Green Tea Extract Ameliorate Liver Toxicity and Immune System Dysfunction Induced by Cyproterone Acetate in Female Rats

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Abstract: Green tea, consumed worldwide since ancient times, is considered beneficial to human health. The present study aimed to evaluate the effect of green tea extract (GTE) on liver damage and immune system function in female rats treated with cyproterone acetate (CPA). Forty healthy female adult albino rats were randomly assigned to four groups. Group (1) was fed on a standard diet as a control. Group (2) was fed on a standard diet and received an intraperitoneally injection of 25mg/Kg/day. Group (3) was fed on a standard diet supplemented with 1 g GTE% and received a daily injection. Group (4) was fed on the supplemented diet for 7 days prior to receiving the daily injection. The experimental duration lasted for 3 weeks initiated from the first injection. The results showed CPA alone led to diminish liver function, hepatic antioxidant enzyme activities and elevated hepatic oxidative stress and serum IgG and IgM levels comparing with the control group of rats. However, the ingestion of GTE either along with or prior to the CPA treatment could significantly improve the function of liver, hepatic oxidative stress and hepatic antioxidant status and elevate the IgG and IgM levels. These data suggested that, GTE possesses a protective effect on the liver against the induced CPA toxicity by increasing auto immunity and countering the hepatic oxidative stress. [Journal of American Science 2010;6(5):179-185]. (ISSN: 1545-1003).

Key words: Cyproterone acetate, green tea extract, liver toxicity, oxidative stress, immunity

1. Introduction

Cyproterone acetate (CPA) is a derivative of 17α-hydroxyprogesterone (Pregnanes) (Fig.1). In addition to the 6, 7 double bond, the 1,2α- methyl group is present.
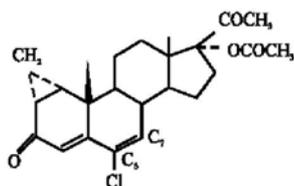

Figure (1): Cyproterone acetate

CPA is a potent steroidal antiandrogen with progestational activity. It is used alone or in combination with ethinylestradiol or estradiol valerate in the treatment of woman suffering from disorders associated with androgenization, e.g., acne or hisuitism. CPA competes with dihydrotestosterone for the ondrogen receptor and inhibits translocation of the hormone receptor complex into the cell nucleus (Siddique and Afzal, 2008). The bioavailability is nearly 100%. CPA has no binding affinity to sex hormone binding globulin and corticosteroid binding globulin in the serum but 93% of compound is bound to serum albumin. It is stored in fat tissue and excreted slowly. The important metabolic steps are hydroxylation reaction and de-acetylation. The metabolite 15β hydroxycyproterone acetate shows only 10% of the progestogenic potency of cyproterone itself. The bio-activation of the CPA involves the reduction of the keto group at carbon-3, which is followed by sulfonation of the hydroxy steroid. The resulting sulfoconjugate is supposed to be very unstable and can decompose to a reactive DNA binding carbonium ion (Schindler et al., 2003).

The International Agency on Cancer (IARC), mainly on the basis of epidemiological studies classifies steroidal estrogens and estrogen progestin combinations among agents carcinogenic to human (Group 1), progestins as possibly carcinogenic (Group 2) and androgenic anabolic steroids as probably carcinogenic (Group 2A). Carcinogenicity to human of sex steroids has been evaluated, and is reported that high dose of estrogen-progestin combinations can cause liver cancer of human (Siddique et al., 2008). In a very recent "Multi Center Study" on oral contraceptives and liver cancer the "Project Team" came to the conclusion that oral contraceptives may enhance the risk of liver carcinomas. CPA is not only a tumor promoting

agent but also a genotoxic chemical (Joosten et al., 2004).

CPA is a tumor initiating agent in the liver of female rats (Deml et al., 1993). It induced micronucleus in rat liver cells, chromosomal aberrations in V79 cells, and human peripheral blood lymphocytes, and also sister chromatoid exchanges in human peripheral blood lymphocyte in vitro (Siddique and Afzal, 2005a). The genotoxic effects of synthetic progestins can be reduced by the use of antioxidants (Ahmad et al., 2002), natural plants products (Siddique and Afzal, 2005b) and herbal health products (Romero-Jimenez et al., 2005).

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or oolong tea. Green tea (GT) is favored in Japan and China, and initial research on the benefits of GT was carried out in these countries because of local customs (Crespy and Williamson, 2004).

Tea contains many compounds, especially polyphenols, a heterogeneous group of chemicals characterized by hydroxylated aromatic rings. Polyphenols contained in teas are classified as catechins, and are collectively reffered to as GT catechins. GT contains six primary catechins compounds: catechin, gallaocatechin, epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate. These constituents posses potent antioxidant action, although to vary degree and are considered as potent scavengers of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals (Khan and Kour, 2007).

GT catechins and their derivatives are shown to contribute beneficial health effects ascribed to tea by their antioxidant, antimutagenic and anticarcinogenic properties. GT consumption has been linked to lowering of various forms of cancer. GT constituents also have been shown to have cardioprotective, neuroprotective, antidibetic, and antimicrobial properties. In addition, GT has been found to be useful in the treatment of arthritis, high cholesterol levels, infection, and impaired immune function. GT consumption also has resulted in improved kidney function in animal models of renal failure (Yokozawa et al., 2005).

The present study is an effort to investigate the role of green tea extract (GTE) in overcoming the hepatotoxic effects of CPA administration of adult female albino rats.

2. Material and Methods

Materials:
- CPA was purchased from Schering S.A., France as compressed tablets each containing 50 mg CPA.
- GTE was obtained from El Obour Pharma (Reg. No. 2958/2002) as tablets, each containing 1000 mg extract.

Animals:
Forty adult female albino rats "Sprague-Dawley strain" weighing 100-126 g were obtained from Research of Bilharizia Institute. Academic of Scientific Research and Technology. Cairo, Egypt. The animals were kept individually in wire cages in an environmentally controlled room (temperature $20\pm2^{o}C$, 12 h dark/ 12 h light cycles), with free access to water and diets.

Experimental protocol:
All animals were allowed to acclimatize for 7 days prior to initiation of the experiment. The rats were divided into four groups with the same average body weight. The rats of group (1) (control group) were fed on a standard diet which is prepared from fine ingredients according to AIN (1993). The rats of groups (2) and (3) (CPA group) and (CPA+GTE group), respectively were injected intraperitoneally 25 mg/Kg/day (Siddique et al., 2006) from the beginning of the experimental duration. Rats of CPA group were fed on a standard diet, whereas those of the CPA+GTE group were fed on a standard diet supplemented with 1 g GTE/100 g diet (Sai et al., 1998). Rats of group (4) (GTE+CPA) were fed on the GTE supplemented standard diet for 7 days prior to receiving an intraperitoneally injection of CPA.

At the end of the experimental duration (3 weeks; initiated from the first injection), animals were scarified after overnight fasting. Blood samples were collected from the hepatic portal veins for serum separation. Liver was removed, rinsed in saline and weighed. All samples were stored at $-80^{o}C$ until analysis be done.

Biochemical measurements:
Serum was analyzed for the following biochemical parameters: alanine transaminase (ALT), asparate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) by using Kits (BioMerieux). Both of IgG and IgM were determined by using radial immunodiffusion plates specific for rats (The binding site Ltd., Birmingham, UK), which contained anti-serum specific to the antigen. The recommended amount of serum was put into the wells of plates and incubated for 72-96 h at room temperature. The diameter of the precipitation

ring was then measured and the concentrations of Igs were determined by using standard calibration curve.

Accurately weighed pieces of liver tissue were treated differently for the separation and estimation of the liver parameters. A portion of liver tissue was per fused with a phosphate buffered saline solution, pH 7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Then tissues were homogenized in 5-10 ml cold 50 mµ potassium phosphate buffer, pH 7.4. Centrifuge at 4000 rpm for 15 min at 4°C, then malondialdehyde (MDA) value was determined in the supernatant colorimetrically by using Kits. A second portion of liver was homogenized in 3% sulfosalicylic acid (5% homogenate), centrifuged at 1000 rpm at 4°C for 20 min and the resultant supernatant was used for the assay of glutathione (GSH). The third portion of liver was homogenized in 5% metaphosphoric acid, centrifuged at 3000 rpm at 4°C for 15 min and the resulted supernatant was used for the estimation of LDH activity. The fourth portion of liver was homogenized in cold 50 mM potassium phosphate buffer pH 7.4 containing 1mM EDTA for catalase activity determination. The homogenate is centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was collected for the assay. The fifth portion of liver was homogenized in Tris-sucrose buffer pH 7.4 (10% homogenate), and centrifuged at 105000 rpm at 4°C for 15 min. Then the supernatant was used for the determination of gamma glutamyl transferase (GGT) activity. Protein concentration of the above supernatants was estimated.

Statistical analysis:

The data were subjected to statistical analysis using one way classification (F-test) and least significant difference (LSD).

3. Results

Table (1): Effect of GTE administration on liver weight and relative weight of adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Relative liver weight (g%)	3.94 ± 0.313	4.86 ± 0.405	4.49 ± 0.234	4.44± 1.41
Liver weight(g)	6.27 ± 0.498	7.58 ± 0.398	7.43 ± 0.62	6.76± 1.07

Data are expressed as means ± S.D.; n= 10.

It is clearly observed from table (1) that, both the absolute and relative liver weights of the female rats treated with CPA were increased significantly compared with the other experimental groups.

Table (2): Effect of GTE administration on liver function parameters (U/L) adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Serum ALT	42.33 ± 4.4	148.34 ± 2.97	63.83 ± 3.71	81.04 ± 3.31
Serum AST	260.70 ± 4.66	386.06 ± 3.59	341.85 ± 4.75	312.2 ± 2.64
Serum ALP	2432.49 ± 4.4	4203.53 ± 6.2	3939.89 ± 3.0	3962.5 ± 3.8
Serum LDH	3834.06 ± 4.5	8702.95 ± 5.7	6324.58 ± 4.2	6520.6 ± 3.1

Data are expressed as means ± S.D.; n= 10

All the estimated liver function parameters; serum ALT, AST, ALP, and LDH activities; illustrated in table (2) shows a highly significant difference ($P< 0.01$) in these parameters in adult female rats treated with CPA, whereas these increments were monitored by the administration of GTE.

Table (3): Effect of GTE administration on hepatic MDA, GSH, LDH, and GGT of adult female albino rats treated with CPA injection.

Groups / Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Hepatic MDA (nmol/g)	5.87± 0.039	9.67± 0.046	5.24± 0.042	5.74± 0.047
Hepatic GSH (mg/g)	25.36± 1.78	34.93± 2.09	31.26± 1.98	27.31± 1.85
Hepatic Catalase (U/g)	8.89± 0.026	7.60± 0.024	8.58± 0.037	8.81± 0.031
Hepatic LDH (µmoles/mg Protein/min	1.34± 0.35	1.32± 0.123	2.18± 0.193	2.26± 0.148
Hepatic GGT (U/mg protein)	2.24± 0.197	5.35± 0.147	2.64± 0.10	2.85± 0.193

Data are expressed as means ± S.D.; n= 10

Table (3) shows increased activities of hepatic MDA and GGT of female rats injected by CPA compared with both the control and treated groups with GTE. Whereas, there was a significant decrease in hepatic GSH catalase and LDH activities of the CPA group comparing with the other groups.

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Table (4): Effect of GTE administration on serum IgG and IgM values of adult female albino rats treated with CPA injection.

Groups Parameters	Control group	CPA group	CPA+ GTE group	GTE+ CPA group
Serum IgG (ng/dL)	652.16 ± 4.84	842.90 ±4.73	874.19 ± 2.74	863.89 ±3.53
Serum IgM (ng/dL)	167.20 ± 4.24	262.89 ±3.94	255.05 ± 3.70	264.80 ± 3.82

Data are expressed as means ± S.D.; n= 10.

Table (4) shows that, there were a significantly elevation in both serum IgG and IgM values in all the experimental groups treated with CPA and administered with GTE either before or along with the treatment comparing with the control group values.

4. Discussions

Hepatocellular carcinoma is considered the main cause of cancer death all over the world. ALT, AST, ALP, LDH, liver weight and relative liver weight are reliable references, widely used in animal study, to indicate poor hepatic function; hepatic damage and malignancy (Cantoni et al., 2003). In the present study, all the previously mentioned parameters were increased significantly in the CPA administered rats, which are an indication of liver cell proliferation. These increases in serum activities of ALT, AST and ALP of female rats injured by CPA were consistent with the results of Ali (2008). Hence, CPA is considered a tumor initiating agent in the liver of female rats by Deml et al. (1993). This was elucidated later that, the bio-activation of the CPA involves the reduction of the keto group at carbon-3, which is followed by sulfonation of the hydroxyl steroid. The resulting sulfoconjugate is supposed to be very unstable and can decompose to a reactive DNA binding carbonium ion (Wolff et al., 2001). GTE at 1g% either before or along with CPA administration gave a high hepatoprotective effect by suppress the increment of serum ALT, AST, ALP and LDH activities, liver weight and relative liver weight values. The observed decrease in these parameters showed that, GTE, to some extent, had liver injury-preventative effects and preserved the structural integrity of the liver from the toxic effect. The hepatoprotective effect of GT polyphenols was confirmed against microcystin-LR (Xu et al., 2007) and chlorpyriphos in rats (Khan and Kour, 2007) as a feature of lowering the increased activities of ALT, AST and ALP induced by the different treatments.

Results of the present study revealed a significant increase in liver MDA level in CPA-received rats. This result could be attributed to

excessive generation of free radicals during the metabolism of CPA. The possible mechanism and cause of the genotoxicity of CPA has been studied by using one of the genotoxic doses (i.e., 30 μM of CPA) with different doses of superoxide dismutase (SOD) and catalase (10 and 20 μg/ml). SODs are family of metal enzymes that covert O^-_2 to H_2O_2 according to the reaction of (Figure 2).Since the treatment with SOD increases the chromosomal aberrations and sister chromatoid exchange frequency, so that there is a possibility somehow CPA is generating oxygen species. Further the treatment with catalase separately and in combination of SOD results in the significant decrease in chromosomal aberrations and sister chromatoid exchange, approving the production of H_2O_2. Because catalase catalyses the decomposition of H_2O_2 to water and oxygen according to the reaction of (Figure 2).In the light of above results, a suggestion can be made for the possible mechanism of generating ROS by CPA (Siddique and Afzal 2008).

Figure (2) shows the structure of CPA with (a) a1,2α-methylene group, (b) a keto group at carbon-3, (c) two double bonds, $C_4=C_5$ and $C_6=C_7$, and (d) C_1 at carbon-6, promote the tendency toward free radical formation. XOOH can also give rise to XOO· (alkoxyl) and ·OOH (peroxyl) radicals. The presence of XOO· appears to be a very remote possibility in view of the highly polar nature of the living system because for that the X has to be essentially an alkyl group (Siddique and Afzal, 2005a).

XOH = Reactive nucleophile within the cell
Cl = Chlorine attached to the carbon atom (C-6) of cyprotene acetate
→ = Enhancing the electrophilic nature of O moiety
OH• = Hydroxyl radical unstable
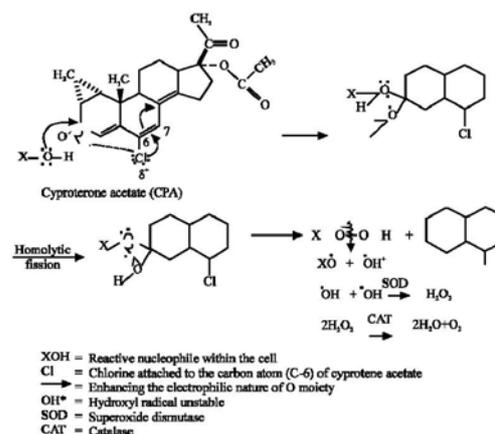SOD = Superoxide dismutase
CAT = Catalase

Figure (2): Possible mechanism of generating free radicals by CPA

The present study revealed significant increase in hepatic GGT and GSH activities accompanied with a significant decrease in hepatic catalase and LDH activities in the CPA administered

female rats. These results are in harmony with those of Ahmad et al. (2002). The ubiquity of elevated GGT level in many rodent and human hepatic and extra hepatic carcinomas have led to the hypothesis that GGT provides a growth advantage to focal cells during carcinogenesis. The advantage may be due to the role of GGT in hydrolysis of GSH to gamma-glutamyl moiety and cysteinylglycine in GSH and GSH conjugate catabolism. This transport in GSH constituents, leading to increase in cellular GSH, which is required for proliferation and resistance to chemotherapy (Csanaky and Gregus, 2005).

The decrease in the hepatic catalase activity in the injured rat group may be explained according to its function as a free radical scavenger enzyme, which suppress the formation of the ROS and/or oppose their action. The by-products of oxygen metabolism initiate different sub cellular outcomes. The superoxide radical has been shown to directly inhibit the activity of catalase. Likewise, singlet oxygen and peroxyl radicals have been shown to inhibit catalase activity (Escobar et al., 1996). These observations manifest and explain the significant inhibition of catalase activity in the CPA administered group of rats.

The result also revealed a significant decrease in hepatic LDH activity in the CPA injured female rats. This may be elucidated according to Sauer and Dauchy (1985) who indicated that tumors in vivo have a large capacity for its production.

Most beneficial health effects ascribed to GT are considered to be mediated by potential antioxidant properties of its constituents that scavenge free radicals and reduce oxidative damage. Several lines of evidence suggest that prooxidant and antioxidant actions of plant polyphenols may be important mechanisms for their anticancer properties. In this study, GTE consumption to female rats either along with or before the treatment with CPA doses exert a noticeable significant decrease in hepatic MDA level, GSH and GGT activities with a significant increase in hepatic catalase and LDH activities. The decrease in hepatic MDA level in the GTE consumed rats is consistent with that of Yokozawa et al. (2002). Also, the increase in hepatic catalase and GSH activities according to the consumption of GTE is consistent with that of Khan and Kour (2007) and Xu et al. (2007). However there is a disagreement of the result of hepatic LDH activity with that of Hasegawa et al. (1995) who found that hepatic LDH activity of rats administered GTE 2% wt/v and injured with 2-nitropropane had a diminished activity.

It has been reported that GT exert its biological effects on the basis of the redox state of a particular cell/tissue and according to the level of GT polyphenols accumulated in the tissues. It is hypothesized that GTP help in the protection against ROS damage by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes (i.e., SOD and catalase), to the total antioxidant defense system (Crespy and William, 2004). GTP, water-soluble antioxidants, have been demonstrated to inhibit iron-induced oxidation of synaptosomes by scavenging hydroxyl radicals generated in lecithin/lipoxidase system. On the other hand, GTP can penetrate the lipid bilayer, decreasing free radicals concentration or influencing antioxidant capability in biomembranes. On the other hand, they could reduce the mobility of free radicals into the lipid bilayers as well. Moreover, GTP can interact with phospholipid head groups, particularly with those containing hydroxyl groups, so they could decrease the fluidity in the polar surface of phospholipid bilayer. In addition, GTP can prevent the loss of the lipophilic antioxidant α-tocopherol, by repairing tocopheryl radicals, and protection of the hydrophilic antioxidant ascorbate (Skrzydlewska et al. 2002). So that, the results of this study made a speculation that GTE administration can modulate the susceptibility to lipid peroxidation coupled with up-regulation of the antioxidant status and block tumor development in liver of female rats treated with CPA doses.

In this study, there is a markedly increase in the serum IgG and IgM of female rats either administered with CPA alone or treated with GTE (either with or before the CPA doses). This elevation in CPA administered group of rats may be due to the increasing of hepatic GSH and GGT activities comparing with control rats. This elevation in activities may activate T-lymphocytes in white blood cells, leading to increase immune parameters. The steroid hormones affect upon cytokine production which is mediated by the nuclear factor-KB (NF-KB). This is an inducible transcription factor that positively regulates the expression of pro-immune and pro-inflammatory genes. It has been shown that the steroid/receptor complex can physically interact with NF-KB and inhibits its transactivation activity (Mc Kay and Cidlowski, 1999). Via this mechanism esterogens, progesterone and testosterone can inhibit pro-inflammatory cytokine expression in immune cells expressing the respective receptor. The mechanism by which steroid binding with membrane receptors affect immune cell function remains obscure. A proposed explanation by their lipophilic nature, sex steroid can integrate into the membrane and alter membrane properties, such as fluidity and thereby changing the function of the immune cells (Lamche et al. 1990). Flavonoids exert a prime function in the most important weapon in the body

defense (i.e., immune system). They stimulated both of the immune branches; the humoral and the cellular. Flavonoids stimulate the production of antibodies in a yet poorly known fashion. However, it is likely that they do so by altering cytokine production, since this is assisted by protein P-kinase cascades, which are known to be under the influence of flavonoids. Besides, flavonoids prevent the synthesis of PGs that suppress the T-cells (Havsteen, 2002). Ingestion of GT improved the depressed immune functions either humoral or cell immunity in mice treated with diethylnitrosamine to basal levels. Moreover, the transplantation of Lewis lung carcinoma cells into mice decreased the CD^+ positive to lymphocytes and CD_4^+: CD_8^+ ratio. So, it could be concluded that GT ingestion improved immune functions and inhibited tumor growth (Zhu et al. 1999).

Conclusion

In conclusion, GTE could potentially attenuate the hepatic injury induced by CPA treatment as evidenced by: (I) Restoration of almost all enzymatic liver function tests. (II) Normalization of the oxidative stress biomarker, MDA. (III) Balancing of the oxidant/antioxidant status of the liver cells. (IV) Improving of the immune system function. Thus daily moderate levels of GT consumption can inhibit the activation of some types of environmentally encountered injures.

References

1. Siddique, Y.H. and Afzal, M. A review on the genotoxic effects of some synthetic progestins. Int. J. Pharmacol. 2008; 4(6): 410-430.
2. Schindler, A.E.; Campagnoli, C.; Drucmann, R.; Huber, J.; Pasqualini, J.R.; Schweppe, K.W. and Thijssen, H.H. Classification and pharmacology of progestins. Mutation 2003; 46: S7-S16.
3. Siddique , Y.H.; Ara, G.; Beg, T.; Faisel, M.; Ahmed, M. and Afzal, M. Antigenotoxic role of Centella asiatica L. extract against cyproterone acetate induced genotoxic damage in cultured human lymphocytes. Toxicol. In vitro 2008; 22: 10-17.
4. Joosten, H.F.P.; van Acker, F.A.A.; van de Dobbelsten; Horbach, D.J. and Kranic, E.I. Genotoxicity of hormonal steroids. Toxicology Letters 2004; 151: 113-134.
5. Deml, E.; Schwarz, L.R. and Oesterle, D. Initiation of enzyme altered foci by the synthetic steroid cyproterone acetate in rat liver foci bioassay. Carcinogenesis 1993; 14: 1229-1231.
6. Siddique, Y.H. and Afzal, M. Genotoxic potential of cyproterone acetate: a possible role of reactive oxygen species. Toxicology in vitro 2005a; 19: 63-68.
7. Ahmad, S.; Hoda, A. and Afzal, M. Additive action of vitamin C and E against hydrocortisone induced genotoxicity in human lymphocytes chromosomes. Int. J. vit. Nutr. Res. 2002; 72: 204-209.
8. Siddique, Y.H. and Afzal, M. Protective role of allicin and L-ascorbic acid against damage induced by chlormadinone acetate in cultured human lymphocytes. Ind. J. Exper. Biology 2005b; 43: 769-772.
9. Romero-Jimenez, M.; Sanchez, J.C.; Analla, M.; Serrans, A.M. and Moraga, A.A. Genotoxicity and antigenotoxicity of some traditional medicinal herbs. Mutation Res. 2005; 285: 147-155.
10. Crespy, V. and Williamson, G. A review of the health effects of green tea catechins in: in vivo animal models. J. Nutr. 2004; 134: 3431S-3440S.
11. Khan, S.M. and Kour, G. Subacute oral toxicity of chlorpyriphos and protective effect of green tea extract. Pesticide Biochem. and Physiol. 2007; 89: 118-123.
12. Yokozawa, T.; Nakagawa, T.; Ova, T.; Okuba, T. and Juneja, L.R. Green tea polyphenols and dietary fiber protect against kidney damage in rats with diabetic nephropathy. J. Pharm. Pharmacol. 2005; 57: 773-780.
13. AIN (1993): By Reeves, P.G.; Nielsen, F.H. and Fahly, G.C. AIN-93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc writing committee on the reformulation of the AIN-76 A rodent diet. J.Nutr. 1993; 123: 1939-1951.
14. Siddique , Y.H.; Ara, G.; Beg, T. and Afzal, M. Effect of vitamin C on cyproterone acetate induced genotoxic damage in mice. Res. J. Biol. Sci. 2006; 1(1-4): 69-73.
15. Sai, K.; Kai, S.; Umemura, T.; Tanimura, A.; Hasegawa, R.; Inoue, T. and Kurokawa, Y. Protective effects of green tea on hepatotoxicity oxidative DNA damage and cell proliferation in the rat liver induced by repeated oral administration of 2-nitropropane. Food Chem. Toxicol. 1998; 36: 1043-1051.
16. Cantoni, L.; Valaperta, R.; Ponsoda, X.; Castell, J.V.; Barelli, D.; Rizzardini, M.;

Mangolini, A.; Hauri, L. and Villa, P. Induction of hepatic heme oxygenase-1 by diclofenac in rodents: role of oxidative stress and cytochrome P-450 activity. J. Hepatol. 2003; 38: 776-786.

17. Ali, E.H.A. Protective effects of Echinacea on cyproterone acetate induced liver damage in male rats. Pak. J. Biol. Sci. 2008; 11(21): 2464-2471.

18. Wolff, T.; Topinka, J.; Deml, E.; Oesterle, D. and Schwarz, L.R. Dose dependent induction of DNA adducts, gene mutations and cell proliferation by the antiandrogenic drug cyproterone acetate in rat liver. Adv. Exp. Med. Biol. 2001; 500: 687-696.

19. Xu, C.; Shu, W.Q.; Qiuz, Z.Q.; Chen, J.A.; Zhao, Q. and Cao, J. Protective effects of green tea polyphenols against sub acute hepatotoxicity induced by microcystin-LR in mice. Enviro. Toxicol. Pharmacol. 2007; 24: 140-148.

20. Csanaky, I. and Gregus, Z. Role of glutathione in reduction of arsenate and of glutamyltranspeptidase in deposition of arsenite in rats. Toxiccology 2005; 207: 91-104.

21. Escobar, J.A.; Rubio, M.A. and Lissi, E.A. SOD and catalase inactivation by singlet oxygen and peroxyl radicals. Free Radic. Biol. Med. 1996; 20: 285-290.

22. Sauer, L.A. and Dauchy Regulation of lactate production and utilization in rat tumors in vivo. J. Biol. Chem. 1985; 260(12): 7496-7502.

23. Yokozawa, T.; Nakagawa, T. and Kitani, K. Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J. Agric. Food Chem. 2002; 50: 3549-3552.

24. Hasegawa, R.; Chujo, T.; Sai-Kato, K.; Umemura, T.; Tanimura, A. and Kurokawa, Y. Preventive effects of green tea against liver oxidative DNA damage and hepatotoxicity in rats treated with 2-nitropropane. Food Chem. Toxicol. 1995; 33: 961-970.

25. Skrzydlewska, E.; Ostrowska, J.; Stankiewicz, A. and Farbiszewski, R. Green tea as a potent antioxidant in clcohol intoxication. Addict. Biol. 2002; 7: 307-314.

26. Mc Kay, L.I. and Cidlowski, J.A. Molecular control of immune/inflammatory responses: Interactions between Kappa B and steroid receptor-singling pathways. Endocr. Rev. 1999; 20: 435-459.

27. Lamche, H.R.; Silberstein, P.T.; Knabe, A.C.; Thomas, D.D.; Jacob. H.S. and Hammerschmidt, D.E. Steroids decrease granulocyte membrane fluidity, while phorbol ester increases membrane fluidity. Studies using electron paramagnetic resonance. Inflammation 1990; 14: 61-70.

28. Havsteen, B.H. The biochemistry and medical significance of the flavonoids. Pharmacl. Therapeutics 2002; 96: 67-202.

29. Zhu, M.; Gong, Y.; Yang, Z.; Ge, G.; Han, C. and Chen, J. Green tea and its major components ameliorate immune dysfunction in mice bearing Lewis lung carcinoma and treated with the carcinogenic NNK. Nutr. Cancer 1999; 35: 64-72.

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