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Protective Effects of *Echinacea* on Cyproterone Acetate Induced Liver Damage in Male Rats

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Abstract: The study was planed to evaluate the effect of *Echinacea* (*E.*) on liver toxicity in rats treated with Cyproterone Acetate (CA). Rats were divided into 5 groups treated for 2 and 4 weeks, 1-control 2- *Echinacea* (63 mg/kg/day), 3-CA (25 mg/kg/day), 4-*E.*+CA and 5-*E.* for 1 week before *E.*+CA. All treatments were administered via an oral tube with the same mentioned doses. Rats treated with CA or *E.*+CA exhibited a significant increase in liver gamma glutamyl transpeptidase and malondialdehyde as compared with the control group. A marked decrease was recorded in all treated groups in comparison with the control with respect to glutathione peroxidase and superoxide dismutase. All treatments caused an increase in serum IGG and IGM in comparison with the control value. WBCs showed an increase after *E.* and CA treatment. While RBCs count and heamatocrit value showed a significant decrease in CA and E+CA treated rats in comparison with the control after four weeks of treatment. These data suggested that, *E.* possesses a protective effect on the liver against the CA toxicity by increasing auto immunity and blood picture components. Also the *E.* antioxidant properties exerted counteracting effects on the CA induced oxidative stress.

Key words: *Echinacea*, cyproterone acetate, liver toxicity, antioxidants, gamma glutamyl transpeptidase

INTRODUCTION

The most serious potential side effect of Cyproterone Acetate (CA), a synthetic steroid, with potent progestational and antiandrogenic actions, is liver toxicity and patients should be monitored for changes in liver enzymes, especially if taking a high dose (200-300 mg day^{-1}) (Watanabe *et al*., 1994; Rüdiger *et al*., 1995; Savidou *et al*., 2006; Miquel *et al*., 2007). There is evidence of liver tumor development in pre-market rodent studies (Brambilla and Martelli, 2002). Toxicity is dose-dependent and the low doses used in birth control pills (2 mg) do not appear to represent a significant risk (Seaman *et al*., 2003; Franks *et al*., 2008).

Echinacea sp. roots were originally used by North American Indians to treat a variety of infections and wounds. In the late 1800s, these *Echinacea* preparations became popular as remedies for the common cold (Simasek and Blandino, 2007) and rhinovirus infection (Barrett *et al*., 2006). There has been renewed interest in *Echinacea* in the United States since the passage of the Dietary Supplement Health and Education Act in 1994 liberalized the regulation of herbal medicines (Turner *et al*., 2005). *Echinacea* roots and derivatives are a good source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects (Pellati *et al*., 2004; Dalby-Brown *et al*., 2005). The objective of this study was to investigate if the used of *Echinacea* with cyproterone acetate or before it, would decrease or prevent the most series side effects of cyproterone acetate on liver through its protective effect.

MATERIALS AND METHODS

Experimental animals: This study was carried out in Zoology Department, University College for Women, Arts, Science and Education, Ain Shams University, on adult male albino rats (*Wister strain*). Eighty five animals were obtained from the animal house of National Organization for Drug Control and Research (NODCAR). Their weights were (130.2±18.5 g). Male rats were housed in iron mesh cages, each cage contained six rats. Animals were kept under controlled temperature of 21±2°C and 12 h light/12 h dark cycle throughout the experiment. A commercial pelleted diet was used during the experiment. The animals were allowed to adapt to the laboratory conditions for two weeks before the beginning of the experiment.

Drugs: Cyproterone acetate ($C_{24}H_{29}ClO_4$) was supplied by Schering, Germany as tablets each containing 50 mg. Cyproterone acetate is a progestogen with anti-androgenic and anti-steroidogenic properties and *Echinacea* was supplied by MEPACO, Egypt as capsules each containing 175 mg from herb dray extract.

Experimental design: The animals were divided into five main groups each one contained 12 rats as follows: (1)-control group, which received 0.1 mL/100 g b.wt. of 0.5 g/100 mL carboxy-methyl cellulose sodium salt CMC/day (2)-*Echinacea* group received 63 mg/kg/day (suspended in CMC). (3)-CA group, which received 25 mg/kg/day (4)-*Echinacea*+CA group which received 63 mg kg^{-1} Echinacea+25 mg kg^{-1} CA/day) and (5)-*Echinacea* W+CA group which received 63 mg kg^{-1} Echinacea/day for 1 week before 63 mg kg^{-1} Echinacea+25 mg kg^{-1} CA/day. All groups were treated for either two or four consecutive weeks from the beginning of the experiment as an oral supplementation. These doses are used as a pharmacological dose calculated from the human equivalent dose according to (Guidance for Industry and Reviewers, 2002). The groups treated with *Echinacea*+Cyproterone acetate received cyproterone acetate first followed by *Echinacea* after 3.5 h. The peak plasma concentration of CA is being achieved in 3 to 4 h from the gastrointestinal tract (Sweetman, 2005).

Biochemical samples under investigation and biochemical analysis: Body weight of the rats recorded weekly throughout the duration of the experiment. Following the completion of the experiments, the rats sacrificed after 12 h from the last dose by rapid decapitation. Blood samples collected in glass tubes and centrifuged for serum separation. Liver dissected out, cleaned and weighed. A tissue portion homogenized in saline containing sodium chloride for the determination of superoxide dismutase (SOD) according to Minami and Yoshikawa (1979), glutathione peroxidase (GPX) by the method of Gross *et al.* (1967) and gamma glutamyl-transpeptidase (GGT) according to Persijn and Van der Slik (1976) and Schumann and Klauke (2003). Malondialdehyde (MDA) was determined by Karatas *et al.* (2002) in tissue homogenized in 75% methanol HPLC grade. The homogenizer placed on ice to avoid the impact of over heating on the enzyme activities. Blood picture was determined using an automated hematologic blood counting system (Coulter Counter; Coulter Electronics Inc, Abacus) using heparinised blood and serum IGG and IGM was determined by using radial immuno-diffusion 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 (end point method).

Statistical analysis: Reported values represent Mean±SE. Statistical analysis was evaluated by one-way ANOVA. Once a significant F test was obtained, LSD comparisons were performed to assess the significance of differences among various treatment groups. Statistical Processor System Support SPSS for Windows software, Release 10.0 (SPSS, Chicago, IL) was used.

RESULTS AND DISCUSSION

The body weight (g) in all the experimental animal groups significantly decreased ($p<0.05$) throughout the experimental period as compared to the control group from (179.83±4.1) to (143.8±4.6) in CA, (146.58±5.99) in *E.*+CA, (150.36±3.7) in *E.* W+CA and (158.16±3.5) in *E.* treated groups at the end of the experiment as represented in Fig. 1. It can be shown from Fig. 2 that, the liver weight (g) showed a numerical, but not statistically significant change in *E.* (6.42±0.40 and 6.16±0.34), CA (6.7±0.36 and 6.19±0.14), *E.*+CA (5.95±0.11 and 6.27±0.25) and *E.* W+CA (6.25±0.26 and 6.77±0.31) groups as compared to the control (6.33±0.13 and 6.40±0.44) after two and four weeks, respectively. Liver/ body weight ratio in Fig. 3 showed a nonsignificant decrease in *E.* treated rats (3.83±0.26) and a significant increase in CA (4.57±0.18), *E.*+CA(4.57±0.18) and *E.* W+CA(4.94±0.24) groups after two weeks of treatment as compared to the control (3.84±0.13) at ($p<0.05$). After four weeks, rats treated with *E.* showed a

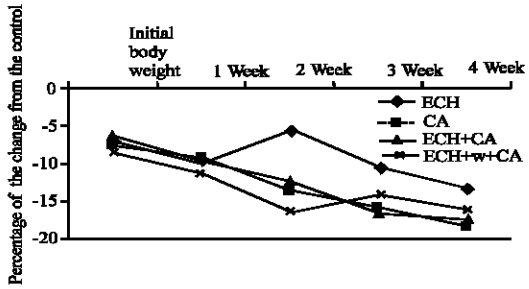

Fig. 1: Percentage of change in body weight gain of rats treated for four weeks

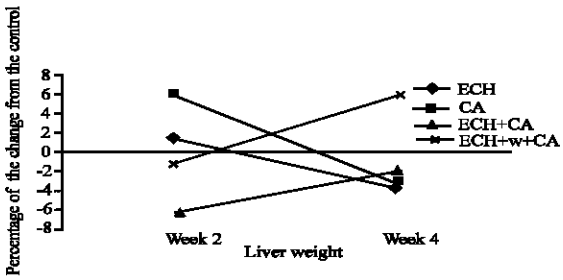

Fig. 2: Percentage of change in liver weight of treated rats

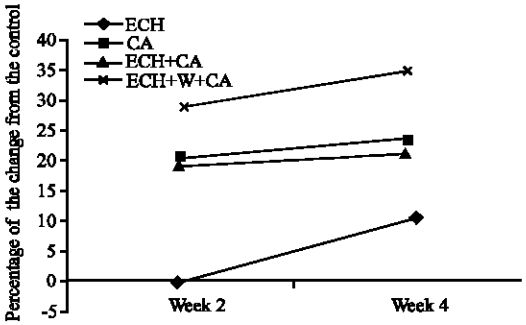

Fig. 3: Percentage of change in relative liver weight of treated rats

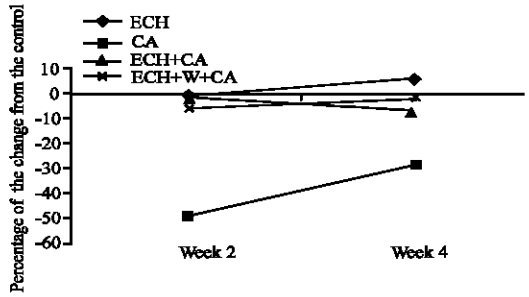

Fig. 4: Percentage of change in liver superoxide dismutase SOD of treated rats

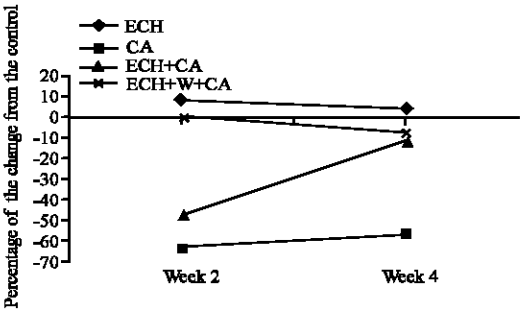

Fig. 5: Percentage of change in liver glutathione peroxidase GPX of treated rats

non significant increase (3.91±0.24) in liver/ body weight ratio as compared to the control (3.56±0.17). While, all the CA treated groups exhibited a significant increase (4.40±0.19) in CA, (4.31±0.19) in *E.*+CA and (4.79±0.21) in *E.* W+CA in liver/ body weight ratio as compared to the control at $p<0.05$.

Data in Fig. 4 showed that liver SOD activity (μg g^{-1} wet tissue) exhibited a significant decrease ($p<0.05$) in CA treated groups of rats (156.7±10.3 and 226.1±7.5 at two and four weeks, respectively). However, SOD activity represented a nonsignificant increase in

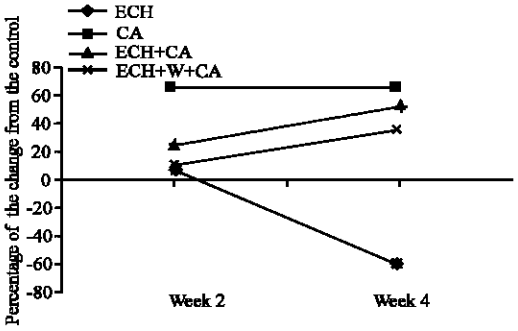

Fig. 6: Percentage of change in liver malondialdhyde MDA of treated rats

E. (302.39±19.2 and 338.81±10.7), *E.*+CA (306.3±3.0 and 295.2±4.3) and *E.* W+CA (290.83±4.2 and 311.3±10.7) groups as compared to the control (307.4±12.4 and 317.9±3.8) throughout the experimental period. As shown in Fig. 5, the liver GPX (mg g^{-1} wet tissue) activity exhibited a significant decrease ($p<0.05$) in CA (62.5±6.7 and 76.7±6.4) and *E.*+CA (93.04±7.0 and 155.95±8.9) at two and four weeks respectively as compared to the control (175.5±2.6 and 175.4±7.7). An insignificant increase was noticed in *E.* treated rats (189.74±9.2 and 183.06±5.3), while a nonsignificant decrease was found in *E.* W+CA (174.99±3.8 and 162.01±4.2) as compared to the control at two and four weeks, respectively.

The results depicted in Fig. 6 showed that liver malondialdhyde (MDA) content (n mol g^{-1} wet tissue) exhibited a nonsignificant increase in *E.* (122.1±8.0) and *E.* W+CA (122.8±12.8) treated rats after two weeks followed by significant decrease in *E.* (48.2±6.0) and a significant elevation in *E.* W+CA (160.4±8.0) after four weeks of treatment. Whereas, CA (185.3±11.6 and 195.3±6.4) and *E.*+CA (142.7±8.9 and 179±11.1) groups exhibited a significant increase in the liver MDA content compared to the control (113.2±2.6 and 118.4±8.2) value after two and four weeks, respectively. The liver GGT activity (U g^{-1} wet tissue) increased significantly ($p<0.05$) in CA (117.2±4.0 and 123.2±6.4), *E.*+CA (74.1±4.8 and 73.1±3.0) and *E.*W+CA (72.1±3.0 and 64.0±4.1) treated groups as compared to the control group (45.8±4.3 and 44.4±1.4). In addition, *E.* treatments induced a nonsignificant increase (51.9±7.5 and 41.8±2.3) in GGT activity in comparison with the control group after 2 or 4 weeks of treatment respectively as shown in Fig. 7.

It is clear from Fig. 8 that there was a significant increase in Serum IGG (ng dL^{-1}) in *E.* (935.3±8.95 and 981±10.96), CA (963.7±23.9 and 940±25.6), *E.*+CA (957.7±24.3 and 956.7±13.4) and *E.* W+CA (980±25.6 and 960.7±21.5) as compared to the control (703±10.6 and 682±21.0) after two and four weeks respectively at $p<0.05$. The data in Fig. 9 indicated that Serum

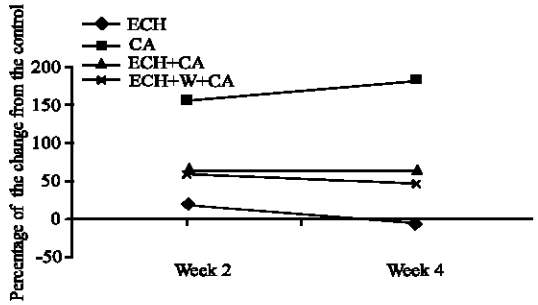

Fig. 7: Percentage of change in liver gamma glutamyl transpeptidase GGT of treated rats

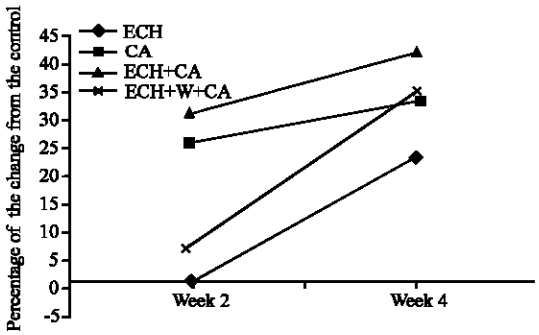

Fig. 8: Percentage of change in serum IGG of treated rats

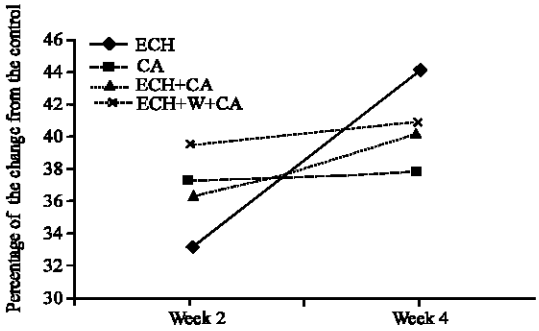

Fig. 9: Percentage of change in serum IGM of treated rats

IGM level (ng dL^{-1}) at two weeks of treatment showed a significant increase in CA (226.3±6.0) and *E.*+CA (236.7±15.6) treated groups. A numerical decrease was noticed in *E.* (179±6.6) and a non-significant increase in *E.* W+CA (193.7±2.4) in comparison with the control (180.2±5.3). At four weeks of treatment, there was a significant increase in Serum IGM level in *E.* (211±4.0), CA (230.7±3.8), *E.*+CA (245.7±14.0) and *E.* W+CA (233.7±16.7) in comparison with the control (172.7±7.2).

Figure 10 revealed that, white blood cells (WBCs) count ($n\times10^3$) showed a non-significant decrease in *E.* treated rats (4.8±0.15), while an insignificant increase was recorded in CA (6.2±0.86) and *E.*+CA (5.9±0.40) rats as compared to the control (4.9±0.12) after two weeks of

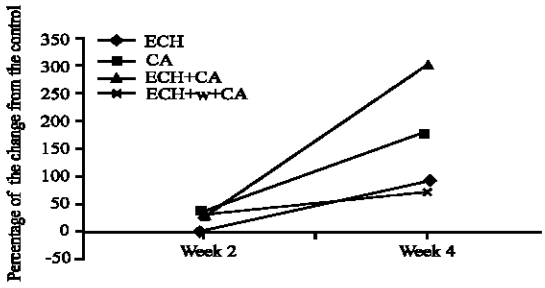

Fig. 10: Percentage of change in white blood cell count WBCS of treated rats

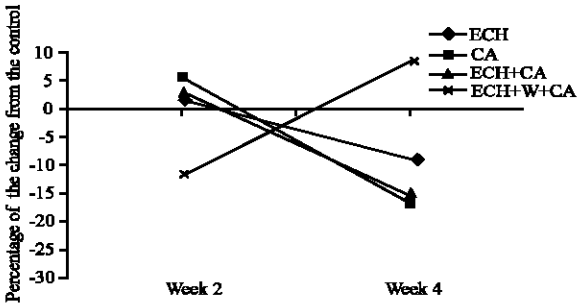

Fig. 11: Percentage of change in red blood cell count RBCS of treated rats

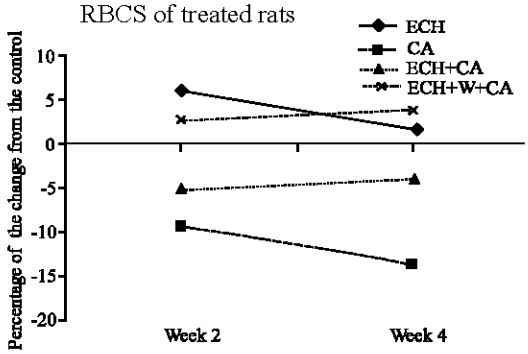

Fig. 12: Percentage of change in hemoglobin Hb of treated rats

treatment. Rats treated with *E.* W+CA exhibited a significant increase in WBCs count (6.3±0.29) as compared to the control after two weeks of treatment at $p<0.05$. After four weeks of treatment, a significant increase was noticed in WBCs count in *E.* (8.3±0.62), CA (12.5±0.79), *E.*+CA (17.6±0.22) and *E.* W+CA (7.6±0.28) in comparison with the control (4.4±0.18). It is evident from Fig. 11 that, at two weeks of treatments, an insignificant elevation was found in red blood cells RBCs count ($n\times10^6$) in *E.* (7.8±0.52), CA (8.1±0.12), *E.*+CA (7.9±0.03) and a significant decrease in *E.* W+CA (6.8±0.39) as compared to the control (7.7±0.16) at $p<0.05$. at four weeks of

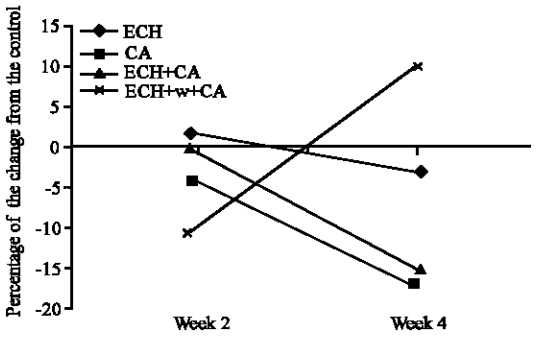

Fig. 13: Percentage of change in hematocrit Ht value of treated rats

treatment, rats treated with *E.* exhibited an insignificant decrease (6.9±0.33) in RBCs count and an insignificant increase in *E.* W+CA (8.2±0.17), while a significant decrease was found in CA (6.3±0.13) and *E.*+CA (6.4±0.06) as compared to the control (7.6±0.35) at $p<0.05$.

Data analysis in Fig. 12 revealed that hemoglobin (Hb) content (g dL^{-1}) insignificantly increased in *E.* (13.5±0.18 and 13.2±0.07) and *E.* W+CA (13.2±0.33 and 13.4±0.19) treated rats, while an insignificant decrease was recorded in *E.*+CA (12.3±0.38 and 12.5±0.43) groups as compared to the control group (12.9±0.11 and 13.0±0.29) after two and four weeks, respectively. Hb content showed a significant reduction in CA treated rats (11.9±0.37 and 11.6±0.41) as compared to the control group (12.9±0.11 and 13.0±0.29) after two and four weeks, respectively. The observed data in Fig. 13 revealed that at two weeks of treatments, an insignificant elevation was found in hematocrit Ht in *E.* (40.4±0.7), CA (38.2±0.3), *E.*+CA (39.6±0.5) and a significant decrease in *E.* W+CA (35.4±2.1) as compared to the control (39.6±0.9) at $p<0.05$. at four weeks of treatment, rats treated with *E.* exhibited an insignificant decrease (37.8±0.7) in Hb content and a significant increase in *E.* W+CA (42.9±0.5), while a significant decrease was found in CA (32.1±0.5) and *E.*+CA (33.1±0.2) as compared to the control (39.0±0.5) at $p<0.05$.

In the present study, *Echinacea* has a positive effect on body weight gain after four weeks of treatment. These results may be due to the antiandrogenic effect of the preparations of *Echinacea purpurea* it is possible to claim that it is associated with the vegetative sterols like sitosterol, campesterol, stigmasterol (Skaudickas *et al.*, 2004). The chemical structure of these compounds is very similar to cholesterol. In the digestive duct vegetative sterols reduce the absorption of cholesterol, creating a certain competition between sterols and cholesterols. The synthesis of testosterone cholesterol, which is converted into pregnenolone, is vital. From it by a progesteronic or dehydroepiandrosteronic methods testosterone is being synthesized (Mukai *et al.*, 2006), therefore, with the decrease effect of cholesterol the concentration of testosterone in blood decreases. Treatment with CA alone or with *Echinacea* caused a significant reduction of body weight, the effect of CA on body weight was explained by Hansson *et al.* (1991) through the lipolytic axis and CA induced a significant reduction of lipid parameters. All the CA treatments induced an increase in liver body weight ratio as compared to the control after four weeks of treatment, this increment may explained in rats treated by CA, *Echinacea*+CA or *Echinacea* W+CA by the liver toxicity of cyproterone acetate. Increased relative liver weight to more than 10% with evidence of hepatotoxicity from increasing GGT enzyme in CA treated rats. The insignificant change in liver weight with significant reduction in body weight and liver body weight ratio is an indicator on liver cell proliferation in all CA treated groups (Wolff *et al.*, 2001).

The antioxidant activity of *Echinacea* was evaluated by many different studies. The mechanism of antioxidant activity of *Echinacea* root extract included free radical scavenging and transition metal chelating (Hu and Kitts, 2000). In Mishima *et al.* (2004) study, they assumed that SOD activity in peripheral blood was increased because of antioxidants such as echinacocide and caffeine acid in *E. purpurea* which eliminate superoxide (O_2) by a free radical scavenging effect. Supporting the antioxidant activity of *E. purpurea.* In addition Pellati *et al.* (2004) indicate that *E.* roots and derivatives are a good source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects. These results are agreed with the results in this study, treatment with *Echinacea* caused a significant reduction in MDA in liver and an insignificant change in SOD. There is a statistically significantly greater antioxidative activity of *Echinacea* tincture compared to that of *Ginkgo* or *Ginseng* tinctures (Masteikova *et al.*, 2007) and they concluded that antioxidative activity was determined not only by phenol compounds of *E.*, but also by a complex of other components of medicinal raw material.

In this study cyproterone acetate caused a significant increase in liver GGT accompanied with significant decrease in liver antioxidants (SOD and GPX). These results were improved by co-treatment with *Echinacea.* The hepatotoxicity and hepato-carcinogenic activity of test substances were assessed by the status of gamma-glutamyl transferase (GGT) enzyme. This enzyme is widely used as a biomarker in preneoplastic lesions of the liver during chemical carcinogenesis (Vodela and Dalvi, 1997).

The metabolism of GSH initiated by GGT may lead to oxidative damage (Stark *et al*., 1993, 1994). Such oxidative damage may be induced *in vivo* by GSH in proximity to GGT-rich preneoplastic foci in rat liver. The reduction of metal ions by extracellular GSH catabolites is capable of inducing the redox cycling processes, leading to the production of reactive oxygen species and other free radicals (Dominici *et al*., 2005). Through the action of these reactive compounds, cell membrane GGT activity can ultimately produce oxidative modifications on a variety of molecular targets, involving oxidation and/or S-thiolation of protein thiol groups in the first place. Increased lipid peroxidation by production of radicals after reperfusion caused the liver cell damage and liver glutathione peroxidase (GPX) activity was significantly decreased and its location altered in the damaged liver (Katayama *et al*., 1997). They findings suggested that GPX contributes significantly to the protection against hepatic reperfusion injuries.

Antioxidants, in general, are free radical scavengers that suppress the formation of ROS and/or oppose their actions. Superoxide dismutase (SOD) and glutathione peroxidase are well-known biological antioxidants that convert superoxide (O_2^-) and peroxide (H_2O_2) radicals to form O_2 and H_2O. SOD protects against spontaneous O_2 toxicity and lipid peroxidation. GGT is the key enzyme that initiates the metabolism and turnover of glutathione. The metabolic function of this cell surface glycoprotein is believed to protect the plasma membrane against oxidant stress and to enable the transfer of glutathione between cells and tissues. GGT is most abundant in epithelial cells and their secretions, inasmuch as these cells line surfaces that are directly exposed to environmental oxidants (Chang-Jean *et al*., 2002). GSH is essential for the activation of T-lymphocytes and polymorphonuclear leukocytes as well as for cytokine production and therefore for mounting successful immune responses when the host is immunologically challenged (Townsend *et al*., 2003). These results are in the same trend of the results in this study as, CA caused an increase in liver GGT accompanied by increase in MDA, IGG, IGM and blood cells and a decrease in liver SOD and GPX.

In the present study, CA treatment alone or combined with *Echinacea* induced an increase in serum IgG and IgM. The effects of steroid hormones upon cytokine production are suggested to be 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 transactivational activity (McKay and Cidlowski, 1999). Via this mechanism estrogens, 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 steroids 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). Also, increasing serum IgG and IgM in CA treated groups may be related with increasing liver GGT activity which increasing intracellular glutathione leading to activation of T lymphocytes in white blood cells. Elevated serum IgG and IgM was recognized in liver diseases (Gonzàlez-Quintela *et al*., 2003).

Moreover, *E.* administration for six weeks increased IgG production in the early to middle term in rats (Rehman *et al*., 1999). He stated that *Echinacea* may enhance immune function by increasing antigen specific immunoglobulin production. Also, a decrease in hemoglobin and hematocrit that seemed to be dependent on the antiandrogenic activity of CA and that would certainly decrease the acceptability of such a regimen (Meriggiola *et al*., 1996, 1998, 2002, 2003). In the present results CA induced a significant decrease in hemoglobin and hematocrit and this reduction was improved by using treatment with Echinacea+CA. this improvement may be due to improvement of hemoglobin levels and number of erythrocytes by *Echinacea* treatment (O'Neill *et al*., 2002). On the other hand cichoric acid and echinacin in *E. purpurea* activate macrophages (Goel *et al*., 2002). The early increased in the leukocyte count in our study appears to be due to the ability of polysaccharides and echinacocide to increase the number of leukocytes and the ability of cichoric acid and echinacin to activate macrophages and to stimulate bone marrow and the reformation of hematopoietic stem cells. So, Echinacea may invoke an immune response through altered expression of 70 kilodalton heat shock proteins (hsp70) and increased white blood cells (Agnew *et al*., 2005).

CONCLUSION

Echinacea possesses a protective effect on the liver against the CA toxicity by increasing auto immunity and blood picture components. Also the *Echinacea* antioxidant properties exerted counteracting effects on the CA induced oxidative stress. So, treatment with *Echinacea*+CA may help in liver protection.

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