E3 Journal of Biotechnology and Pharmaceutical Research Vol. 4(5), pp. 89-93, May 2013
Available online at http://www.e3journals.org/JBPR
ISSN 2141-7474 ©2013 E3 Journals

Full Length Research Paper

Effects of aqueous extract of *Camellia* sinensis (l.) O.kuntze on liver markers of cadmium treated rats

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Accepted 1 April, 2013

The effects of oral feeding of aqueous extract of green tea leaf on alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT), aspartate transaminase (AST), sugar and total proteins were studied in the liver of cadmium treated rats. In cadmium control group sugar and protein levels were decreased and ALP level was significantly ($p<0.001$) decreased, whereas ACP, ALT and AST levels showed increase. In the other two experiment groups, treated with cadmium and aqueous extract of green tea, the decreased level of sugar, proteins and ALP came back to normal level in both 2 mg and 4 mg *Camellia sinesis* dose groups. Decrease was observed in the activity of ACP, ALT and AST in 2 mg *C. sinensis* dose group but with higher dose of *C. sinensis,* that is 4 mg dose, levels of ACP, ALT and AST were normal. The results of this study indicate that the aqueous extract of green tea restores the normal activity of these marker enzymes and antioxidant present in the green tea prevent the liver injury as indicated by the levels of enzymes status.

Key words: Liver, green tea, cadmium, alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT), aspartate transaminase (AST).

INRODUCTION

In recent times, attention has been focused on the physiological importance of a wide variety of naturally occurring polyphenol compounds that act as antioxidants. These compounds are found abundantly in plants including ginger, garlic and cabbage. They exert profound chemo-preventive activities due to their ability to scavenge and reduce the production of free radicals and act as transition metal inhibitors (Bhakta et al., 1999). Metals, when concentrated can be quite toxic and can result in death of organisms. Numerous hazardous heavy metals are inhaled and absorbed by humans and animals every day (Pentyala et al., 2010). Some of heavy metals such as the alkaline earth metals and particularly trace elements are essential for survival because they help build molecules that sustain life. Other metals such as lead (Pb), mercury (Hg) and cadmium (Cd), which are examples of heavy metals are very toxic at even minute quantities and serve no purpose of sustaining life (Cockerham and Shane, 1994). Cd toxicity has been proposed to involve the generation of reactive oxygen species (Obianime and Roberts, 2009). Antioxidant nutrients such and as vitamin C, E and Selenium have been found to counter free radical generation by Cd (Dai and Mumper, 2010).

The tea plant *Camellia sinensis* (L.) O. Kuntze, family Theaceae (commonly known as green tea in English) has antioxidant, anticarcinogenic, antiviral, and bactericidal properties (Hertog et al., 1997). Natural antioxidants, such as polyphenols from green tea extracts, have recently attracted considerable attention for preventing oxidative stress-related diseases including cancers, cardiovascular diseases and degenerative diseases (Ogura et al., 2008). Tea polyphenols showed a protection role against liver injury in many animal models of liver diseases, liver fibrosis, and hepatic ischemia-reperfusion injury (Zhong et al., 2002; Chen et al., 2002). Histological and hepatic examination revealed that Epigallocatechin gallate (EGCG) significantly arrested the progression of hepatic fibrosis and partially the formation

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of lipid peroxidative products (Zhen et al., 2007). Green tea is a popular beverage consumed worldwide, and it also possesses chemopreventive effects in a wide range of target organs in rodent carcinogenesis models (Safe et al., 1999; Yokozawa et al., 2004). Ethanol consumption is related with peroxidation of membrane lipids and there are evidences that green tea protects phospholipids from enhanced peroxidation and prevents changes in biochemical parameters and morphologic changes observed after ethanol consumption. The present study also supports the suggestion that green tea protects membranes from lipid peroxidation (Ostrowaska et al., 2004). Green tea extract can potentiate acetaminophen-induced hepatotoxicity in mice (William et al., 2012).

The present study was designed to evaluate the protective role of green tea against Cd toxicity and an attempt has been made to find out the effects of C. *sinensis* on some liver markers against cadmium toxicity.

MATERIALS AND METHODS

Preparation of extract

Tea was procured from Tea State of Tata Group of Company, TALAT, Assam. Green tea is prepared by picking, lightly steaming and allowing the leaves to dry. The dried material was ground into powder using mortar and pestle and sieved with a sieve of 0.3 mm aperture size. 100 g of the powdered plant material was steeped in 600 ml of distilled water and heated in water bath for 3 h at 90^{o}C. The mixture was allowed to cool to room temperature. Preparation of aqueous extract of *C. sinensis* was done according to the method described by (Dahiru et al., 2007). The dose of 2.0 or 4.0 mg/100g body weight of the extract was given orally to the experimental rats.

Experimental animals

Male Wistar strain albino rats (7-8 weeks old) procured from Animal Division of IVRI, Izatnagar, were maintained in the animal facility of the Zoology Department of Meerut College, Meerut with standard food pellets and tap water *ad libitum.* All animals were cared for according to guidelines of the Institutional Animal Ethics Committee (IAEC) and experiments were also approved by IAEC.

Experimental design

Animals were acclimatized for laboratory conditions and kept on normal diet for two weeks. Experimental animals were divided into the following 4 groups of 18 animals each:

Group I: normal control group.
Group II: cadmium control group. Each rat received a dose of 1 mg/100 g body weight cadmium chloride by oral route.
Group III: animals were given a dose of 2.0 mg/100 g body weight aqueous extract of *C. sinensis* leaves orally along with cd.
Group IV: animals were given a dose of 4.0 mg/100 g body weight aqueous extract of *C. sinensis* leaves orally along with cd.

Animals of each group were further divided into three sub-groups. Animals of sub-group (a) were treated for 15 days, of sub-group (b) for 30 days, and of sub-group (c) were treated for 30 days and then kept on normal diet for 15 more days (total duration of experiment – 45 days) to study the reversible effect of the treatment.

Biochemical studies

Six rats were sacrificed from each group (I, II, III and IV) after 15 days (sub-group a), 6 after 30 days (sub-group b) and six after 45 days (sub-group c) under light ether anesthesia and dissected. The liver was removed and placed in iced beakers. A 1 g portion of the liver was used to prepare homogenate of the liver (10%) in ice cold KCl solution (1.15% w/v) using Teflon homogenizer. The homogenate was centrifuged at 4000 g for 10 min to remove cell debris. The supernatant was used for the estimation of sugar, total proteins, alkaline phosphatase (ALP), acid phosphatase (ACP), alanine transaminase (ALT) and aspartate transaminase (AST) (Dahiru et al., 2007).

Statistical analysis

Results are expressed as mean ± SD. The Student's t-test was used for calculating level of significance. Statistical significance was accepted at $p < 0.05$.

RESULTS

The levels of all biochemical parameters studied are given in detail in Table 1. In general the sugar level was decreased in liver after 1 mg dose of cadmium chloride. All doses of *C. sinensis* used during the present study were able to increase level of sugar in the liver. Protein level showed slight decrease with 1 mg cadmium, but treatment with 2 mg dose of C. *sinensis* was able to bring back the decreased level of protein to normal level.

ALP level showed highly significant decrease in cadmium treated group. In 2 mg *C. sinensis* dose, the level of ALP started increasing, but this increase was not highly significant. In 4 mg *C. sinensis* dose group, increase in ALP was highly significant in comparison to cadmium control groups and it came back to normal. Similarly, in reversibility groups some increase in ALP level was observed in 2 mg *C. sinensis* group and these levels came back to normal after treatment with 4 mg dose of *C. sinensis*. These levels remained unaffected even after discontinuation of the extract feeding for 15 days. ACP level was increased with 1 mg dose of cadmium treatment. Some decrease was observed in ACP activity in 2 mg *C. sinensis* dose group but with higher dose of *C. sinensis*, level of ACP was normal. Likewise, in reversibility group, ACP level remained increased in both cadmium control groups even after discontinuation of cadmium treatment for 15 days. In 2 mg dose groups of *C. sinensis* some decrease in the

Table 1. Effects of *C. sinensis* on liver markers of $CdCl_2$ treated albino rats

Parameters	Duration	Control (N=6)	$CdCl_2$ group 1mg/100g (N=6)	$CdCl_2$ + GT group 1mg + 2mg/ 100g B wt. (N=6)	$CdCl_2$ + GT group 1mg + 4mg/ 100g B wt. (N=6)
Sugar (mg/dl)	15days	21.83±2.86	16.50±4.18[d]	17.83±2.48[d]	22.83±3.92[d]
	30 days	21.67±3.26	17.33±2.94[d]	18.33±2.03[d]	20.83±3.82[d]
	45 days	22.33±2.66	17.17±2.86[a]	19.00±1.79[d]	20.17±1.47[d]
ALP (U/L)	15 days	425±31.87	256±14.95[c]	393±46.70[c]	430±37.45[c]
	30 days	436±24.30	210±22.61[c]	359±39.62[c]	420±44.66[c]
	45 days	425±34.66	294±21.56[c]	389±70.22[a]	412±60.02[b]
Acid Phtase (KA Unit)	15 days	1.37±0.46	1.93±0.33[d]	1.60±0.18[d]	1.13±0.37[b]
	30 days	1.20±0.38	1.23±0.34[d]	1.37±0.45[d]	1.27±0.27[d]
	45 days	1.13±0.33	1.65±0.44[d]	1.37±0.15[d]	1.23±0.08[d]
Total Protien (g/dl)	15 days	3.52±0.70	2.97±0.88[d]	3.33±0.30[d]	3.68±0.29[d]
	30 days	3.93±0.35	2.17±0.45[c]	3.40±0.25[c]	3.80±0.47[c]
	45 days	4.17±0.56	2.87±0.70[a]	3.17±0.29	3.27±0.69
ALT U/L	15 days	41.17±2.23	80.83±35.22[d]	68.00±19.06	43.17±2.32
	30 days	32.33±7.94	95.00±13.54[c]	66.50±3.39[c]	38.33±2.94[c]
	45 days	35.17±8.68	92.00±37.80[b]	64.33±3.01	36.33±5.28[b]
AST U/L	15 days	26.33±10.23	43.00±2.83[b]	32.17±4.12[c]	27.17±2.71[c]
	30 days	24.17±1.72	46.00±4.56[c]	37.17±2.64[b]	31.83±8.45[c]
	45 days	22.83±1.47	49.33±3.08[c]	38.50±6.44[b]	24.00±3.58[c]

Values are mean ± SD. Significance as per Student's "t" test. a = P<0.01, b = P<0.005, c = P<0.001 d = non significant

elevated ACP level was observed. More significant increase was observed in the ALT and AST levels after Cd treatment. Both doses of *C. sinensis* were able to decrease the increased level of ALT and AST.

DISCUSSION

The major organ involved in cadmium toxicity was liver (Renugadevi and Prabu, 2010; Al-Attar, 2011). Sarker and Moitra (1976) suggested that cadmium manifested these changes by modifying the activity of several membrane bound enzymes. The mechanism behind these changes includes phosphorylation, adenylation, ADP-ribosylation and oxidation of thiol groups as well as the respective reverse reactions. The protection offered by antioxidants would therefore be attributed to improvement in these biochemical mechanisms. Polyphenols and epicatechins present in *C. sinensis* are able to protect liver. Sugiyama et al. (1998, 1999) tested the liver injury induced by D-galactsamine (GalN) alone could be suppressed effectively by dietary green tea.

The major findings of this hypothesis was that green tea also had a protective effect against LPS+GalN-induced liver injury when fed to rats for 14 days and when force-fed once before injection of the drugs, indication that effect of green tea is elicited quickly. Yang et al. (1998) demonstrated that the enhanced mortality and serum TNF-α concentration induced a large amount of LPS alone (40 mg/kg body) could be depressed by single force-feeding of tea polyphenols (catechins) in mice. In this study also, fraction II, which contained tea catechins as exclusively major constituents, slightly but significantly suppressed LPS+GalN-induced liver injury. Verma (1980) reported association of ALP with the formation of fibrous protein and passage of metabolites through cell membranes. Nair and Bewgade (1989) clearly specified that ALP is correlated with amino acid pool movement through plasma membrane and transportation of phosphatase through Golgi elements.

It is an essential component of carbohydrate metabolism, with the help of which the glycogen is converted into glucose or fructose-6-phosphatase in order to participate in glycolysis and energy liberation processes. Kornblatt et al. (1983) observed that decrease in ALP activity reflects decreased synthetic activity of cell due to the impairment of mitochondrial function. Inhibition of this enzyme indicates that transphosphorylation reactions and absorption of glucose from the brush border is adversely affected by the heavy metal poisoning. Renugadevi and Prabu (2010) and Al-Attar (2011) have also reported decrease in ALP activity after Cd treatment. Inhibition of ALP activity noted in the present study may be due to the disintegration of the affected cells or direct binding of metal ions with enzyme protein.

The important finding of the present study is that ALP is known to play a role in the transport of phosphatase

through cellular membrane. Present results show that cadmium inhibits ALP activity in liver as indicated by decreased level of ALP in cadmium control group. Co-treatment with antioxidants restored their activity in liver. ALP being the key enzyme of metabolic pathway has to respond to a greater number of different controlling sites in addition to the state of plasma membrane. Several physiological factors have been reported to affect ALP activity.

Loss of acid phosphatase activity indicates lysosomal damage since it is the most important characteristic hydrolases of the lysosomes. Secondly, some metals are known to form inclusion bodies. These inclusion bodies cause cell injury by altering lysosomal structure and functions. Ress and Sinha (1960) are of the opinion that the damaged organs might produce an augmented quantity of enzymes.

The level of acid phosphatase, alanine transminase (ALT) and asparate transaminase (AST) showed increase in all durations of 2 mg groups. High level of acid phosphatase in liver indicates liver damage. High levels of the alanine transminase (ALT) and asparate transaminase (AST) in the liver are also confirmatory of the liver damage. Their high concentration indicates extent of liver damage (Sherlock and Dooley, 1993). Renugadevi and Prabu (2010) and Al-Attar (2011) have also reported increase in the levels of ALT and AST after treatment with Cd. Renugadevi and Prabu (2010) observed decrease in ALT and AST levels after naringenin treatment whereas Al-Attar (2011) reported same results with vitamin E.

Assessment of liver function can be made by estimating the activities of serum ALT, AST and acid phosphatase which are enzymes originally present in higher concentration in cytoplasm. When there is any liver damage, these enzymes leak into the blood stream in confirmatory with the extent of liver damage.

Our results are also in accordance with the work of above mentioned researchers. Normal level of acid phosphatase, alanine transminase (ALT) and asparate transaminase (AST) in the liver with 4 mg dose of *C. sinensis* indicate normal function and structure of liver, although we have not studied liver histology.

Venukumar and Latha (2004) had reported decline in the level of ALT and AST (that was increased in CCl_4 control groups) in CCl_4+*Coscinium fenestratum* treated rats is indicative of hepatoprotective effect of *C. fenestratum*. Liver histology of such experimental animals also showed improvement. These findings are in agreement with our findings, although histological studies were not conducted during the present project.

Increased level of acid phosphatase in cadmium control group indicates tissue damage by cadmium toxicity, whereas normal level of acid phosphatase after treatment with *C. sinensis* indicates protective effect of *C. sinensis* against tissue damage. Acid phosphatase is a marker for lysosomes. Experimental evidence also suggests that it has not been restricted to lysosomal fraction but has been found in Golgi cisternae and specialized region of endoplasmic reticulum known as GERL (Farquhar et al., 1974).

Loss of acid phosphatase activity indicates prevention of lysosomal damage since it is one of the most characteristic hydrolases of the lysosomes. Secondly some metals are known to form inclusion bodies. These inclusion bodies cause cell injury by altering lysosomal structure and functions. Rees and Sinha (1960) are of the opinion that the damaged organs might produce an augmented quantity of enzymes. Al-Kubaisy and Al-Noaemi (2007) also associated catalytic activity in cells with increased level of ACP after treatment with CCl4. Treatment with *Nigellia sativa* oil was able to prevent the tissue damage caused by CCl4, hence reduced level of ACP. Present results are also almost parallel to these findings. Increased level of acid phosphatase activity in cadmium control groups showed decrease in its activity after co treatment with green tea extract.

Conclusion

It can be concluded that as C. sinensis contains many flavanols and polyphenols which probably quenched the ROS produced by cadmium hence showing protection against cadmium toxicity. The protective effect of flavanols and polyphenols present in C. *sinensis* might be produced indirectly by modifying the enzyme activity thus preventing tissue damage.

ACKNOWLEDGEMENT

The authors are grateful to the Director, Scientific and Applied Research Center, Meerut for providing research facilities and encouragement to P.R.

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