1 induced liver damage as evident from hepatic architectural pattern with absence or mild hepatic necrosis. In post-treatment group, rats treated with BT, KBT and Liv52 (Figs. 2C, 2D and 2E, respectively), after the establishment of toxic injury showed mild to moderate focal or bridging necrosis.

Discussion

In our experiment, single dose of AFB1 caused increase in the level of lipid peroxidation by 18 fold when compared to control. Pretreatment with KBT and Liv52 (group 8 and 9, respectively) resulted in the reduction of TBARS content to the basal level. Rastogi *et al*. [2001] found two-fold increase in lipid peroxide level or MDA concentration in the liver of rats 6 week after AFB1 administration. In the present study, it was observed that administration of AFB1 to rats caused a marked depletion of hepatic glutathione concentration by 79%. This is in consistent with previous finding that the active metabolite exo-AFB1-8,9-epoxide is detoxified largely by GST-mediated conjugation with glutathione [Jodynis-Liebert *et al*., 2006]. Pretreatment with BT and KBT (group 7 and 8, respectively) prevented glutathione depletion. Thus, BT and KBT provided the significant protection against toxicity of AFB1-derived epoxide. BT and KBT (group 4 and 5) when given alone increased the hepatic

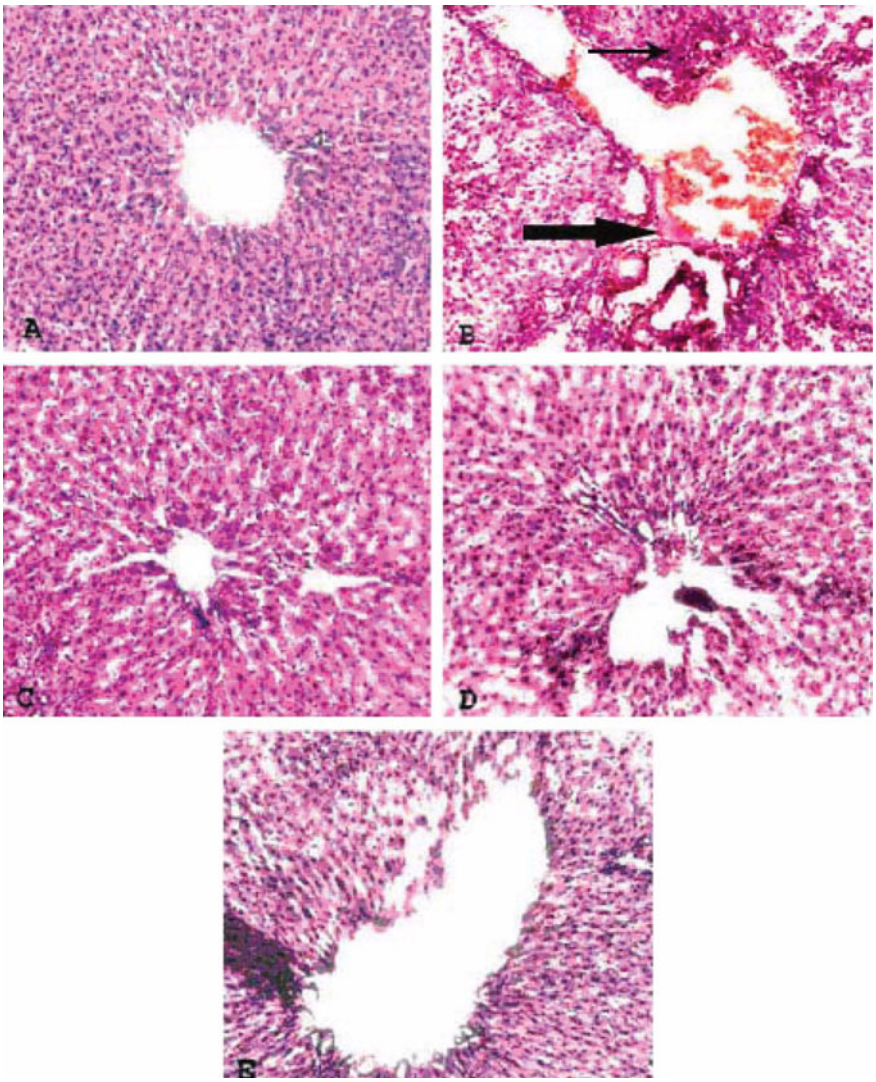

Fig. 1. Cross section of liver in rats treated (hepatoprotective effect) with AFB_1, BT, KBT and Liv52 (100×). Liver from control (A) shows normal architecture. Liver from AFB_1 treated (B) reveals degenerative changes in the hepatocytes with necrosis. Small and big arrow marks show focal necrosis and bridging necrosis, respectively. The liver of rats treated with AFB_1 and BT (C), AFB_1 and KBT (D), AFB_1 and Liv52 (E) shows mild degenerative changes and recovery of the hepatocytes.

glutathione content by 13% and 23%, respectively. It can be explained that BT and KBT induce enzymes involved in glutathione synthesis. Several reports are available for the effect of green and black tea on prevention of depletion of glutathione concentration induced by hepatotoxin and microbial infection [Skrzydlewska *et al*., 2002]. In accordance with our findings, it was demonstrated that substances with known hepatoprotective activity, picroliv and silymarin [Rastogi *et al*., 2001] as well as extracts from garlic, onion and cabbage [Abdel-Wahhab and Ahy, 2003] given to rats pretreated with AFB1 restored glutathione level. Generally, the activity of antioxidant enzymes was affected mildly by AFB1. In our study, only GST was found to be increased (45%) by AFB1 administration and catalase, SOD, GPx and GRx were reduced (15-40%) in AFB1 treated rats. Earlier reports have also stated a mild decrease in all antioxidant enzymes activity in the liver of rats treated with AFB1, by 15-40%. They have also noticed a remarkable change in SOD (35-40%) and GST (27-35%) activity. It could be suggested that the reduction of the activity of these enzymes may be due to denaturation of these enzyme-proteins by free radicals as evidenced by the increase in the level of lipid peroxides or MDA level as well as glutathione depletion [Rastogi *et al*., 2001]. Similarly, 8-week administration of AFB1 (50 μg/kg b.w.) resulted in the decrease in GPx and GRx activity [Meki *et al*., 2004]. AFB1 (2 mg/kg) administration to rats did not change hepatic glutathione level and increased the GRx (15%) activity [Ip *et al*., 1996]. AFB1 is metabolized by cellular

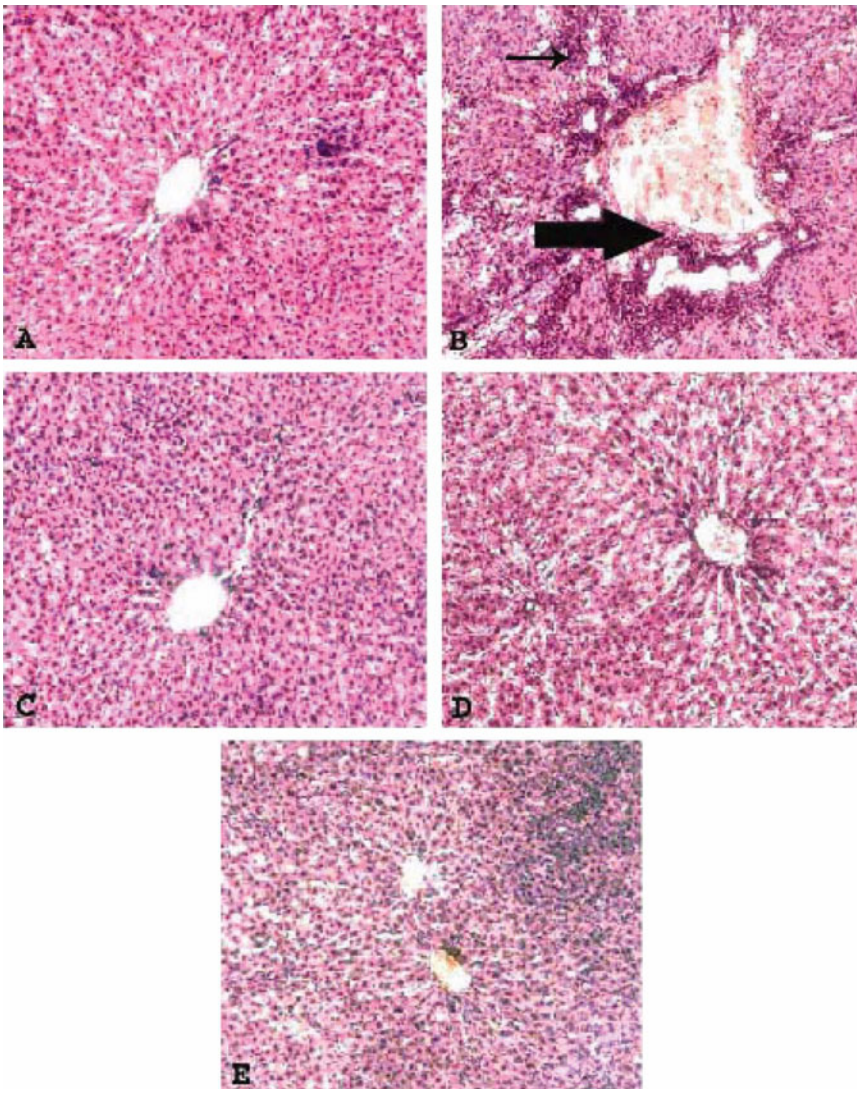

Fig. 2. Cross section of liver in rats treated (hepatocurative effect) with AFB_1, BT, KBT and Liv52 (100×). Liver from control (A) shows normal architecture. Liver from AFB_1 treated (B) reveals degenerative changes in the hepatocytes with necrosis. Small and big arrow marks show focal necrosis and bridging necrosis, respectively. The liver of rats treated with AFB_1 and BT (C), AFB_1 and KBT (D), AFB_1 and Liv52 (E) shows mild degenerative changes and recovery of the hepatocytes.

cytochrome P450 enzyme system to form the reactive intermediate, AFB1 8,9-epoxide, which in turn reacts with macromolecules such as lipid and DNA, leading to lipid peroxidation and cellular injury [Stresser *et al*., 1994].

The AFB1 induced alteration in hepatic antioxidant status may therefore be a manifestation of increased tissue oxidative stress caused by AFB1 metabolism. Since the detoxification of AFB1 can be mediated by the GST catalyzed conjugation with glutathione in the liver [Raney *et al*., 1992], the increased hepatic GST activity induced by KBT and Liv52 treatment can therefore reduce the acute AFB1 hepatotoxicity. The increase in hepatic GRx activity in rats treated with KBT and Liv52, can increase hepatic glutathione level by facilitating the regeneration of glutathione from its oxidized form. This postulation is consistent with the increase in hepatic glutathione level in rats treated only with KBT and Liv52. The changes in antioxidant enzymes activity after AFB1 administration were in consistent with previous findings [Rastogi *et al*., 2001; Meki *et al*., 2004]. Hepatoprotective agents (BT, KBT and Liv52) (group 4, 5 and 6) when given alone to rats increased the activities of GRx (KBT >BT>Liv52) and catalase (Liv52>KBT>BT). KBT and Liv52 (group 5 and 6) were found to increase the activity of GST but no change was observed in GPx. BT, KBT and Liv52 could induce the synthesis of antioxidant enzymes as revealed by significant increase in activity of antioxidant enzymes (Table 3). Increased activities of serum SGOT, SGPT and ALP are well known diagnostic

Table 3. Hepatoprotective effect of kombucha black tea on antioxidant enzymes changed by AFB_1 administration

Groups	Glutathione peroxidase nmol NADPH /min/mg/protein	Glutathione reductase nmol NADPH /min/mg/protein	Glutathione S transferase nmol CDNB /min/mg/protein	Catalase μmol H_2O_2 /min/mg/protein	SOD U/g Hb
Normal (Group 1)	410±2.28	24.0±2.09	409.00±3.40	44.25±1.21	2.33±0.30
DM (Group 2)	38.5±1.04	22.5±0.83	404.00±2.52	42.75±1.20	1.99±0.19
AF (Group 3)	34.0±2.28	19.0±1.78	593.41±3.65	31.30±1.73	1.44±0.15
BT (Group 4)	40.5±1.04	31.0±1.41	410.75±2.25	48.85±0.73	2.31±0.09
KBT (Group 5)	41.5±1.51	35.5±1.04	451.70±1.44	50.45±1.08	2.31±0.10
Liv52 (Group 6)	41.0±1.78	27.5±1.04	649.35±2.24	59.15±2.55	2.40±0.15
BTAF (Group 7)	32.0±1.26[c]	30.5±1.04[c]	306.90±2.27[c]	32.35±1.33[c]	1.56±0.13[c]
KBTAF (Group 8)	35.5±1.51[b]	32.5±1.51[b]	329.75±2.84[b]	34.60±1.11[b]	1.91±0.10[b]
Liv52AF (Group 9)	38.5±1.51[a]	31.5±1.04[a]	608.40±2.21[a]	51.80±1.60[a]	2.17±0.15[a]

SOD, Superoxide dismutase; Hb, Haemoglobin.
Values are mean±S.D., n=6.
The letters mean the statistically significant difference compared with the group 3 ($p<0.05$).

Table 4. Hepatocurative effect of kombucha black tea on antioxidant enzymes changed by AFB_1 administration

Groups	Glutathione peroxidase nmol NADPH /min/mg/protein	Glutathione reductase nmol NADPH /min/mg/protein	Glutathione S transferase nmol CDNB /min/mg/protein	Catalase μmol H_2O_2 /min/mg/ protein	SOD U/g Hb
Normal (Group 1)	41.00±2.28	24.00±2.09	409.00±3.40	44.25±1.21	2.33±0.30
DM (Group 2)	38.50±1.04	22.50±0.83	404.00±2.52	42.75±1.20	1.99±0.19
AF (Group 3)	34.00±2.28	19.00±1.78	593.41±3.65	31.30±1.73	1.44±0.15
BT (Group 4)	38.30±3.56	23.30±0.99	405.75±1.02	44.51±3.20	2.18±0.14
KBT (Group 5)	39.05±1.08	22.95±1.05	406.05±1.07	42.72±0.53	2.21±0.12
Liv52 (Group 6)	40.87±0.79	23.70±0.90	407.85±1.23	43.91±1.45	2.28±0.18
BTAF (Group 7)	35.65±0.94[c]	19.35±0.56[c]	569.65±2.10[c]	31.71±1.02[c]	1.53±0.17[c]
KBTAF (Group 8)	36.05±1.00[b]	20.00±0.33[b]	558.95±2.62[b]	35.96±0.79[b]	1.72±0.13[b]
Liv52AF (Group 9)	37.85±0.40[a]	21.10±1.15[a]	520.00±1.58[a]	46.37±0.61[a]	1.80±1.10[a]

SOD, Superoxide dismutase; Hb, Haemoglobin.
Values are mean±S.D., n=6.
The letters mean the statistically significant difference compared with the group 3 ($p<0.05$).

indicators in hepatic injury. In cases, such as liver damage with hepatocellular lesions, these enzymes are released from the liver into the blood stream. In our study, elevation of SGOT and SGPT activities were observed in rats treated with AFB1 treatment. Similar elevation in SGOT activity (26 fold) and SGPT activity (39 fold) were reported after intraperitoneal administration of AFB1 (1 mg/kg b.w.) to rats [Souza *et al*., 1999]. The results of the present study indicate a significant increase in the activities of these markers enzymes in serum, which is in accordance with the previous reports [Sai Ram *et al*., 2000]. In rats treated with AFB1 (3 mg/kg feed) for 15 days, SGPT and SGOT activities were increased by 5.2 and 3.8 fold respectively [Abdel-Wahhab and Ahy, 2003]. Pretreatment with BT and KBT (group 7 and 8) significantly lowered the levels of these enzymes and the values were comparable with that of the control animals and Liv52 (group 9) pretreated group. This suggests the hepatoprotective role of BT and KBT. Decrease in SGOT and SGPT activities by BT and KBT (group 7 and 8, respectively) in AFB1 treated rats could be due to the presence of tea flavanoids-the known antioxidants. GGT which is a biomarker for hepatocarcinogenesis was also found to be significantly elevated from 1.5 to 9.2 U/L in AFB1 treated rats. In BT, KBT and Liv52 (group 7, 8 and 9, respectively) treated groups, GGT was found to be reduced to 4.95, 4.31 and 3.63 U/L respectively. The protection offered by BT, KBT and Liv52 may be due to their antioxidant effects and their ability to act as a free radical scavenger and thereby protecting membrane

permeability.

Tea contains polyphenols, flavanols (theaflavins and thearubigins), catechins, caffeine, adenine, theobromine, theophylline, xanthine, gallotannin and small amounts of aminophylline. Theaflavin and thearubigins are products of the enzymatic oxidation of polyphenols [Xie *et al*., 1993]. Most of the beneficial effects of tea have been attributed to the antioxidant and free-radical scavenging properties of tea components such as polyphenols and flavanols. Fadhel and Amran [2002] reported that black tea components (0.7%) exhibit antioxidative effects against carbon tetrachloride induced toxicity in liver of male and female rats. Black tea might act to prevent tissue damage by shielding cells from toxic agents and protect them against cytotoxic agents, carbon tetra chloride and its free radicals. Protection to AFB1 induced liver damage is in the order of Liv52>KBT>BT. From the results of the present study, it is interesting to note that KBT prevents the liver damage more effectively than BT. The difference in the effect of BT and KBT on inducing GPx and GST may be attributed to their chemical composition. Kombucha tea is claimed to be natural detoxifier due to the presence of D-glucuronic acid. In the liver, glucuronic acid binds up toxins from both environmental and metabolic processes through UDP-glucuronyltransferase, and brought them to the excretory system [Blanc, 1996]. The presence of higher concentration of glucuronic acid in KBT than BT was reported by Jayabalan *et al.* [2007] which could be an explanation to the high hepatoprotective property of KBT. In this study, the daily intake of EGCG by rats was calculated to be 9 mg/kg/day and 8.2 mg/kg/day when drinking BT and KBT, respectively, instead of tap water. Though KBT contains less concentration of EGCG than BT, fermentation with tea fungal consortium for 14 days yield KBT with more potential antioxidant compounds. Dufresne and Farnworth [2000] proposed that some curative effects of kombucha tea might come from fermentation process but the mechanism remained unclear.

Chu and Chen [2006] reported that the average antioxidant potentials of kombucha tea after fermenting for 15 days were raised to about 17, 40, 49%, respectively, as determined by the assays of DPPH, ABTS radical scavenging and inhibition of linoleic acid peroxidation. They have also reported that phenolic content was decreased which implied that thearubigin might be subjected to biodegradation during fermentation, resulting in the release of smaller molecules with higher antioxidant activities. In our laboratory, it was observed that 5% of theaflavin and 11% of thearubigin were lost when kombucha fermentation was extended upto 18 days [Jayabalan *et al*., 2007]. The brown color of the black tea was mainly from the chromophoric group of thearubigins. Since the color intensity of the kombucha broth is decreasing during fermentation period, it is suggested that thearubigins undergo microbial transformation. It has been reported that kombucha starters secreted some unknown enzymes, which are capable of catalyzing the biodegradation of thearubigin and the hydrolysates were potent antioxidant molecules [Chu and Chen, 2006; Jayabalan *et al*., 2007].

From the results of our study, it can be concluded that pretreatment with plain black tea and kombucha black tea attenuated AFB1 induced hepatic injury as evidenced by the inhibition of lipid peroxidation, preventing reduced glutathione depletion and the decrease in transaminase enzymes. Thus black tea and kombucha black tea may play protective role against AFB1-mediated liver injury. It is interesting to note that efficiency of kombucha black tea in preventing AFB1 induced liver injury is more than black tea. The present study indicates that KBT pretreatment can enhance hepatic glutathione antioxidant/detoxification system. This effect is important in removing free radicals and reactive intermediates generated from the metabolism of environmental toxins. The mechanism of hepatoprotection offered by KBT treatment may involve the facilitation of both antioxidant and detxification processes in the liver.

Evaluation of hepatoprotective and hepatocurative effects against AFB_1 induced toxicity revealed that pretreatment of BT and KBT attenuated AFB_1 induced hepatic injury. KBT is more effective in protecting than curing AFB_1 mediated liver toxicity. It is interested to note that KBT is efficient than BT in protecting the liver. The study also indicated that KBT treatment enhances the hepatic glutathione antioxidant/detoxification system. The efficiency of KBT over BT in protecting liver from AFB_1 toxicity may be due to the presence of higher concentrations of glucuronic acid and the biotransformed products produced during fermentation.

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