EFFECT OF GREEN TEA ON SOME BIOCHEMICAL PARAMETERS IN RATS TREATED WITH AFB1-CCl4 AS CANCER INDUCERS

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ABSTRACT

In the present work 160 male albino rats were randomly divided into four groups (40 rats each): Group I (control group), Group II (AFB_1-CCl_4), Group III (AFB_1-CCl_4, followed by green tea extract (GTE) as the sole source of drinking fluid and Group IV GTE followed by AFB_1-CCl_4. The effect of GTE on the relative weight of liver and kidney, and some biochemical parameters were determined in rats treated with AFB_1-CCl_4.

Treating rats with AFB_1+CCl_4 (Group II) for 12 weeks increased the relative weight of liver together with kidney compared with thoseof untreated rats (Group I, control). The enlargement of these organstended to decrease by the end of the experiment (16 weeks), but stillhigher than those of control. The harmful effect of AFB_1+CCl_4 on therelative weight of the above mentioned organs, has been markedly remediated with applying GTE either after or before applying AFB_1-CCl_4.

Changes in ALT level in serum of rats of Group III was similar to those recorded in Group II during the first 12 week, but tend to decrease sharply to attend the levels recorded in serum of the animal of control group by the end of week 16. Treating rats with GTE either after or before AFB_1+CCl_4 treatments depressed the level of ALT to values similar to those recorded in control animals (Group I). The changes in AST in serum of rats of the four groups during the experimental period were similar to those demonstrated for ALT.

The serum total bilirubin in rats treated with AFB_1 followed by CCl_4 Group II and III was significantly higher than those recorded for control animals especially during the first 12 weeks of the experiment. Treatment with GTE before AFB_1-CCl_4 revealed total bilirubin levels very close to those observed for the control animals during the whole experemintal period.

Results indicated that both urea and creatinine (except for the first 2 weeks), increased steadily in rats treated with only AFB_1+CCl_4 (Group II and III) for 12 weeks, then their concentration tend to decrease during the last four weeks of the experiment. Treating rats with GTE before AFB_1+CCl_4 treatment (Group IV) decreased the elevation in both urea and creatinine concentration throughout the experimental period (16 weeks). Pretreating with GTE seemed to be more effective as chemopreventive agents (Group IV) rather than post-treating (after AFB_1+CCl_4) (Group III).

Data reveal that treating rats with AFB_1+CCl_4 (GroupII) resulted in gradual increases in GSH up to the twelfth week, and then it tended to decrease. On the other hand, GST behaved in opposite trend shown in case of GSH. Post-treating rats with GTE exerted such decreases in GSH values, but to less extent compared with GTE pretreated rats, which seemed to be more effective than post-treating GTE did.

INTRODUCTION

Contamination of the foods with aflatoxin B1 (AFB1) is one of the important risk factors responsible for human liver cancer (Wogan, 1992). Carbon tetrachloride (CCl4) a well known hepatotoxin , plays an important role in the promotion of hepatic carcingenesis induced by exposure to various chemicals, including AFB1 and administration of CCl4 and AFB1 has been reported to accelerate carcinogenesis (Hiruma et.al., 1996 and Aziz et.al., 2005).

The harmful effects of AFB1 have been attributed to the metabolism of this mycotoxin to reactive metabolites that can bind to cellular macromolecules. Initially, an epoxidation reaction catalyzed by certain cytochrome P450 (CYP450) enzymes especially hepatic CYP1A2 and CYP3A4 convert AFB1 to the DNA-reactive AFB1 exo 8,9-oxide (Essigman et.al., 1977 and Iyer et.al., 1994). Glutathione S-transferase (GSTs) can conjugate this reactive epoxide with reduced glutathione. Thus preventing the formation of DNA adducts which can

lead mutation (Neal et.al., 1986). Alternatively, this epoxide can hydrolyse to form the AFB1 dihydrodiol. Under physiological conditions, AFB1 dihydrodiol can rearrange, forming a reactive dialdehyde configuration that can bind to primary amine group in proteins by Schiff base reaction (Patterson and Roberts, 1970; Neal and Colley, 1979 and Sabbioni et.al., 1987). Another detoxication enzyme designated AFB1 aldehyde reductase (AFAR), was isolated and characterized by Judah et.al., (1993), from the liver of rats. AFAR catalyzes the reduction of AFB1 dialdehyde to dialcohol.

Carbon tetrachloride (CCl4) is a toxic agent used in experimental liver damage. It is metabolized by the mitochondrial monooxygenase (P450 2E1) system. During the metabolism, an unstable trichloromethyl (CCl3) free radical is formed, and rapidly converted to trichloromethyl peroxide (Cl3COO-) (Recknagel et.al., 1989). These free radicals lead to the peroxidation of fatty acids found in the phospholipids making up the cell membranes. Consequently, cell membrane structures and intracellular organelle membrane structures are completely broken down. Structural damage spreads. Chronic administration results in fibrosis and cirrhosis (Brattin et.al., 1985). Lipid peroxidation is important in liver damage associated with CCl4 (Gasso et.al., 1996). Kuzu et.al., (2007) investigated the protective effect of genistein in experimental acute liver damage induced by CCl4.

Tea is broadly classified according to the production method as unfermented tea (green tea), half-fermented tea (oolong tea), and full-fermented tea (black tea). Green tea (GT) is produced from freshly harvested leaves of the tea plant, Camellia sinensis. Exposure of fresh tea leaves to hot steam and air inactivates polyphenol oxidase and results in a brilliant green color, from which it gets the name of green tea which is polyphenol-rich (Loest et.al., 2002)).

Commercially prepared green tea extracts (GTEs) contain 60% polyphenols (USDA, 2003). These polyphenols are the source of bioflavonoids, which have strong antioxidant activity. The major bioflavonoids in green tea are epicatechins. Like all bioflavonoids, the tea catechins have three hydrocarbon rings; hydroxyl molecules are found at the 3, 5, and 7 positions. The four major tea catechins are epicatechin (EC), EC 3-gallate (ECG), epigallocatechin (EGC) and EGC 3-gallate (EGCG). The relative proportions of EC, ECG, EGC and EGCG in non-decaffeinated green tea are 792 ± 3, 1702 ± 16,

1695 ± 1 and 8295 ± 92 mg 100 g_1 dry wt, respectively; corresponding proportions in non-decaffeinated black tea are 240 ± 1, 761 ± 4, 1116 ± 24 and 1199 ± 0.12 mg 100 g_1 dry wt (USDA, 2003).

Green tea is being widely studied for its alleged beneficial properties in the treatment or prevention of human diseases. To date, more than 1,500 articles referencing "green tea" are listed in Medline. Green tea is reported to delay or prevent certain forms of cancer, arthritis, and cardiovascular and other disorders (Higdon and Frei, 2003 and Mustata et.al., 2005) Epidemiological studies show that polyphenolic compounds present in tea reduce the risk of a variety of diseases (Zhang et.al., 2002; Wu et.al., 2003 and Jian et.al., 2004).

The most abundant and biologically active green tea catechin, (-) -epigallocatechin-3-gallate or (–)-EGCG, has been shown to act as a proteasome inhibitor and tumor cell death inducer (Landis et.al., 2007). However, (–)-EGCG is unstable under physiologic conditions and has poor bioavailability. Previously, in an attempt to increase the stability of (–)-EGCG, they introduced peracetate protections to its reactive hydroxyl groups and showed that this peracetate-protected (–) -EGCG [Pro-EGCG (1)] (Landis et.al., 2007) increases the bioavailability, stability, and proteasome-inhibitory and anticancer activities of (–)-EGCG in human breast cancer cells and tumors, suggesting its potential use for cancer prevention and treatment.

To consider whether consumption of black tea has a positive or negative impact on health. Databases were searched by Gardner et.al., (2007) for relevant epidemiological and clinical studies published between 1990 and 2004. They (Gardner et.al., 2007) concluded that there was sufficient evidence to show risk reduction for coronary heart disease (CHD), at intakes of more than 3 cups per day and for improved antioxidant status at intakes of one to six cups per day. They added that Black tea generally had a positive effect on health.

The goal of this study was to examine the effects of green tea extract (GTE) on Aflatoxin B1 (AFB1)-initiated and carbon tetrachloride (CCl4)-promoted hepatic carcinogenesis in rats. The effects of green tea extracts (GTE) on the relative weights of liver and kidney and some biochemical parameters in rats treated with AFB1-CCl4 were performed.

MATERIALS AND METHODS

The pure crystalline AFB1 toxin was dissolved in an adequate volume of dimethylsulfoxide (DMSO) as a solvent then completed to the required volume by 0.9 NaCl saline, pH 7.2 to perform the final concentration 300 µg/ml. (i.e. 1/20 of LD50 in male rat; 6 mg / kg b.wt. as determined by Butler and Neal, (1973).

Carbon tetrachloride (5ml) was dissolved in corn oil (45ml) to obtain a solution of CCl4 which were used for treating rats (0.08ml/100gm B.W) as recommended by Qin et.al., (1998) and Aziz et.al., (2005).

GTE (1.5%) was prepared daily as described by Loest et.al., (2002) with some modifications. GT leaves (7.5g) were soaked in tap water (980C) for 5 min. The extract was filtered and the volume was adjusted to 500 ml in a volumetric flask with tap water. The extract (GTE) was used as the sole source of drinking fluid.

Animals and treatments:

One hundred and sixty five male wistar rats (*Rattus norvegicus*) 5-6 weeks old (120-140g body weight) were purchased from a private sector breeding laboratory in Giza, great Cairo, Egypt.

The rats were housed in plastic cages in air condition room at 25 ± 2 (with a 12 hr light/dark cycle). A commercial balanced diet and tap water *ad libitum* were provided for at least one week before starting the experiment. The rats (160 rats) were randomized divided into four groups (40 rats each), and five rats were scarified at the beginning of the experiment (week 0).

The control group (Group I) received vehicle only (injected intraperiteonaly, i.p., with 0.9 NaCl saline, pH 7.2 or corn oil). The rats of second group (Group II) were injected with AFB_1 (0.5 mg / kg B.W i.p.) each third days for four weeks and followed by injection with CCl_4 dissolved in corn oil (0.8 ml / kg B.W i.p.) once per week for 8 weeks and left without treatment for another four weeks. The third group (Group III) received AFB_1 and CCl_4 as described in group II, except that dirking water was replaced with green tea extract (GTE) as the sole source of drinking fluid for another four weeks. The fourth group (Group IV), received green tea extracts (GTE) as the sole source of drinking fluid for four weeks. Then treated with AFB_1 and CCl_4 as described in group II.

Sampling of Blood and Tissues:

At two weeks intervals, the diets were removed from the cages at 8 am. The animals (5 from each group) were lightly anesthetized with ether, killed between 2 p.m. and 4 p.m. and the blood samples were collected from animals, through decapitation avoiding first drops. Suitable volumes of fresh blood were immediately taken after addition of EDTA as anticoagulant for hematological and immunological examinations. The other parts of blood samples were allowed to coagulate at room temperature, and then centrifuged at 4^0C and the clear non-haemolysed sera were separated and stored at -20^0C till used in biochemical analysis.

Animals were dissected as quickly as possible and the liver, kidney, spleen and testes were excised, wiped with filter paper and weight. Parts of these organs were fixed in 10 % neutral buffered formalin and stored in 70 % ethanol for histopathological examinations. The rest of liver parts were homogenized in ice-cold 100 mM phosphate buffer, pH 7.4. Homogenates were centrifuged at 7000 r.p.m for 20 min at 4^0C and the resulting supernatant were stored at -80^0C.

Biochemical Assays:

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in serum were determined according to the method of Reitman and Frankel, (1957) using reagent kits purchased from BioMerieux Chemical Company (France).

Total bilirubin was determined by a coupling reaction with diazotized sulfanilic acid, in the presence of caffeine to give an azo dye. The same reaction was used to measure direct bilirubin, but in the absence of caffeine (Jendrassik and Grof, 1938; Gambino, 1956 and Tietz, 1983).

Serum total protein was determined by the biuret reagent as described by Gornall et.al., (1949). Serum albumin concentration was determined according to the method of Doumas et.al., (1971) using reagent kits purchased from Spinreact Company (Spain). Serum globulin concentration and albumin/globulin ratio were calculated according to Doumas et.al., (1971) using the following equations:-

Globulin (g/L) = Total protein (g/L) – Albumin (g/L)

The determination of glucose concentration was performed according to the method of Siest and Schielef, (1981), using reagent kits purchased from Bio Merieux Chemicals (France).

Serum urea was quantified with the urease-glutamic dehydrogenase reaction as described by Eisenweiner, (1976). Creatinine was determined by its reaction with picric acid in alkaline medium, the colored complex was proportional to the concentration of creatinine in the sample, which was measured at 510 nm (Larsen, 1972 and Bartles et.al., 1972).

C- Extraction and assay of Caspase-3:

Pieces of fresh liver were homogenized in 100 mM phosphate buffer, pH 7.4 containing 25 mM HEPPS (4-(2-hydroxy ethyl-1-piperazine propane sulfonic acid), pH 7.5, 5 mM $MgCl_2$, 1mM EDTA (ethylene diamine tetra acetic acid), 0.1 % (w/v) CHAPS (3-(3-chloramido propyl dimethyl ammonio)-1-propane sulfonate) and 10 µg / ml aprotinin. After centrifugation at 7000 r.p.m for 20 min at 4^0C, the supernatant was used for assaying Caspase-3 according to the procedure described by **(Jaeschke *et.al.*,1998 and Kim *et.al.*, 2000),** using Acetyl-Asp-Glu-Val-Asp-p-nitroaniline as a substrate.

D-Estimation Of Glutathione (GSH) In Liver Tissues:

The level of reduced glutathione (GSH) in liver extract was determined as described by **Mitchell *et.al.*, (1973)** using 5,5-Dithio-bis (2-nitrobenzoic acid) (3-carboxy-4-nitro-phenyl disulfide; DTNB; Elman's reagent) as a substrate.

E- Assay of Glutathione S-transferase (GST):

Hepatic GST activity was determined by the method of Habig et.al., (1974) using 1-chloro-2,4-dinitro benzene and glutathione as substrares.

RESULTS AND DISCUSSION

This work was conducted to investigate the effect of GTE on rats treated with AFB_1 followed by CCl_4. Several models for hepatic carcinoma have been developed and we used AFB_1-initiated and CCl_4-promoted hepatic carcinogenesis in rats as reported by many investigators (Cupid et.al., 2004; Park et.al., 2004 Shupe and sell, 2004 and Aziz et.al., 2005). In the present work 160 male albino rats were randomly divided into four groups (40 rats for each):

Group I (control group), Group II (AFB1-CCl4 treated group), Group III (AFB1-CCl4, followed by GTE as the sole source of drinking fluid) and Group IV (GTE as the sole source of drinking fluid followed by AFB1-CCl4 treated group). Various treatments are described in materials and methods. During the present study, the effect of treatment with AFB1-CCl4 and GTE on the relative weight of liver and kidney, and some biochemical parameters were determined.

Changes in relative weight of liver and kidneys:

Treating rats with AFB_1+CCl_4 (Group II) for 12 weeks increased the relative weight of liver **(Fig 1)** together with kidney **(Fig 2)** compared with those of untreated rats (Group I, control). The enlargement of these organs tended to decrease by the end of the experiment (16 weeks), but still higher than those of control.

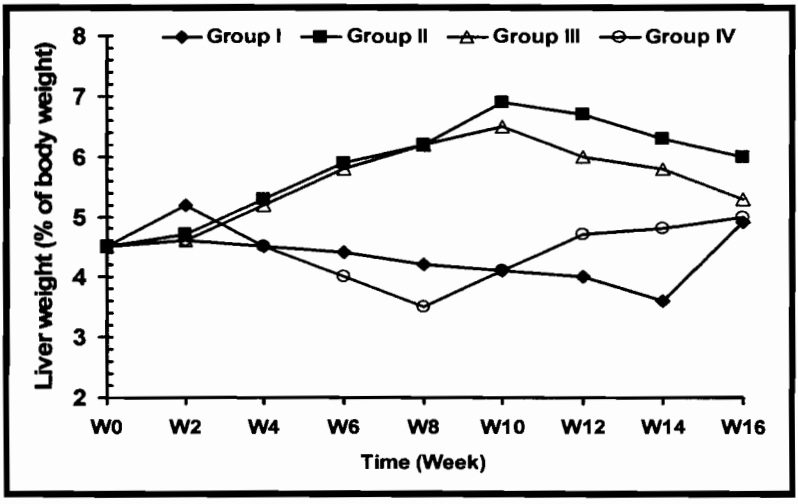

Fig (1): Effect of GTE on weight of liver (expressed as relative weight) in male albino rats treated with AFB1+ CCl4

The harmful effect of AFB_1+CCl_4 on the relative weight of the above mentioned organs, has been markedly remediate with applying GTE either after or before applying AFB_1-CCl_4.

Hepatic and kidney damage due to CCl4 ingestion or inhalation has been reported (ATSDR, 1992 and U.S. EPA, 1995). Also DHS,

(1987) indicated that both sub-chronic and chronic exposure affected the same targets as acute exposure on nervous system and liver and kidney. Moreover liver / body weight ratios are dose dependant in mice received several CCl4 doses ranged between 12 up 1200 mg/kg for 90 days (Hayes et.al., 1986).

Several compounds have been demonstrated to have such chemoprotective effect against AFB1 and CCl4. Abd El-Rahman et.al., (2007) dealing with inisitol hexa phosphate (IP6) which depressed the increase in hepatic weight, Yasseen (2007) reported that Bowman-Birk protease inhibitor (BBI) depressed the increase of relative weight of liver and kidney in rats treated with AFB1-CCl4 compared with control animals.

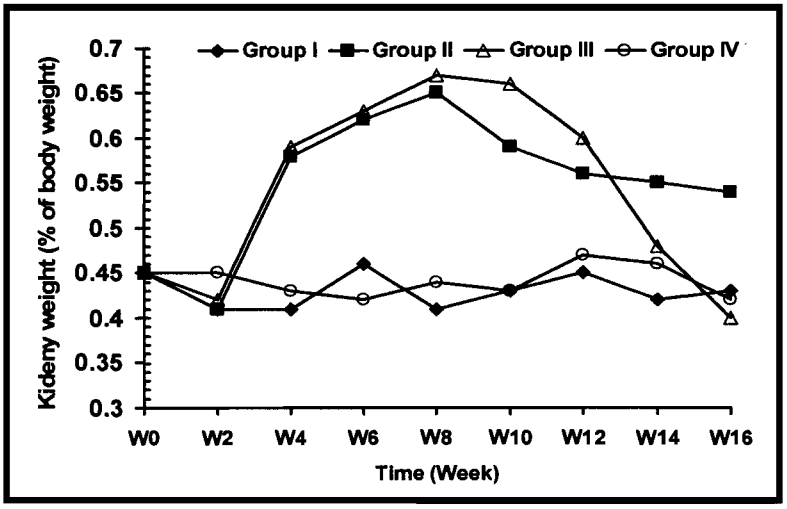

Fig (2): Effect of GTE on weight of kidney (expressed as relative weight) in male albino rats treated with AFB1+ CCl4

Changes in some biochemical parameters in serum of rats treated with AFB1 + CCl4:

Changes in serum transaminases (ALT and AST) activity in rats of different group are shown in Fig 3 and 4. Treating rats with AFB$_1$+CCl_4 for 12 weeks resulted in serious liver damage, as ALT activities increased significantly and reached maximum after 12 weeks (from 28.00 to 112.00 U/L) but tend to decrease to (80.00 U/L) by the end of week 16th, but still higher than the value recorded in the control animal (33.00 U/L) Changes in ALT level in serum of rats of Group III

was similar to those recorded in Group II during the first 12 week, but tend to decrease sharply to attend the levels recorded in serum of the animal of control group by the end of week 16. Treating rats with GTE either after or before AFB_1+CCl_4 treatments depressed the level of ALT to values similar to those recorded in control animal (Group I)

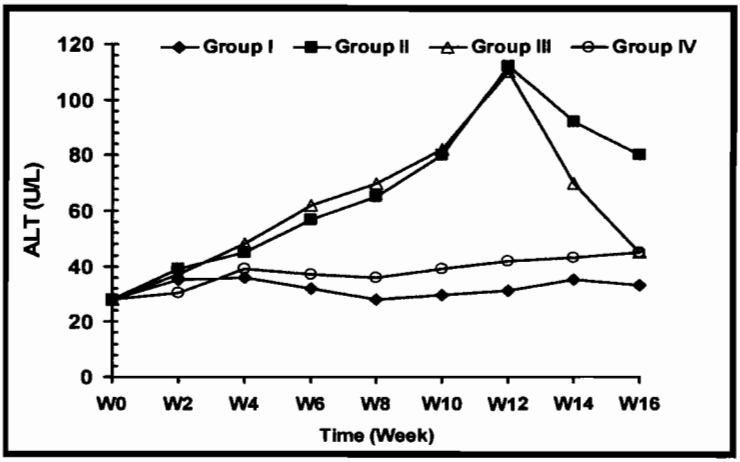

Fig (3): Effect of GTE on level of ALT (U/L) in serum of male albino rats treated with AFB1+ CCl4

As shown in Fig 4 the changes in AST in serum of rats of the four groups during the experimental period were similar to those demonstrated for ALT but the levels of AST were higher than those recorded for ALT.

Depending upon the above mentioned findings we can conclude that GTE, effectively relieved liver damage induced by AFB1+CCl4, whether they were administrated before or after AFB1+CCl4 treatment; as GTE effectively remediated the elevation of serum transaminases (ALT and AST). Similar finding was reported by Kuzu et.al., (2007) who observed protective role of Genistein in acute liver damage induced by CCl4. Serum enzymes including ALT and AST are used in evaluating hepatic diseases. An increase in these enzyme activities reflects liver damage, either chronic or acute. Acute inflammatory hepato cellular disorders resulted in elevated s transe aminases levels (Kaplan, 1972 and Forstan et.al., 1995).

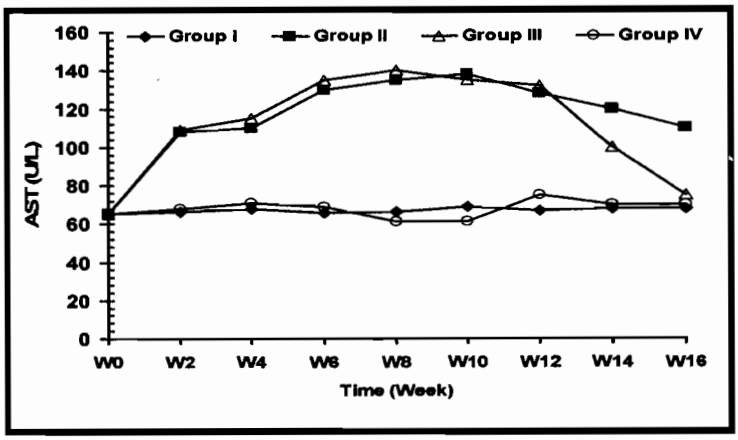

Fig (4): Effect of GTE on level of AST (U/L) in serum of male albino rats treated with AFB1+ CCl4

Data presented in Fig 5 indicated that the serum total bilirubin in rats treated with AFB_1 followed by CCl_4 Group II and III was significantly higher than those recorded for control animals especially during the first 12 weeks of the experiment. Treatment with GTE before AFB_1-CCl_4 revealed total bilirubin levels very close to those observed for the control animals during the whole experemintal period (Fig 5). A large decrease in total bilirubin was found in rats of Group III during the last 4 weeks of the experiment, but still higher and lower than the values recorded in rats of Group I and II respectively (Fig 5).

It is well known that accumulation of total bilirubin in blood is also a second marker of liver damage and or metabolic disturbance in liver. If the liver is unable to form bilirubin glucuroniods, which is secreted into bile, or if there is excessive destruction of red cell, bilirubin may accumulate in blood plasma. In this connection Cheesborough, (1992) suggested that the rise in serum levels of ALT, AST and total bilirubin in rats treated with AFB1+CCl4 compared with control may be due to liver cell damage, or metabolic disturbance in liver involving defective conjugation and / or excretion of bilirubin.

Results shown in Fig 6 and 7 indicated that both urea and creatinine (except for the first 2 weeks), increased steadily in rats treated with only AFB_1+CCl_4 (Group II and III) for 12 weeks, then their concentration tend to decrease during the last four weeks of the experiment. Treating rats with GTE before AFB_1+CCl_4 treatment

(Group IV) resulted in such chemopreventive impact, as decreased the elevation in both urea and creatinine concentration throughout the experimental period (16 weeks).

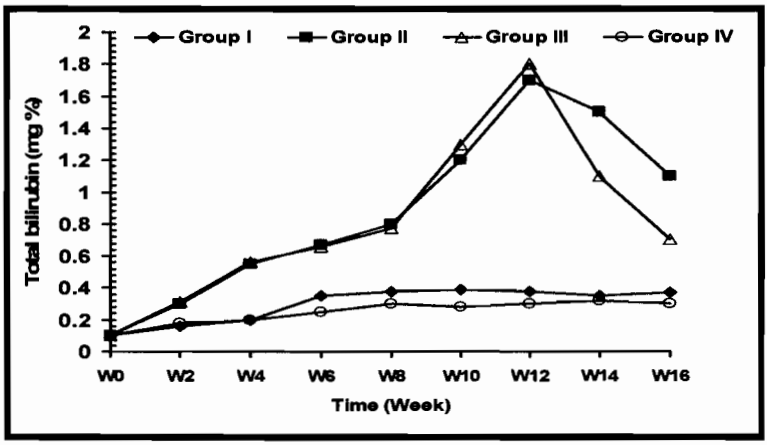

Fig (5): Effect of GTE on level of total bilirubin (mg %) in serum of male albino rats treated with AFB1+ CCl4

Data in Fig 6 and 7 proved that addition of GTE before AFB_1+CCl_4 application showed the highest efficiency in regulating both urea and creatinine concentration, which nearly approached that of normal level in control rats. On the other hand; post treating rats with GTE after AFB_1+CCl_4 mediated significantly the elevation in urea but its values still far from its normal value.

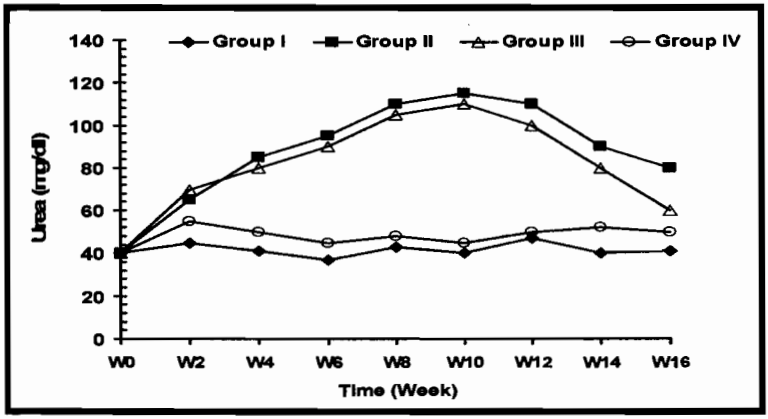

Fig (6): Effect of GTE on level of Urea (mg/dl) in serum of male albino rats treated with AFB1+ CCl4

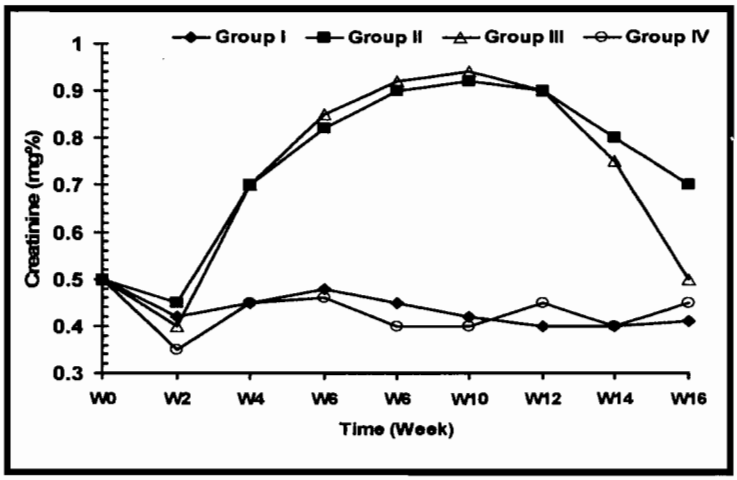

Fig (7): Effect of GTE on level of Creatinine (mg %) in serum of male albino rats treated with AFB1+ CCl4

Changes in serum urea and creatinine have been used as important indices for evaluating the impact of chemicals on kidney functions. Increasing of urea and creatinine concentration in blood suggest the inability of kidney to excerete these waste products, and consequently further suggest a decrease in glomerular filtration rate (GFR). A common manifestation on nephritic damage in acute renal failure is characterized by decline in GFR, which may have been induced by toxic chemicals including AFB_1 and CCl_4 (Daves and Berndt, 1994).

Changes in Caspase-3 activity in rat liver:

The changes in Caspase-3 activity due to hepatotoxicity of AFB_1+CCl_4, and to what extent GTE has relieved these deleterious effect, are illustrated in **Fig 8.** Caspase-3 activity elevated steadily due to AFB_1+CCl_4 application up to the 12 week, then it tended to decrease up to the 16 week as rats kept out any further AFB_1+CCl_4 treatment (Group II). Treating with GTE, showed very effective remedial influence in decreasing Caspase-3 activity towards the normal levels in control rats. Pre-treating with GTE seemed to be more effective as chemopreventive agents (Group IV) rather than post-treating (after AFB_1+CCl_4) (Group III).

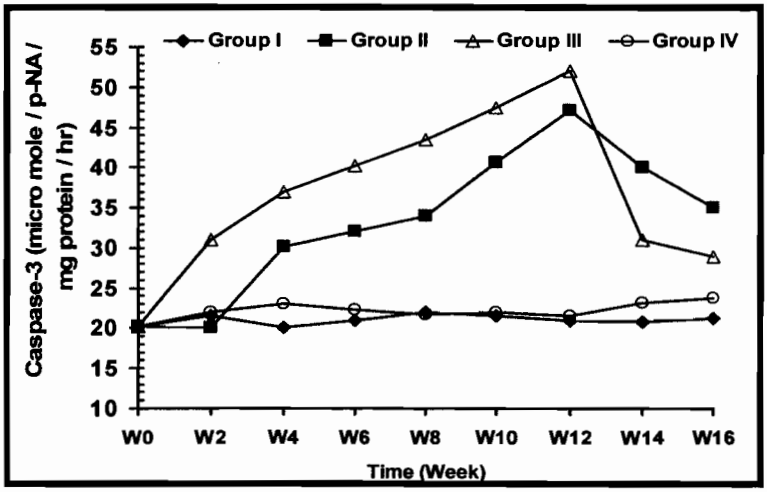

Fig (8): Effect of GTE on Caspase-3 activity (µmol p-Nitro aniline/mg protein/h) in liver tissues of male albino rats treated with AFB1+ CCl4

Caspase-3 enzyme is apoptosis effector, therefore its activity has been determined in rat livers of the different groups examined in the present study. This enzyme is one of cysteine proteases, which plays a major role in the execution of apoptosis (Nicholson, 1999). A number of genetic and biochemical studies have suggested that Caspase-3 activation is essential for the occurrence of the apoptotic phentype of cell death (Janicke *et.al.*, 1998). Apoptosis is a programmed mode of cell death and depends on the subsequent activation of different processes, which ultimately leading to the proteolyic activation of cysteine protease (Caspases) which in turn catalyze the cleavage of the cellular macromolecules, i.e., protein and DNA (Ferreira *et.al.*, 2002).

Changes in glutathione (GSH) and glutathione-S-transferase (GST) in rat livers:

Changes in GSH and GST in livers of rats of all groups used in this work are shown in Fig 9 and 10, which revealed that GSH and GST are inversely related to each other, i.e., an increase in GST activity may mean low levels of GSH in rat liver, and vise versa. Therefore it is better to discuss the influence of feeding rats with GTE on GSH (Fig 9) together with GST (Fig 10) in liver tissues of rats treated with AFB_1+CCl_4. Data reveal that treating rats with

AFB_1+CCl_4 (Group II) resulted in gradual increases in GSH up to the twelfth week, then it tended to decrease by stopping further AFB_1+CCl_4 application up to the sixteenth week (Fig 9). On the other hand, GST behaved in opposite trend shown in case of GSH (Fig 10). Post-treating rats with GTE exerted such decreases in GSH values, but to less extent compared with GTE pretreated rats, which seemed to be more effective than post-treating GTE did (Fig 9).

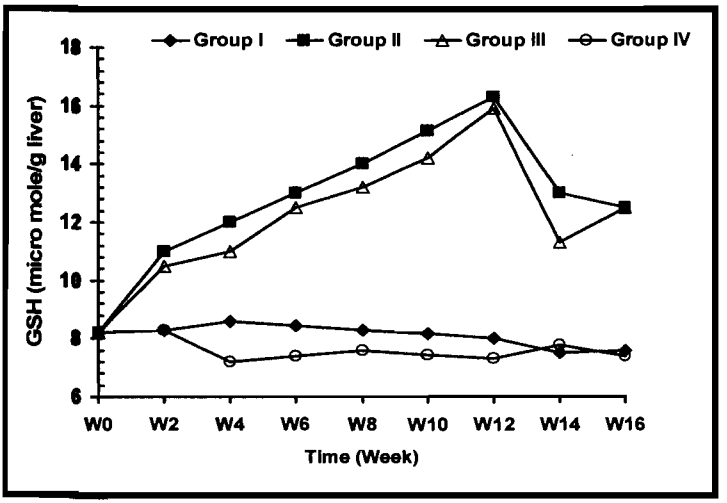

Fig (9): Effect of GTE on Glutathione (GSH) level (μmol of reduced GSH/g liver) in liver tissues of male albino rats treated with AFB1+CCl4

Behavior of GST are demonstrated in Fig 10 which revealed that pre-treating rats with GTE exerted marked elevation in GST activity compared with control up to the twelfth week. On the other hand, after 12 weeks, GTE used before or after AFB_1+CCl_4 exerted significant high activities compared with both control and rats treated only with AFB_1+CCl_4 as the latter treatment showed the lowest GST activity during the course of experiment (Fig 10).

Glutathione-S-transferases (GSTs) are family of phase II detoxification enzymes that catalyze the conjugation of GSH to a wide variety of toxic endogenous and exogenous electrophilic compounds (Tounsend and Tew, 2003). GSH and GST aid in the protection of cells from the lethal effects of toxic carcenogenic compounds (Ketlerer, 1988). GSH and GST can reduced the covalent binding of epoxides to DNA (Forming DNA adducts) and other macromolecules.

This reduction in DNA binding is effective in decreasing the incidence of hepatocarcenogensis caused by AFB1 (Lotlikar et.al., 1980).

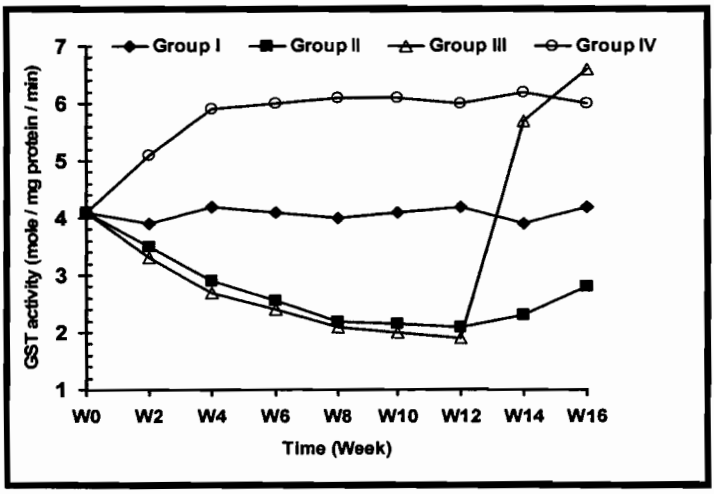

Fig (10): Effect of GTE on Glutathione-S-transferase (GST) activity (μmol/mg protein/min) in liver tissues of male albino rats treated with AFB1+ CCl4

GSH and its related enzymes have numerous functions including protection of tissues against damage to toxic metabolites through conjugation via:

(a) GST catalyzed reactions. (b) Oxidation of GSH by H2O2 (c) Glutathione peroxidase catalyzed reactions. These reactions lead to the generation of oxidized glutathione (GSSG), which is reduced back to GSH by glutathione reductase and thus maintain the redox state (Cartana et.al., 1992).

In conclusion, the role of tea in protection against cancer has been supported by ample evidence from studies in cell culture and animal models. However, epidemiological studies have generated inconsistent results, some of which associated tea with reduced risk of cancer, whereas others found that tea lacks protective activity against certain human cancers. These results raise questions about the actual role of tea in human cancer that needs to be addressed. Thus more work should be performed to clear the role of green tea in cancer prevention.

REFERENCES

Abd El-Rahman, A.A.; Abd El-Naem, G.F.; El-Shafei M.A. and El-Morsi, E.A. (2007). Effect of Phytic acid on liver and kidneys weight and some Biochemical parameters in Albino rats treated with Aflatoxin B1 In the proceeding of 8th African crop Science Society (ACSS) and Faculty of Agriculture, Minia University conference. 27-31 October, 2007, Minia, Egypt 1765-1772.

ATSDR (1992). Carbon tetrachloride toxicity. American Family Physician. 46: 1199-1207.

Aziz, A.T.; Abd El-Aziz, M.; Fouad, H.H.; Rashed, L.A.; Salama, H.; Abd-Alla, S.; Abdel wehab, M.A. and Ahmed, T. (2005). Interferon-α·gene therapy prevents aflatoxin and carbon tetrachloride promoted hepatic carcinogenesis in rats. Inter. J. Molecul. Med. 15: 21-26.

Bartles, H.; Bohmer, M. and Heirli, C. (1972). Clin.Chem. Acta. 37: 193.

Brattin, W.J.; Glende Jr., E.A. and Recknagel, R.O. (1985). Pathological mechanisms in carbon tetrachloride hepatotoxicity, Journal of Free Radicals in Biology and Medicine.1: 27-38..

Butler, W.H. and Neal, G.E. (1973). The effect of aflatoxin B1 on the hepatic structure and RNA synthesis in rats fed a diet marginally deficient in choline. Cancer. Res. 33: 2878-2885.

Cartana, J.; Romiu, A. and Arola, L. (1992). Effect of copper, cadmium and Nickel on liver and kidney Glutathione redox cycle of rats. (Rattus. Sp.) Comb. Biochem. Physiol. 101: 209-216.

Cheesborough, M. (1992). Medical Laboratory Manual for tropical countries. Vol I pp.472-505. Butter worth-Heinemanm Ltd., Hakkey court, Jordan Hill.

Cupid, B.C.; Lightfoot, T.J.; Russell, D.; Gant, S.J.; Turner, P.C.; Dingley, K.H.; Curtis, K.D.; Leveson, S.H.; Turteltaub, K.W.; and Garner, R.C. (2004). The formation of AFB1-macromolecular adducts in rats and humans at dietary levels of exposure. Food and Chemi. Toxicolo. 42: 559-569.

Davis, M.E. and Berndt, W.D. (1994). Renal methods for toxicology. In Hayes A.W. (eds). Principles and Methods of toxicology 3rd Ed. New York Raven, pp 871-894.

DHS (1987). Part B. Health effects of carbon tetrachloride. Report to the Air Resources Board on Carbon Tetrachloride Submitted to the

Scientific Review Panel for Review. California Department of Health Services. Currently the Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Doumas, B.T.; Watson, W.A. and Biggs, H.G. (1971). Determination of serum albumin. J. Clin. Chem. Acta. 31: 87-89.

Eisenweiner, H.G. (1976). Kinetishe Bestimung des Harnstottes mit dem LKB-System. J. Clin. Chem. Clin. Biochem. 14: 261-264.

Essigman, S.M.; Croy, R.G.; Nadzan, A.M.; Busby, W.F.; Reinhold, V.N. and Wogan, G.N. (1977). Stricture identification of the major DNA adducts formed by aflatoxin B1 in vitro. Proc. Natl. Acad. Sci. USA. 74: 1870-1874.

Ferreira, C.G.; Epping, M.; Kruyt, F.A. and Giaccone, G. (2002). Apoptosis: target of cancer therapy. Clin. Cancer Res., 8: 2024–2034.

Fortsan, W.C.; Tedesco, F.J. and Starens, F.C. (1995). Marked elevation of serum transaminase activity associated with extra hepatic biliary tract disease. J. clin. Gastroenterol. 7: 502-515.

Gambino, S.R. (1956). In standard methods of clinical chemistry.S. Meites, (Ed), New York, Academic press. 5: 55-64.

Gardner, E.J.; Ruxton, C.H.S. and Leeds, A.R. (2007). Black tea – helpful or harmful? A review of the evidence. Euro. J. Clin. Nutr. 61: 3–18

Gasso, M.; Rubio, M.; Varela, G.; Cabre, M.; Caballeria, J.; Alonso, E.; Deulofem, R.; Camps, J.; Gimenez, A.; Pajares, M.; Pares, A.; Mato, J. M. and Rodes, J. (1996). Effects of S-adenosylmethionine on lipid peroxi-dation and liver fibrogenesis in carbon tetrachloride-induced cirrhosis. J. Hepatology 25: 200-205

Gornall, A.G.; Bardawill, C.J. and David, M.M. (1949). Determination of serum protein by means of the biuret reagent. J. Biol. Chem. 177: 751-766.

Habig, W.H.; Pabst, M.J. and Jakoby, W.B. (1974). Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

Hayes, J.R.; Condie, L.W. and Borzelleca, J.F. (1986). Acute, 14-day repeated dosing, and 90-day subchronic toxicity studies of carbon tetrachloride in CD-1 mice. Fundam Appl Toxicol, 7: 454–463.

Higdon, J.V. and Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions. Crit Rev Food Sci. Nutr. 43: 89-143.

Hiruma, S.; Qin, G.Z.; Gopalan-Kriczky, P.; Shinozuka, H.; Sato, K. and Lotlikar, P.D. (1996). Effect of cell proliferation on initiation of aflatoxin B1-induced enzyme altered hepatic foci in rats and hamsters. Carcinogenesis. 17: 2495-2499

Iyer, R.S.; Coles, F.F.; Raney, K.D.; Their, R.; Guengerich, F.B. and Harris, T.M. (1994). DNA adduction by the potent carcinogen Aflatoxin B1: Mechanistic studies. J. Am. Chem. Soc. 116: 1603-1609.

Jaeschke, H.; Fisher, M.A.; Lawson, J.A.; Simmons, C.A.; Fahood, A. and Jones, D.A. (1998). Activation of caspase-3 (CPP32)-like protease is essential for TNF-J induced hepatic parenchymal cell apoptosis and neutrophil-mediated necrosis in a murine endotoxin shock model. J. Immunol. 160: 3480-3486.

Janicke, R.U.; Spregart, M.L.; Wati, M.R. and Porter, A.G. (1998). Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J. Biol. Chem. 273: 9357-9360.

Jendrassik, L. and Grof, P. (1938). Biochem. 81: 7297.

Jian, L.; Xie, L.P.; Lee, A.H. and Binns, C.W. (2004). Protective effect of green tea against prostate cancer: a case-control study in southeast China. Int. J. Cancer 108: 130-135.

Judah, D.J.; Hayes, J.D.; Yang, J.; Lian, L.; Roberts, G.C.K.; Farmer, F.B.; Lamb, J.H. and Neal, G.E. (1993). A novel aldehyde reductase with activity towards a metabolite of aflatoxin B1 is expressed in rat liver during carcinogenesis and following the adminstration of an antioxidant. Biochem. J. 292: 13-18.

Kaplan, M. (1972). Alkaline phosphatase. Gastroenerology. 62: 452-465.

Ketterer, B. (1988). Protective role of Glutathione and Glutathione-S-transferase in mutagensis and carcinogensis. Mutation Res. 202: 343-361.

Kim, H.S.; Hausman, D.B.; Comton, M.M.; Dean, R.G.; Martin, R.J.; Hausman, G.J.; Hartzell, D.L. and Baile, C.A. (2000). Induction of apoptosis by all-trans-retinoic acid and C2-ceramide treatment in rat stromal-vascular cultures. Biochem. Biophys. Res. Commun. 270: 76-80.

Kuzu, N.; Metin, K.; Dagli, A.F.; Akdemir, F.; Orhan, C.; Yalniz, M.; Ozercan, I.H.; Sahin, K.; and Bahcecioglu, I.H. (2007). Protective Role of Genistein in Acute Liver Damage Induced by Carbon Tetrachloride. Mediators of Inflammation 2007: Article ID 36381, 6 pages doi:10.1155/2007/36381

Landis-Piwowar, K.R.; Huo, C.; Chen, D.; Milacic, V.; Shi, G.; Chan, T.H. and Dou, Q.P. (2007). A Novel Prodrug of the Green Tea Polyphenol (–)-Epigallocatechin-3-Gallate as a Potential Anticancer Agent. Cancer Res. 67: 4303-4310.

Larsen, K. (1972). Clin. Chem. Acta. 41: 209.

Loest, H.B.; Noh, S.K. and Koo, S.I. (2002). Green Tea Extract Inhibits the Lymphatic Absorption of Cholesterol and α-Tocopherol in Ovariectomized Rats. J. Nutr. 132: 1282–1288.

Lotlikar, P.D.; Insetta, S. and Lyons, P. (1980). Inhibition of microsomes mediated binding of aflatoxin B1 to DNA by glutathione S-transferase. Cancer Lett. 9: 1143-1149.

Mitchell, J.R.; Jollow, D.J.; Patter, W.Z.; Davis, D.C.; Gillette, J.R. and Brodie, B.B. (1973). Acetaminophen – induced hepatic necrosis: Role of drug metabolism. J. Pharmacol. Exper. 187: 185-194.

Mustata, G.T.; Rosca, M.; Biemel, K.M.; Reihl, O.; Smith, M.A.; Viswanathan, A.; Strauch, C.; Du, Y.; Tang, J.; Kern, T.S.; Lederer, M.O.; Brownlee, M.; Weiss, M.F. and Monnier, V.M. (2005). Paradoxical Effects of Green Tea (Camellia Sinensis) and Antioxidant Vitamins in Diabetic Rats: Improved Retinopathy and Renal Mitochondrial Defects but Deterioration of Collagen Matrix Glycoxidation and Cross-Linking. Diabetes., 54: 517–526.

Neal, G.E. and Colley, P.J. (1979). The formation of 2,3-dihydro-2,3-dihydroxy aflatoxin B1 by the metabolism of aflatoxin B1 in vitro by rat liver microsomes. FEBS Lett. 101: 382-386.

Neal, G.E.; Judah, D.G. and Green, J.A. (1986). The in vitro metabolism of AFB1 catalyzed by hepatic microcosms isolated from control or 3-MC stimulated rats and quail. Toxicol. Appl. Pharmacol. 82: 454-460.

Nicholson, D.W. (1999). Caspase structure proteolytic substrates and function during apoptotic cell death. Cell Death. Diff. 6: 1028-1042.

Park, D.H.; Lee, M.S.; Kim, H.J.; Kim, H.S.; Lee, Y.L.; Kwon, M.S.; Jang, J.J. and Lee, M.J. (2004). Chronic hepatotoxicity of carbon tetrachloride in hsp-70 knock out mice. Exp Anim. 53: 27- 30.

Patterson, D.S.P. and Roberts, B.A. (1970). The formation of aflatoxins B2a and G2a and their degradation products during the in vitro detoxification of aflatoxin by livers of certain avian and mammalian species. Food Cosmet. Toxicol. 8: 527-538.

Qin, G.; Ning, Y.; Sul, J.; Shinozuka, H. and Lotlikar, P.D. (1998). Enhancement of Aflatoxin B1-induced enzyme altered hepatic foci in rats by treatment with carbon tetrachloride. Experimen. and Molecul. Med., 30:186-191.

Recknagel, R.O.; Glende Jr., E.A.; Dolak, J.A. and Waller, R.L. (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol. and Therapeuti. 43:139–154.

Reitman, S. and Frankel, S. (1957). Colourimeteric of serum transaminases. Am. J. Clin. Pathol. 68: 28:56.

Sabbioni, G.; Skipper, P.L.; Buchi, G. and Tannenbaum, S.R. (1987). Isolation and characterization of the major serum albumin adduct formed by aflatoxin B1 in vivo in rats. Carcinogenesis. 8: 819-824.

Shup, T. and Sell, S. (2004). Low hepatic glutathione S-transferase and increased hepatic DNA adduction contribute to increased tumorigenicity of aflatoxin B1 in newborn and partially hepatectomized mice. Toxicol. Lett. 148: 1-9.

Siest, G. and Schielef, H.J. (1981). Interpretation des examen de laboratoire, Karger ed. Pp: 206-223.

Tietz, N.M. (1983). Textbook of clinical chemistry. W.B. saunders Co. Try. 64: 1033-1043

Townsend, D.M. and Tew, K.D. (2003). The role of glutathione-Stransferase in anticancer drug resistance, Oncogene. 22: 369–7375.

U.S. EPA (1995). Technical fact sheet. Part of Drinking Water and Health, U.S. Environmental Protection Agency. EPA 811-F-95-004-7, PB 95-140232.

USDA (2003). Database for the Flavonoid Content of Selected Foods, Prepared by the Nutrient Data Laboratory, Food Composition Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, US Department of Agriculture, Belsville, MD, 2003

Wogan, G.N. (1992). Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res., 52: S2114–S2118.

Wu, A.H.; Yu, M.C.; Tseng, C.C.; Hankin, J. and Pike, M.C. (2003). Green tea and risk of breast cancer in Asian Americans. Int. J. Cancer 106: 574–579.

Yassien, Eman, E. (2007). Effect of plant protease inhibitor on rats treated with a cancer inducer. M.Sc. thesis. Faculty of Agriculture, Minia university.

Zhang, M.; Binns, C.W. and Lee, A.H. (2002). Tea consumption and ovarian cancer risk: a case-control study in China. Cancer Epidemiol. Biomarkers Prev. 11: 713–718.

تأثير الشاي الأخضر على بعض الوظائف الحيوية في فئران التجارب المعاملة بالأفلاتوكسين ورابع كلوريد الكربون

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أستخدم في هذه الدراسة 160 فأر متجانسة الأوزان والأعمار قسمت عشوائيا إلى أربعة مجاميع كل منها 40 فأر وقد اشتملت على المعاملات والفترات التالية :-

المجموعة الأولى:- مجموعة المقارنة (بدون معاملات)

المجموعة الثانية:- حقنت بالأفلاتوكسين لمدة 4 أسابيع متبوعا بالحقن برابع كلوريد الكربون لمدة 8 أسابيع ثم استمرت بدون معاملة لمدة 4 أسابيع أخرى.

المجموعة الثالثة:- عوملت بالأفلاتوكسين ورابع كلوريد الكربون مثل المجموعة الثانية إلا أن ماء الشرب استبدل بمستخلص الشاي الأخضر بداية من الأسبوع الثالث عشر حتى نهاية السادس عشر.

المجموعة الرابعة:- استخدم مستخلص الشاي الأخضر كمصدر وحيد للشرب خلال الأربع أسابيع الأولى ثم عوملت بالأفلاتوكسين ورابع كلوريد الكربون حتى نهاية التجربة مثل ما تم في حيوانات المجموعة الثانية.

وقد تم أخذ عينات من الفئران (5 فئران من كل معاملة من المعاملات الأربع) على فترات منتظمة كل أسبوعين من بداية التجربة ، وبعد وزن الفئران وتخديرها تم تجميع عينات الدم وفصل بعض الأعضاء (الكبد – الكليتين) ووزنها ثم حفظها ، ولقد أجريت بعض التقديرات البيوكيميائية في كل من سيرم الدم ومستخلص الكبد ، ويمكن تلخيص بعض نتائج هذه الدراسة فيما يلي :-

1- التغير في الأوزان النسبية لكبد وكلى الفئران:-

أدت معاملة الفئران بالمواد المسرطنة (الأفلاتوكسين ب1 – رابع كلوريد الكربون) إلى زيادة في الأوزان النسبية للكبد والكلى (حدوث تضخم) وبتناول الفئران لمستخلص الشاي الأخضر قبل أو بعد المعاملة بالمواد المسرطنة أدى إلى الحد من التأثير الضار لها على الأوزان النسبية للأعضاء سالفة الذكر أي حماية الفئران من حدوث تضخم للأعضاء.

2- التغيرات في القياسات الكيميائية الحيوية:-

- معاملة الفئران بالأفلاتوكسين ب1 ، رابع كلوريد الكربون أدى إلى حدوث زيادة في نشاط الإنزيمات الناقلة لمجموعة الأمين (ALT,AST) مما يشير إلى احتمال حدوث تلف في أنسجة الكبد. استخدام الفئران لمستخلص الشاي الأخضر قبل أو بعد المعاملة بالأفلاتوكسين ب1 ، رابع كلوريد الكربون أدى إلى انخفاض مستوى هذه الإنزيمات إلى قيم مماثلة للمجموعة الضابطة.

– لوحظ حدوث زيادة في تركيز البيليروبين الكلي نتيجة المعاملة بالأفلاتوكسين ب1 ، رابع كلوريد الكربون مقارنة بالكونترول إلا أن تناول الفئران لمستخلص الشاي الأخضر قبل أو بعد المعاملة بالمواد المسرطنة كان له تأثير ايجابي في إفراز البيليروبين الكلي من أجسام الفئران وهذا يؤدي إلى انخفاض تركيزه في سيرم الفئران المعاملة حيث اقتربت تركيزاته من المستوى الطبيعي في فئران المجموعة المقارنة.

– تشير النتائج إلى زيادة تركيز اليوريا والكرياتينين في الفئران المعاملة بالمواد المسرطنة وتؤدي المعاملة بمستخلص الشاي الأخضر إلى نقصها إلى المستوى الطبيعي في حيوانات المجموعة المقارنة خاصة عند تناوله كجرعة وقائية قبل التعرض للمواد المسرطنة.

– معاملة الفئران بالمواد المسرطنة ادى إلى زيادة معنوية في نشاط انزيم الكاسباس -3 حتى الأسبوع الثاني عشر من التجربة وأمكن الحد من هذه الزيادة باستخدام مستخلص الشاي الأخضر كجرعة وقائية قبل التعرض للمواد المسرطنة حيث أدت إلى تماثل نشاطه مع نشاط الإنزيم في مجموعة الفئران المقارنة وهذه المعاملة ذات تأثير أقوى من استخدام الشاي بعد المعاملة بالمواد المسرطنة.

– تشير النتائج إلي حدوث زيادة في تركيز الجلوتاثيون حتى الأسبوع الثاني عشر في الفئران المعاملة بالأفلاتوكسين ب1 ، رابع كلوريد الكربون ، وكان هذا متزامنا مع انخفاض نشاط إنزيم الجلوثاثيون الناقل للكبريت ، ويؤدي تناول الفئران لمستخلص الشاي الأخضر قبل أو بعد التعرض للمواد المسرطنة إلى الحد من التأثير الضار للمواد المسرطنة على مستوى كلا من الجلوتاثيون والجلوتاثيون الناقل للكبريت حيث اقتربت مستوياتهما من المستوى الطبيعي في فئران المجموعة المقارنة ، إلا أن تناول مستخلص الشاي قبل التعرض للمواد المسرطنة يكون ذو تأثير أقوى من تناوله بعد التعرض للمواد المسرطنة.