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# EFFECT OF GREEN TEA EXTRACTS (*CAMELLIA SINENSIS*) ON ALCOHOLIC- INDUCED LIVER DISEASE IN RABBITS

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## Abstract

Alcohol is currently recognized as the most prevalent known cause of abnormal human development. Our aim of this study was to investigate the effect of green tea extracts on alcoholic- induced liver disease in rabbits.5rabbits were give 10% ethanol alcohol in drinking water only for 60 days, while 20 rabbits were give 10 % ethanol in drinking water and oral supplementation of green tea extracts (100 mg /kg Bw) two times daily for 60 days and 5 healthy rabbits were untreated with alcohol as control groups. Both alcoholic rabbits and normal rabbits were subjected to detailed clinical examination and laboratory investigations. Blood samples were collected and the liver abnormality function assessed by measuring the activities of liver marker enzymes such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH) which were elevated in alcoholic rabbits. Increased lipid peroxidation in alcoholic rabbits was accompanied by decreased activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx). Significantly lowered the activities of liver marker enzymes, decreased the levels of lipid peroxidation and enhanced the antioxidant status to near normal. Thus, the data of the present study suggest that green tea extracts offers protection against oxidative stress and antioxidant activities in alcoholic liver disease.

***Keywords*:** Alcoholic liver disease, Liver marker enzymes, green tea, Lipid peroxidation, Antioxidants, Oxidative stress.

## Introduction

Alcoholic livers disease is one of the most serious consequences of chronic alcohol abuse. The disease is often progressives and is considered to be a major cause of morbidity and mortality (Shah, 2009). Frees radicals and oxidative stress have been implicated in the pathogenesis of ethanol induced livers injury in humans and experimental animals (Lin *et al.,* 1998; Zima *et al.,* 2001). Basically, ethanol is metabolized into cytotoxic acetaldehyde by alcohols dehydrogenases in the liver and acetaldehyde is oxidized to acetates by aldehyde oxidases or xanthine oxidases giving rise to Reactive oxygen species (ROS) vias Cytp450 (Berg *et al.*, 2007). Thus, excess intakes of alcohols resulted in the oxygen radicals which leads to lowering the body's normal defenses mechanisms thereby altered enzyme activity, decreased DNA repair and impaired utilizations of oxygen, lipid peroxidation and proteins oxidations. Some of these alterations induced by oxidative stress can eventually causes necrosis and subsequently leads to oxygen damages (Ogutcu *et al.,* 2008).

*Camellia sinensis* commonly known as greens tea herbs of Theaceae family (Hertog *et al.*, 1997). Green tea is the oldest cultivated plants and has been used as a spices, foods and folklore medicines for over 4000 years. It has been used as a traditional medicines in the treatments of hearts diseases, tumors and headaches and exhibits medicinal properties including immune modulation, hepato protection, antioxidant, antimutagenic, antibacterial and anticarcinogenic effects (Chen, 2002). Moreover, it has also been reported to possess antifungals (Halliwell *et al.,* 1992), antihypertensive effect (Negishi *et al*., 2004), hypolipidemic, anti-atherosclerotic properties (Bursill *et al*., 2001). Green tea (*Camellia sinensis*) is a widely consumed beverages in the worlds, and contains antioxidants such as catechins, ascorbics acid, α-tocopherol and β-carotenes(Geleijinse *et al.*, 2002). The active principle presents in green teas leaves are rich in flavanol monomers known as catechins such as epicatechins which are 13.6 g/100 g in green tea and 4.2 g/100 gm dry weights in blacks tea (Ahmed, 2009). These actives compounds are mainly responsible from tissues damage and various disorders. However, the inhibition of lipidsperoxidation and free radicals scavenging activity has been suggested as a possible mechanism of hepato protective action. Thus, the present study was under-taken to establish the hepato protective effects of green tea on alcohol livers disease.

## Material and Methods

**Plant material:** *Camellia sinensis* L. was purchased from the local market. It was classified according to plant classification references related to medicinal plants (Lawerace, 1951). Also a vouchers specimens of the plant was identified and authenticated at the herbariums of the College of Education, University of Mosul.

**Preparation of extracts:** Preparation of flavonoids, glycosides and alkaloids extract of *Camellia sinensis* was done according to the method described by (Abed Al-Saadon, 2005) while isoflavonoid extract of *Camellia sinensis* was done according to the method described by (Hutabarat *et al*., 2001).

**Animalused:** Male local rabbits weights between 750-850 gm were used. The animals were reared in cages with feed diet and given tap water ad labium, housed in at(22-25ºC) temperature environment with (12h. light and 12h. dark cycle).

**Animal grouping:** Thirty adult male rabbits5 each in all group were taken for the studies and dosing protocol for different groups were as follows.

- **Group I**: served as control and was administered tab water only for 60 days.
- **Group II**: Served 10 % ethanol in drinking water only for60 days. [12] was prepared daily. over entire 60 days period of the experiment .
- **Group III**: Served 10 % ethanol in drinking water for 60 days and100 mg/Kg body weight of flavonoid extract from 30 days with ethanol till the end experiment
- **Group IV**: Served 10 % ethanol in drinking water for 60 days and 100 mg/Kg body weight of isoflavonoid extracts from 30 days with ethanol till the end experiment.
- **Group V**: : served 10% ethanol in drinking water for 60 days and 100 mg/Kg body weight of alkaloids extract from 30 days with ethanol till the end experiment.
- **Group VI** : served 10 % ethanol in drinking water for 60 days and 100 mg/Kg body weights of glycosides extracts from 30 days with ethanol to completes 60 day still the ends experiments.

### Biochemical Analysis

### Estimation of liver marker enzymes

The activities of serum aspartates aminotransferases (AST, E.C.2.6.1.1) and serum alanine aminotransferase (ALT, E.C.2.6.1.2) were assayed by the method of (Reitman's and Frankel, 1957). Serum alkaline phosphatase (ALP, E.C.3.1.2.3.1) was estimated using (King, 1965). The activity of lactate dehydrogenase (LDH, E.C1.1.27) was estimated by the method of (King 1965).The serum gammas glutamyl transferees (GGT, E.C.2.3.2.2) was assayed according to the method of( Rosalki and Rau 1972). Serum total protein, albumin were estimated by Biuret method (Reinhold, 1953).

### Lipid peroxidation and enzyme assays

The rabbits were killed then the livers were immediately isolated, cleaned and weighed for biochemical investigations. Small pieces of liver were cut and fixed 10% of neutral buffered formalin dehydrated in a series of increasing concentration of ethanol, clarified inxylol then embedded in paraffin sections of (5-6µm) and stained with hematoxylin and Eosins then the slides examined under light microscopes for histological studies. Other sections of livers washed with saline solution, weighed, cuts in small parts, homogenizedin 10% (w/v) ice-cold 100 mM phosphate buffer (pH 7.4). Homogenates were centrifuged at 10.000xg at 4°C for 15 min, then the supernatants were used for the measurements of antioxidant enzyme activities (CAT, SOD and GPx), the activities of enzymatic antioxidants CAT, SOD and GPx were assayed by the methods of (Cohen *et al*.,1984), (Brown and Goldstein, 1983) and (Rotruck *et al.,* 1984) respectively. Estimation the levels of malondialdehyde (MDA) by using Thiobarbituric acid reactions method. Thiobarbituric acid (TBARS) in the serum was estimated by the method of (Guidet & Shahs1989) & estimating the of GSHs by the method of (Sedlak and Lindsay, 1968).

### Statistical analysis

The values were expressed as mean ±S.D. Statistical evaluation was done using one way analysis of variance (ANNOVA) which is followed by Duncantest (DT). The level of statistical significances was set at $p< 0.05$.

## Results

Table 1 shows the effects of alcohol on the hepatic markers enzyme that alcoholic rabbits have severe liver damage which was indicated by the increase in marker enzymes such as AST, ALT, ALP, GGT and LDH. However, administration of greens tea significantly decreased the activity of these enzymes which was compared to that before treatment. The level of serum total protein was increased and the albumin level were decreased in alcoholic rabbits, while on treatment with green tea it significantly improved both protein level and albumin level to near normal which was also compared to that of the normal rabbits.

Table-2 shows that the levels of lipid peroxidation indicated by TBARS were significantly higher in Serum of alcoholic rabbits as compared with normal subjects. TBARS level was lowered significantly in the serum of rabbits treated with green tea extracts. Also the result showed a significant increase in GSH level in the serum of rabbits treated with green tea extracts compared with alcoholic rabbits. Further, more the activities of hepatic SOD, CAT and GPx were observed in normal and alcoholic rabbits. In alcoholic rabbits, the activity of hepatic SOD, CAT and GPx were significantly lower than the normal subjects. Treatment of alcoholic rabbits with green tea significantly elevated the antioxidant defense activity compared with that before treatment.

Table 3: shows that the levels of lipid profile (total cholesterol, triglyceride, LDL-c, VLDL-c) were significantly higher in Serum of alcoholic rabbits as compared with normal subjects. While lipid profile level was lowered significantly in the serum of rabbits treated with green tea extracts. Also the results shows to significant increase in HDL-c level in the serum of rabbits treated with green tea extracts compared with alcoholic rabbits.

**Table 1:** Effect of Green tea extracts treatment on hepatic marker enzymes and serum proteins in alcoholic rabbits.

| Parameters | Control rabbits | Alcoholic rabbits before treatment | Alcoholic rabbits after flavonoid treatment | Alcoholic rabbits after isoflavonoid treatment | Alcoholic rabbits after alkaloids treatment | Alcoholic rabbits after glycoside treatment |
|---|---|---|---|---|---|---|
| **AST (IU/L)** | 48±3.4 | 100±5.1 | 70±3.3 | 63±7.3 | 69±4.3 | 60±4.4 |
| **ALT (IU/L)** | 38±2.1 | 125±4.7 | 63±4.6 | 61±4.4 | 61±4.0 | 62±3.4 |
| **ALP (IU/L)** | 82±3.4 | 165±2.3 | 85±4.7 | 82±4.4 | 120±5.3 | 92±5.5 |
| **LDH (IU/L)** | 100±4.3 | 240±8.5 | 154±3.9 | 167±4.8 | 159±5.6 | 177±8.4 |
| **GGT (IU/L)** | 50±2.9 | 200±8.8 | 144±6.1 | 187±6.3 | 180±7.0 | 169±9.2 |
| **Total protein (g/dl)** | 5.95±0.48 | 7.44±1.1 | 5.02±0.65 | 5.49±1.1 | 6.3±0.44 | 6.64±0.61 |
| **Albumin (g/dl)** | 4.12±0.41 | 3.06±0.42 | 3.53±0.32 | 3.27±0.22 | 4.5±0.23 | 4.86±0.45 |
| **Globulin (g/dl)** | 1.83±0.12 | 4.38±0.32 | 1.49±0.22 | 1.22±0.22 | 1.8±0.21 | 1.78±0.19 |

Blood samples were taken after 60 days of oral administration, the value refer to MEAN ± SD, number of rabbit each group = 5.Significantly different from control P< 0.05.

**Table 2:** Effect of Green tea extracts treatment on hepatic lipid peroxidation and enzymatic antioxidants in alcoholic rabbits.

| Parameters | Control rabbits | Alcoholic rabbits before treatment | Alcoholic rabbits after flavonoid treatment | Alcoholic rabbits after isoflavonoid treatment | Alcoholic rabbits after alkaloids treatment | Alcoholic rabbits after glycoside treatment |
|---|---|---|---|---|---|---|
| **MDA (mM/100 g tissue)** | 0.52±0.02 | 0.92±0.02 | 0.65±0.03 | 0.87±0.03 | 0.48±0.02 | 0.61±0.02 |
| **GSH (mg/100mg tissue)** | 85±3.21 | 47±2.8 | 78±3.3 | 69±3.3 | 61±2.9 | 76±3.8 |
| **CAT (mmol/l)** | 0.170±0.01 | 0.09±0.01 | 0.132±0.02 | 0.149±0.02 | 0.136±0.02 | 0.134±0.01 |
| **GPx (U/mg protein)** | 6.80±0.51 | 3.43±0.32 | 5.55±0.42 | 5.43±0.49 | 4.98±0.49 | 4.60±0.56 |
| **SOD** | 2.30±0.2 | 1.23±0.2 | 1.87±0.12 | 1.41±0.13 | 1.56±0.12 | 1.35±0.13 |

Liver samples were taken after 60 days of oral administration with alcohol and treatment , the value refer to MEAN ± SD , number of rabbit each group = 5.Significantly different from control P < 0.05.

**Table 3:** Effect of Green tea extracts treatment on hepatic lipid profile in alcoholic rabbits.

| Parameters | Control rabbits | Alcoholic rabbits before treatment | Alcoholic rabbits after flavonoid treatment | Alcoholic rabbits after isoflavonoid treatment | Alcoholic rabbits after alkaloids treatment | Alcoholic rabbits after glycoside treatment |
|---|---|---|---|---|---|---|
| **Cholesterol mmol/L** | 4.45±0.65 | 8.58±0.68 | 5.79±0.65 | 6.23±0.54 | 5.79±0.44 | 4.63±0.45 |
| **Triglyceride mmol/L** | 0.98± | 1.83±0.34 | 1.78±0.32 | 2.41±0.19 | 2.07±0.19 | 1.56±0.21 |
| **HDL-c mmol/L** | 1.47±0.21 | 0.59±0.11 | 1.18±0.18 | 2.06±0.19 | 2.06±0.21 | 2.0±0.32 |
| **LDL-c mmol/L** | 2.52±0.51 | 7.17±0.54 | 3.81±0.49 | 3.09±0.49 | 2.80±0.45 | 1.93±0.13 |
| **VLDL-c mmol/L** | 0.44±0.04 | 0.82±0.04 | 0.80±0.05 | 1.08±0.09 | 0.93±0.09 | 0.70±0.11 |

Blood samples were taken after 60 days of oral administration, the value refer to MEAN ± SD , number of rabbit each group = 5.Significantly different from control P < 0.05.

**Histopathological Changes:**

**Normal liver animals :**

The liver of control group (1) showed the normal architecture of liver tissue which consisting of central vein surrounded by radiating hepatocyte plate and kupffer cells with normal architecture the liver exhibiting normal rabbit (Fig.1).

**Liver of alcoholic treated animals :**

Histopathological examination of the hepatic tissue of animals treated with alcohol demonstrated that alcohol induces vacuolar degeneration of hepatocytes, with macrovesicular steatosisin addition to in addition to coagulative necrosis of hepatic cells around central vein (Fig 2). Additionally other sections of liver illustrated focal infiltration of inflammatory cells especially mononuclear cells (lymphocytes and macrophages) (Fig. 3).

**Liver of alcoholic and plant extracts treated animals:**

Histopathological examination of the hepatic tissue treated with alcohol and isoflavonaid extract showed dilatation and congestion of central vein with infiltration of inflammatory cell ( lymphocytes and macrophages) (Fig4). However, induced only fatty change and coagulative necrosis of hepatocytes with thrombus of central vein (C.V) when the co-administered with alkaloid extract and glycoside (Fig. 5,6).

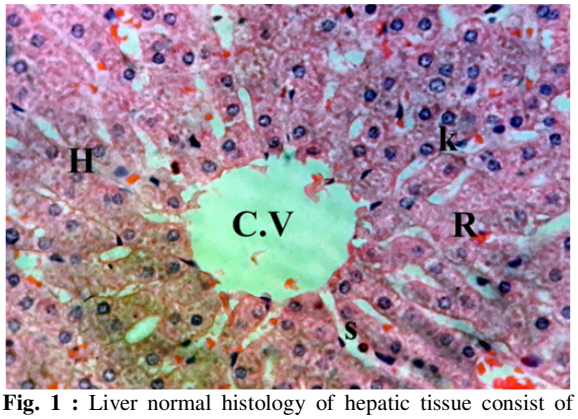


**Fig. 1 :** Liver normal histology of hepatic tissue consist of cords of hepatocytes arranged in single cell layer (H) which is separated from other hepatic cords by vascular sinusoid note(S) the presences of red blood cells (R)and kupffer cells (K) running through sinusoid( H&E 400x).

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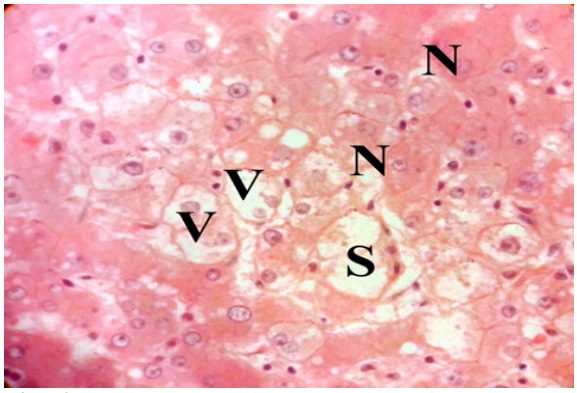



**Fig. 2 :** Histopathological evolution of the liver of animal treated with alcohol showed vacuolar degeneration (V) with macro vesicular steatosis (S) and coagulative necrosis of hepatocyte (N) ( H&E 400x)

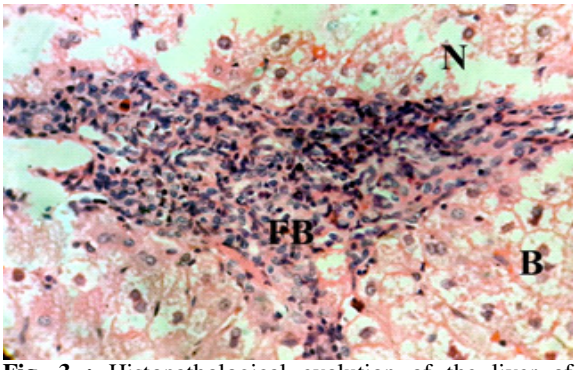



**Fig. 3 :** Histopathological evolution of the liver of animal treated with alcohol showed focal infiltration of inflammatory cells (I) with coagulative necrosis of hepatocyte (N) and vacuolar degeneration of other hepatocytes (V) ( H&E 400x).

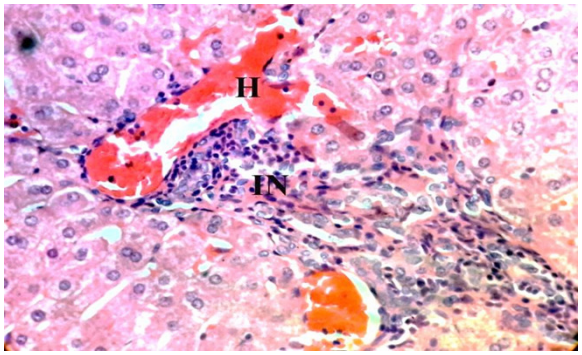
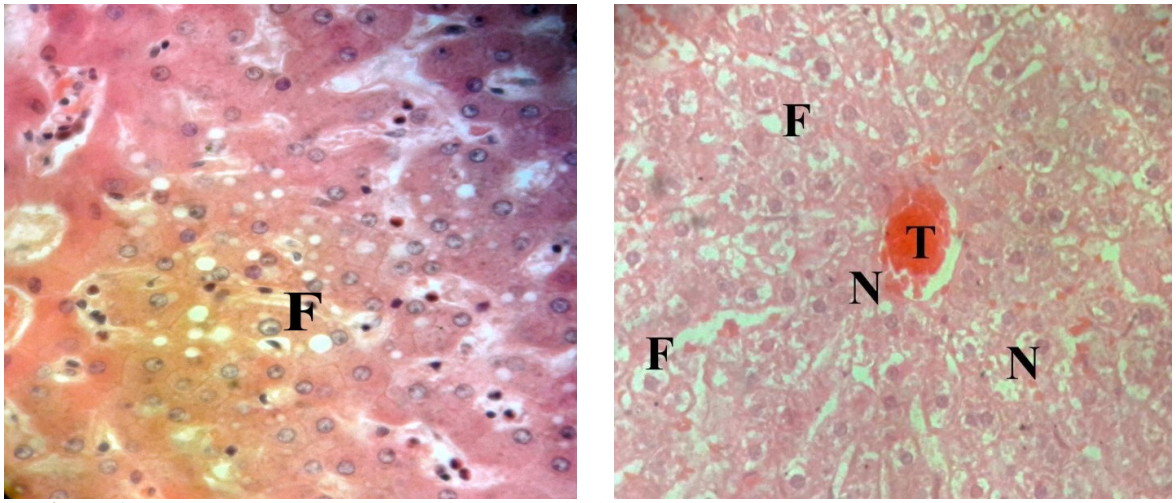



**Fig. 4 :** Histopathological evolution of the liver of animal treated with alcohol & flavonoid extract showed dilatation & congestion of blood vessels (H) in addition to infiltration of inflammatory cells lymphocytes and macrophages. (H&E, 400x)




**Figs. 5,6 :** Histopathological evolution of the liver of animal treated with alcohol & with alkaloid & glycoside extract showed fatty change (F), thrombus (T) & coagulative necrosis of hepatocytes (N) (H&E, 400x)

## Discussion

Free radical mediated damage to macromolecule plays a crucial role in the pathophysiology of atherosclerosis, inflammation, carcinogenesis, aging, drug reaction and toxicity (Rajeshwari *et al.,* 2011). When the liver gets damaged after consumption of alcohol, it leads to leakage of cellular enzymes into the plasma (Baldi *et al.,* 1993). The increased levels of serum enzymes such as (AST), (ALT), (ALP), (GGT) and (LDH) observed in alcoholic rabbits, resulted in liver damage, increased permeability and necrosis of hepatocytes (Mirunalini *et al.,* 2010). In our study, administration of green tea extract to alcoholic rabbits alleviates the increased the activities of serum enzymes AST, ALT and ALP to near normal. Serum GGT is a sensitive marker enzyme widely used as a laboratory test for the hepatobilliary diseases especially alcoholic liver disease and alcohol induced liver damage (Nakanishi *et al.,* 2006). In the present study, we observed that GGT has invariably elevated while AST and ALP are slightly increased in alcoholic rabbits. Green tea supplementation significantly lowered in the activities of GGT demonstrating reduced liver damage following Green tea extracts administration.

Albumins and globulins are two key components of serum proteins. As albumin is synthesized in the liver, it can be used as a biomarker to monitor liver function (Aly *et al.,* 2010). In serum total proteins, albumin contents were reduced in alcoholic rabbits. Hence a significant decrease in the serum total protein and increase in serum albumin was observed in alcoholic rabbits treated with green tea extracts. This stabilization of serum protein level is a clear indication of green tea being related to an improvement in the functional status of the liver cells.

Lipid peroxidation mediated by free radicals is considered to play a pivotal role in the mechanism by which ethanol may exert its toxic effects on the liver and other extra hepatic tissues (Nordmann, 1994). Increase in the levels of TBARS indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanism to prevent the formation of excess free radicals (ALharbi and Azmat, 2011). In our study we observed an increase in TBARS and a decline in antioxidant status in serum of alcoholic rabbits. However, treatment with green tea significantly decreased the levels of lipid peroxidation.

Free radical scavenging enzymes such as SOD, CAT, and GPx are the major defense enzymes against oxidative injury. SOD is a ubiquitous chain breaking antioxidant, plays an important role in protection against deleterious effects of lipid peroxidation (Krishan and Chakkarwar, 2011). It converts the highly reactive superoxide radical to hydrogen peroxide, which in turn either metabolized by catalase or by glutathione peroxidase. The primary role of catalase is to scavenge $H_2O_2$ and convert it into $H_2O$. It plays an important role in the acquisition of tolerance to oxidative stress in adaptive response of cells. Studies have shown that decrease in catalase during alcohol consumption may be due to the decreased protein synthesis. Thus, there is an increased utilization of CAT during alcohol consumption.

Gpx is a selenium dependent enzyme found primarily in the cytoplasm and also found in the mitochondria. It catalyses the detoxification of

endogenous metabolic peroxides and hydroperoxides that leads to the oxidation of GSH. It has a high potency in scavenging reactive free radicals in response to oxidative stress.

The antioxidant defense systems SOD, CAT and GPx activity is significantly decreased in alcoholic rabbits. This decrease could be due to a feedback inhibition or oxidative inactivation of enzyme protein because of excess ROS generation. The generation of α-hydroxyethyl radical may lead to inactivation of these enzymes (Pourghassem-Gargari *et al*., 2011) and accumulation of highly reactive free radicals also lead to deleterious effects such as loss of cell membrane integrity & membrane function (Krishnakanth and Lokesh, 1993).

There was a significant increase in the activity of these enzymes after green tea extracts administration. It is reported that green tea suppresses the formation of superoxide anion and hydrogen peroxide by increasing the activity of SOD, CAT and GPx (Borek 2001). Therefore, green tea increases antioxidant action by scavenging ROS, enhancing the cellular antioxidant enzymes and increasing glutathione in the cells. Moreover, it has also been reported that green tea modulates the levels of lipid peroxidation (Hussein *et al.,* 2007). Although multiple actions may take place during hepatoprotective activity, modulation of lipid peroxidation and antioxidant status may be one of the important mechanisms by which green tea exerts its toxic inhibitory effect.

Thus, our results suggest that, oral administration of green tea protects tissue damage by increasing the antioxidant status against oxidative stress. Hence, green tea plays a promoting role in antioxidant and it can be considered as a potent drug for the treatment of alcoholic disorders. Further studies are needed to unravel the mechanism of action of green tea and its active components.

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