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Full Length Research Paper

The protective effect of some polyphenolic compounds on lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats

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To study the hepatoprotective effect of tea and cocoa extracts against liver injury and to know the potent effect of each in protecting the liver from Lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats. Eighteen healthy male rats were used in this study and divided into eight groups; group I and V received saline, groups II and VI received black tea, groups III and VII received green tea, groups IV and VIII received cocoa extracts orally for one month, then groups V, VI, VII and VIII received D-galactosamine and Lipopolysaccharide for induction of hepatitis. Serum liver enzymes, liver oxidants/antioxidants profile and tumor necrosis factor-α were estimated. The data showed that all used extracts had a protective effect against liver injury. Although, green tea extract showed the maximum improvement in liver enzymes and antioxidants levels, cocoa extract showed the maximum improvement in tumor necrosis factor-α level compared to green and black tea prophylactic groups. We concluded that tea and cocoa had a protective effect against liver injury which attributed to their free radical scavenging antioxidants that reduced both the production and release of tumor necrosis factor – α and protected the liver from oxidative damage.

Key words: D-galactosamine, polyphenolic compounds, black tea, green tea, cocoa, hepatitis.

INTRODUCTION

Hepatitis infection is a major cause of chronic liver disease which infects more than 170 million persons worldwide, often leading to cirrhosis, hepatic failure and hepatocellular carcinoma *(*Zuo et al., 2007). D-galactosamine (DGa1N) together with Lipopolysaccharide (LPS) can lead to pronounced secretion by Kupffer cells of pro-inflammatory mediators, which have been shown to be early and important mediators of liver injury (Osman et al., 2007).

LPS is known to activate macrophages, including Kupffer cells and stimulate the synthesis and release of inflammatory cytokines such as tumor necrosis factor-*α* (TNF-*α*), interleukin-1*β* and interleukin-6. Of these cytokines TNF-*α* plays a critical role in the pathogenesis of LPS-induced hepatitis (Bradham et al., 1998). Food derived antioxidants have a strong potential for long term use as chemo-preventive agents in disease states involving oxidative stress such as hepatitis and alcoholic liver diseases (Mckim et al., 2002).

Tea and cocoa are food sources rich in phenolic compounds which have many important functions including antioxidant, antimutagenic and antitumor activities (Othman et al., 2007). Thus, the aim of this study is to investigate the protective effect of some polyphenolic compounds heavily present in tea and cocoa, against lipopolysaccharide-induced hepatitis in D-galactosamine sensitized rats.

MATERIALS AND METHODS

Materials

Male albino rats weighing 180 - 200 g were obtained from the animal house of National Research Center, Giza, Egypt. D-galactosamine Hydrochloride (DGa1N) and LPS were purchased from Sigma-Aldrich Company, St. Louis, MO, USA. Black tea, green

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tea and cocoa were purchased from local market.

Methods

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of the National Research Centre (NRC).

Plant extraction

Water extracts of the plant were prepared by adding 200 ml deionized water at 90°C into a glass containing 2 g of plant (Tea or Cocoa) (Majchrzak et al., 2004).

Induction of hepatitis

LPS was dissolved in normal saline 0.9% w/v. The solution containing (42 µg/180 g body weight), pH was adjusted at 7.4 and intraperitonealy injected in rats and after 2 h DGa1N dissolved in normal saline (0.9% w/v). The solution containing (105 mg/180 g body weight), pH was adjusted at 7.4 and intraperitonealy injected in rats; blood samples were withdrawn after 22 h to check induction of hepatitis (He et al., 2001).

Experimental design

Eighty healthy male albino rats weighing 180 - 200 g were divided into eight groups (10 rats in each).
Group I (normal control): healthy rats received normal saline (1 ml/kg b.w. /day) orally for thirty days (Najmi et al., 2005).
Group II (black tea group): healthy rats received black tea extract (36 mg/rat twice a day) orally for thirty days (Majchrzak et al., 2004).
Group III (green tea group): healthy rats received green tea extract (36 mg/rat twice a day) orally for thirty days (Majchrzak et al., 2004).
Group IV (cocoa group): healthy rats received cocoa extract (36 mg/rat twice a day) orally for thirty days (Majchrzak et al., 2004).
Group V (hepatitis control group): healthy rats received normal saline (1 ml/kg b.w. /day) orally for thirty days before induction of hepatitis.
Group VI (prophylactic black tea group): healthy rats received black tea (36 mg/rat twice a day) orally for thirty days before induction of hepatitis.
Group VII (prophylactic green tea group): healthy rats received green tea (36 mg/rat twice a day) orally for thirty days before induction of hepatitis.
Group VIII (prophylactic cocoa group): healthy rats received cocoa (36 mg/rat twice a day) orally for thirty days before induction of hepatitis.

Blood sampling

After 22 h of DGa1N administration (He et al., 2001), fasting blood samples were withdrawn from the retro-orbital plexus vein of each animal, under light anesthesia by diethyl ether, according to the method of Madway et al. (1969). Blood samples were clotted and serum was separated by centrifugation at 3000 rpm for 15 min, then divided into aliquots and stored at -20°C for biochemical assays.

Preparation of tissue homogenate

Liver was removed quickly and placed in iced normal saline, perfused with normal saline solution to remove blood cells, blotted on filter paper and frozen at -80°C. The frozen tissues were cut into small pieces and homogenized in 5 ml cold buffer (0.5 g of Na_2HPO_4 and 0.7 g of NaH_2PO_4 per 500 ml deionized water (pH 7.4) per gram tissue, then centrifuged at 4000 rpm for 15 min at 4°C and the supernatant was removed for oxidant/antioxidant parameters estimation (Manna et al., 2005).

Biochemical analysis

Serum liver enzymes alanine amino-transferase (ALT) and aspartate amino-transferase (AST) were measured according to the method of Reitman and Frankel (1957), alkaline phosphatase (ALP) was determined according to the method of Kind and King (1954) and gamma glutamyl-transferase (γGT) activity was measured by the method of Whitefield et al. (1973). All oxidant/antioxidant parameters were determined by Commercial kits (Purchased from BioMed Diagnostics). Liver superoxide dismutase (SOD) was measured according to the method of Nishikimi et al. (1972), catalase (CAT) was determined according to the method of Aebi (1984), reduced glutathione (GSH) was determined according to the method of Beautler et al., (1963) and malonedialdehyde (MDA) was measured according to the method of Uchiyama and Mihara, (1978). Serum tumor necrosis factor-α (TNF- α) was measured by ELISA kit (purchased from orgenium laboratories Finland) according to the method described by Seriolo et al. (2006).

Statistical analysis

All data were expressed as mean ± standard error. Data were analyzed using one-way ANOVA by SPSS (Version 12). Duncan's new multiple-range test was used to assess differences between means. Pearson's correlation test was used to assess correlations between means. A significant difference was considered at the level of $P < 0.05$.

RESULTS

The results shown that, the mean values of serum AST, ALT, ALP and γGT levels were significantly increased in DGa1N / LPS group compared to control group. Although, it significantly decreased in all prophylactic groups (black tea, green tea and cocoa) compared to DGa1N / LPS group (Table 1). As shown in Table 2, the mean values of liver SOD, CAT and GSH were significantly decreased in DGa1N / LPS group while the mean value of liver MDA was significantly increased compared to control group. In prophylactic groups, the mean values of liver SOD, CAT and GSH were significantly increased while the mean value of MDA was significantly decreased compared to DGa1N / LPS group indicating the reduction of oxidative stress by all used extracts. The mean value of serum TNF-α in DGa1N / LPS group was significantly increased compared to control group, while it significantly decreased in prophylactic groups compared to DGa1N / LPS group (Table 3). From these results, it was observed that there was a positive correlation between serum TNF-α and liver enzymes (AST, ALT, ALP and γGT). Also, there was a

Table 1. Serum liver enzymes in different studied groups.

Groups	AST U/L	ALT U/L	ALP U/L	γGT U /L
Control	91.5 ± 1.6 [b]	15 ± 1.2 [b]	88.3 ± 6.2 [b]	24.2 ± 2.3 [b]
Black tea	99.5 ± 1.5 [b]	13.4 ± 0.8 [b]	109 ± 10 [b]	13.7 ± 2.8 [a, b]
Green tea	93.2 ± 4.6 [b]	11 ± 1.1 [b]	94 ± 5.8 [b]	10.5 ± 1.6 [a, b]
Cocoa	89.6 ± 4.6 [b]	9.15 ± 0.9 [b]	110.1 ± 7.1 [b]	15.4 ± 1.3 [a, b]
DGa1N / LPS	155 ± 15.2 [a]	53.25 ± 6.9 [a]	296.5 ± 26.4 [a]	46.5 ± 7.8 [a]
Black tea + DGa1N / LPS	101 ± 1.6 [b]	17.25 ± 3.2 [b]	133.1 ± 8.5 [a, b]	21.4 ± 2.9 [b]
Green tea + DGa1N / LPS	96.2 ± 4 [b]	19.85 ± 3.5 [b]	105 ± 9 [b]	13.7 ± 1.6 [a, b]
Cocoa + DGa1N / LPS	104.5 ± 3.7 [b]	20.68 ± 0.6 [b]	134 ± 1.2 [a, b]	16 ± 0.5 [b]

Significant P value < 0.05, a = significant difference compared to control group, b = significant difference compared to DGa1N / LPS group, n = number of animals = 10.

Table 2. Liver oxidant and antioxidant parameters in different studied groups.

Groups	SOD U/g tissue	CAT U/g tissue	GSH mg/dl	MDA Mmol/L
Control	439.5 ± 34 [b]	0.319 ± 0.017 [b]	4.7 ± 0.44 [b]	12.88 ± 0.13 [b]
Black tea	538.7 ± 35 [a, b]	0.354 ± 0.025 [b]	6.5 ± 0.48 [a, b]	12.21 ± 0.31 [a, b]
Green tea	517.5 ± 8.2 [a, b]	0.322 ± 0.011 [b]	5.8 ± 0.86 [a, b]	12.95 ± 0.13 [b]
Cocoa	437.3 ± 3.3 [b]	0.332 ± 0.002 [b]	4.6 ± 0.032 [b]	12.64 ± 0.11 [b]
DGa1N / LPS	143 ± 9.3 [a]	0.158 ± 0.020 [a]	3.1 ± 0.12 [a]	21.74 ± 0.52 [a]
Black tea + DGa1N / LPS	375 ± 5.6 [a, b]	0.232 ± 0.015 [a, b]	3.3 ± 0.032 [a]	12.68 ± 0.14 [b]
Green tea + DGa1N / LPS	318 ± 1.0 [a, b]	0.265 ± 0.014 [a, b]	3.5 ± 0.16 [a]	13.04 ± 0.11 [b]
Cocoa + DGa1N / LPS	350 ± 8.4 [a, b]	0.176 ± 0.009 [a]	3.7 ± 0.28	13.17 ± 0.016 [b]

Significant P value < 0.05, a = significant difference compared to control group, b = significant difference compared to DGa1N / LPS group, n = number of animals = 10.

Table 3. Serum tumor necrosis factor-α in different studied groups.

Groups	TNF-α Pg/ml
Control	40.5 ± 0.96 [b]
Black tea	42.6 ± 2.3 [b]
Green tea	40.3 ± 4.2 [b]
Cocoa	24.6 ± 3.5 [a, b]
DGa1N / LPS	78.9 ± 12.4 [a]
Black tea + DGa1N / LPS	45.5 ± 9.6 [b]
Green tea + DGa1N / LPS	46.4 ± 2.03 [b]
Cocoa + DGa1N /LPS	30.1 ± 3.3 [b]

Significant P value < 0.05, a = significant difference compared to control group, b = significant difference compared to DGa1N / LPS group, n = number of animals = 10.

directly proportional between serum TNF-α and liver antioxidant parameters (SOD, CAT and GSH) as well as inversely proportional between serum TNF-α and liver MDA (Figures 1 - 8)

DISCUSSION

D-galactosamine-induced experimental model system in rats recognized to be much like viral hepatitis in humans

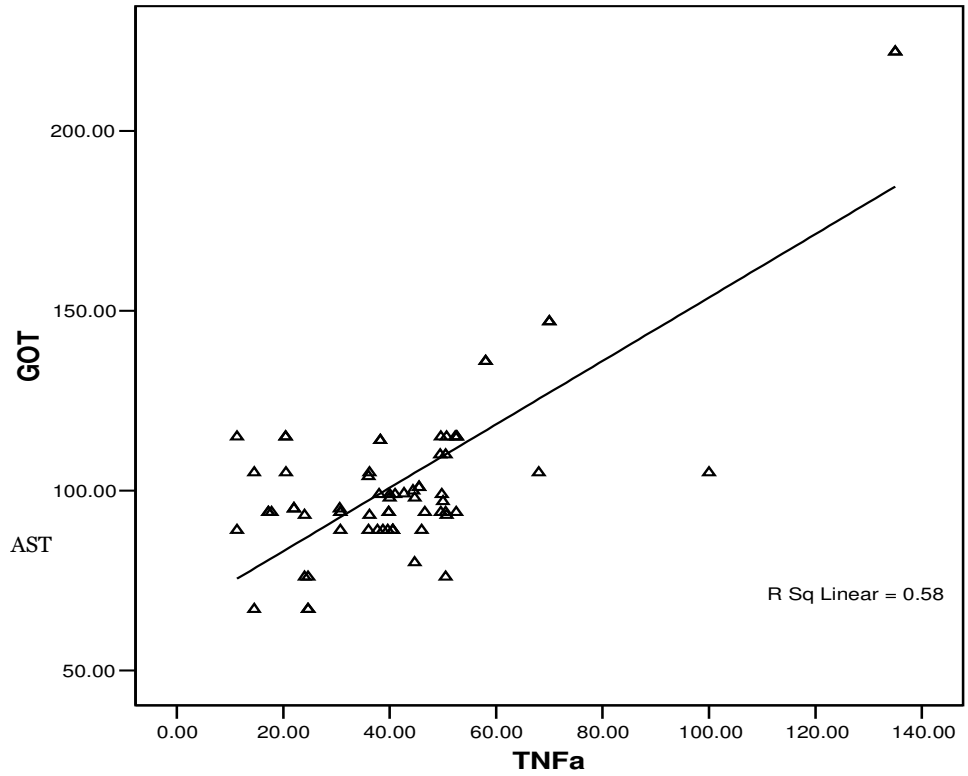

Figure 1. Correlation between TNF-α and AST in different studied groups.

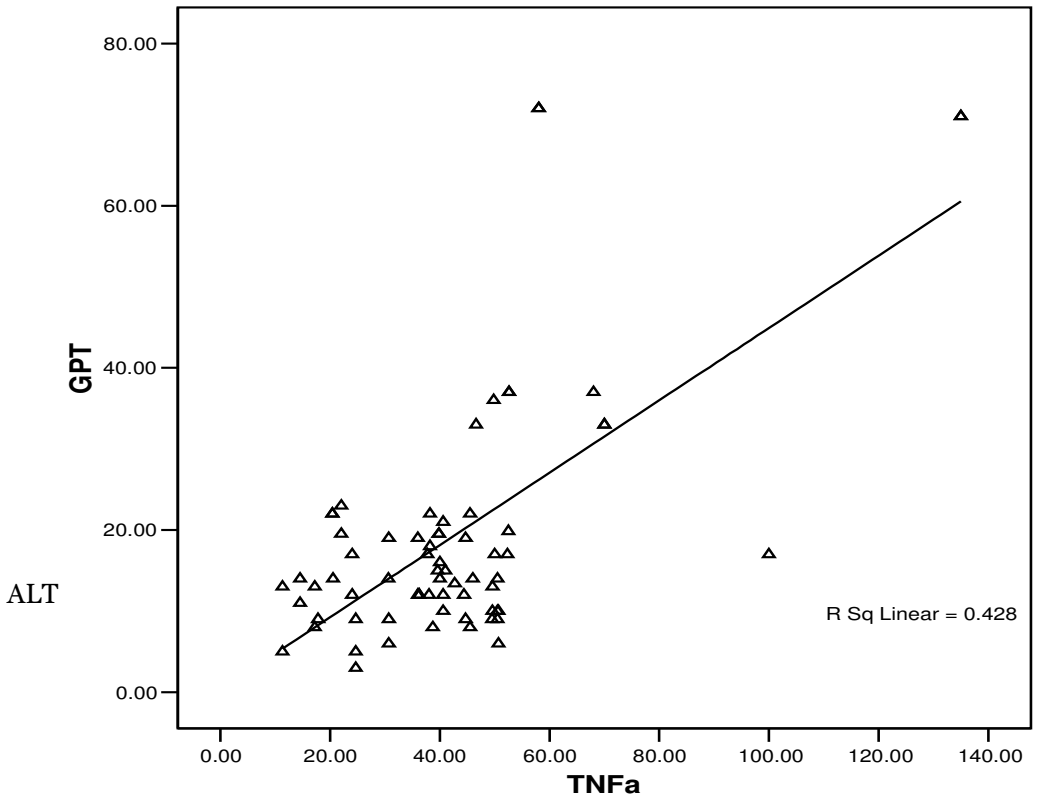

Figure 2. Correlation between TNF-α and ALT in different studied groups.

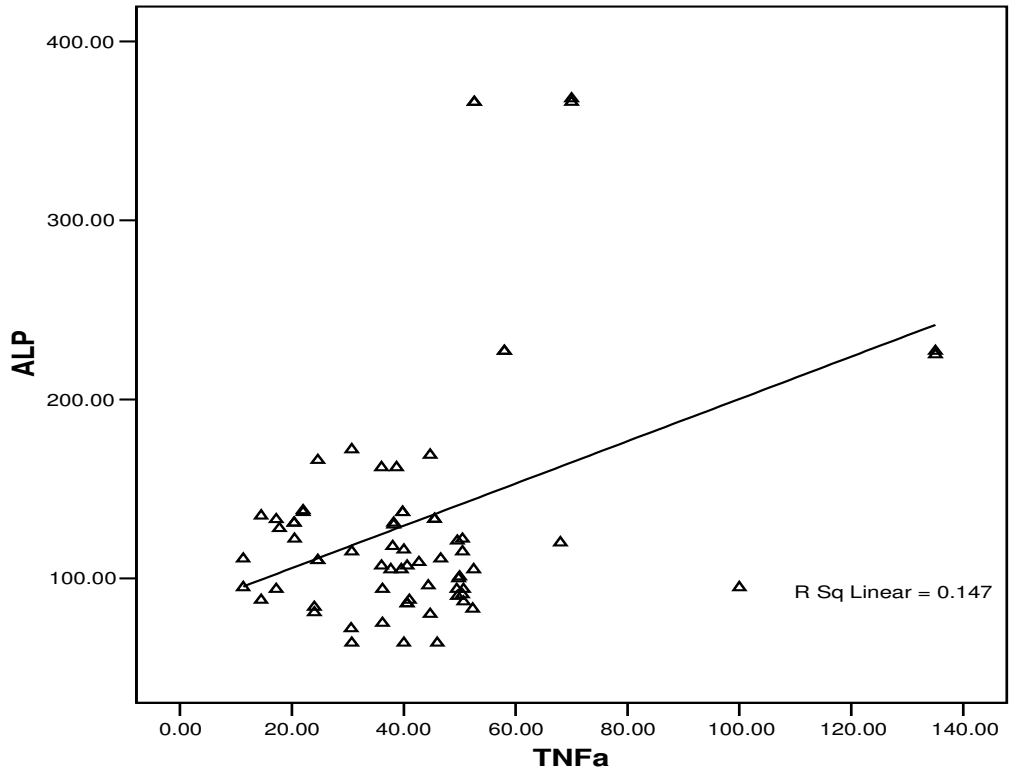

Figure 3. Correlation between TNF-α and ALP in different studied groups.

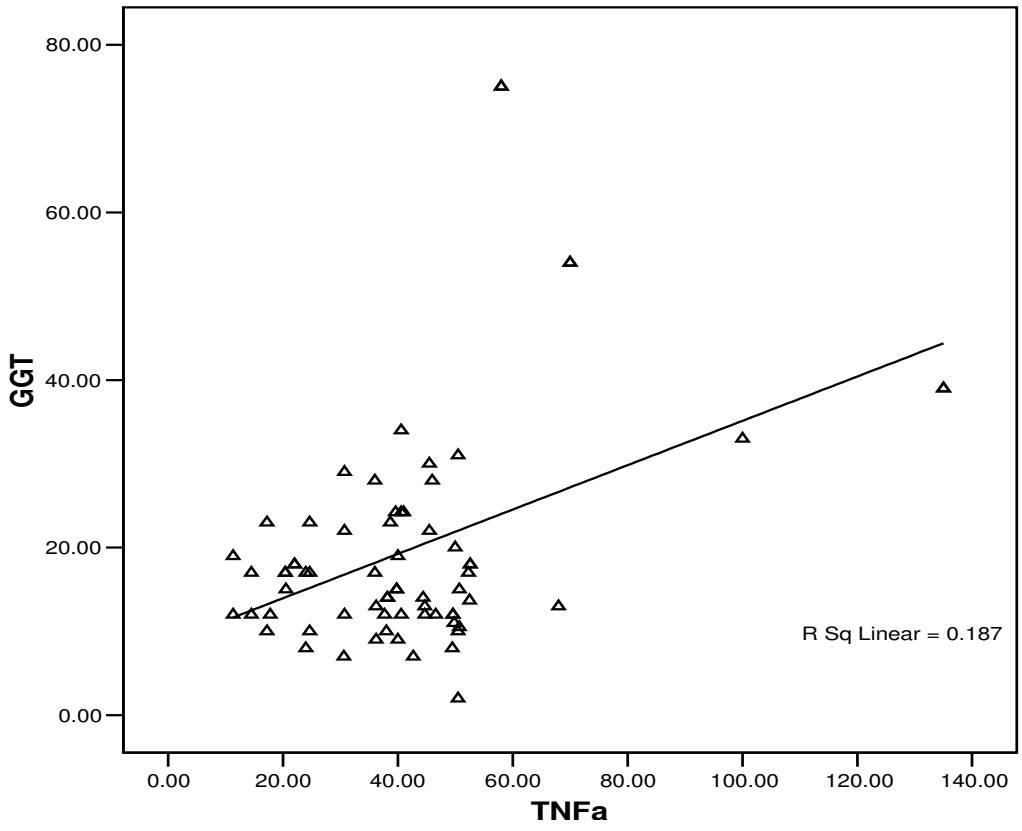

Figure 4. Correlation between TNF-α and γGT in different studied groups.

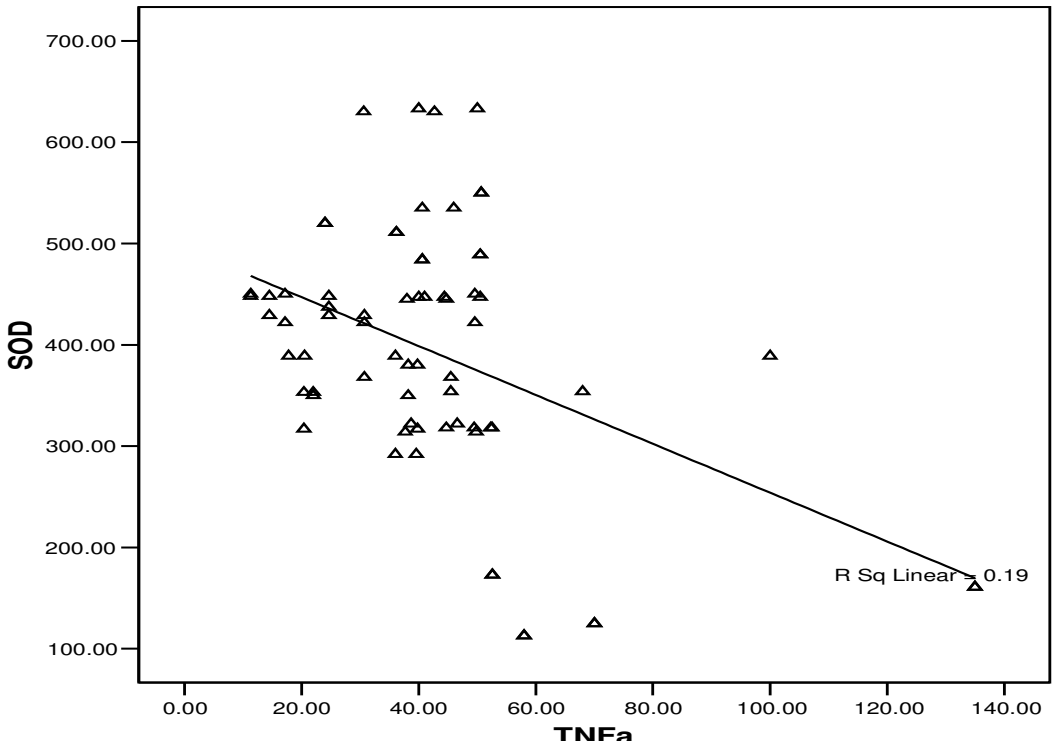

Figure 5. Correlation between TNF-α and SOD in different studied groups.

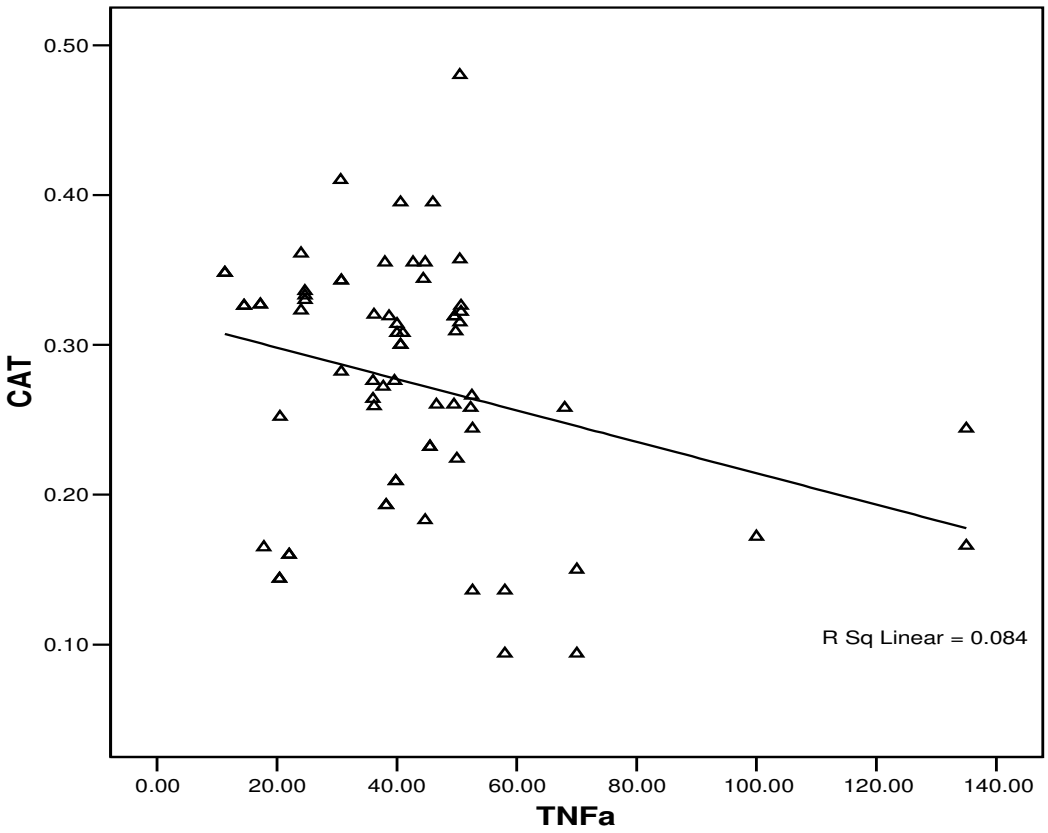

Figure 6. Correlation between TNF-α and CAT in different studied groups.

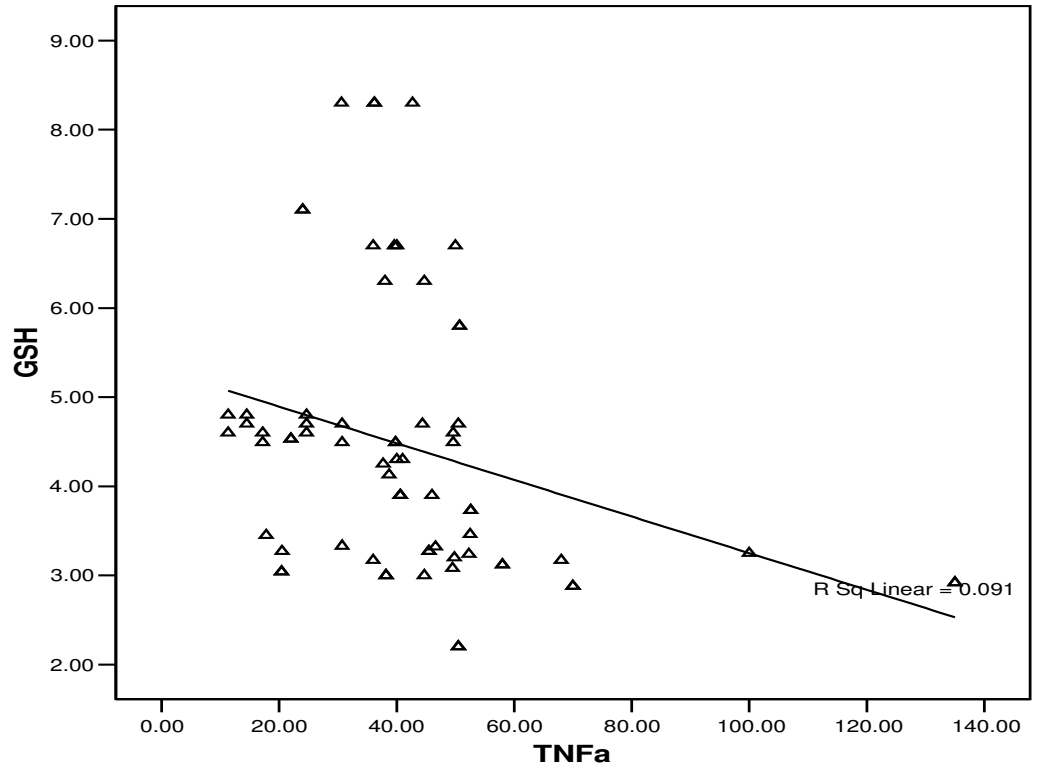

Figure 7. Correlation between TNF-α and GSH in different studied groups.

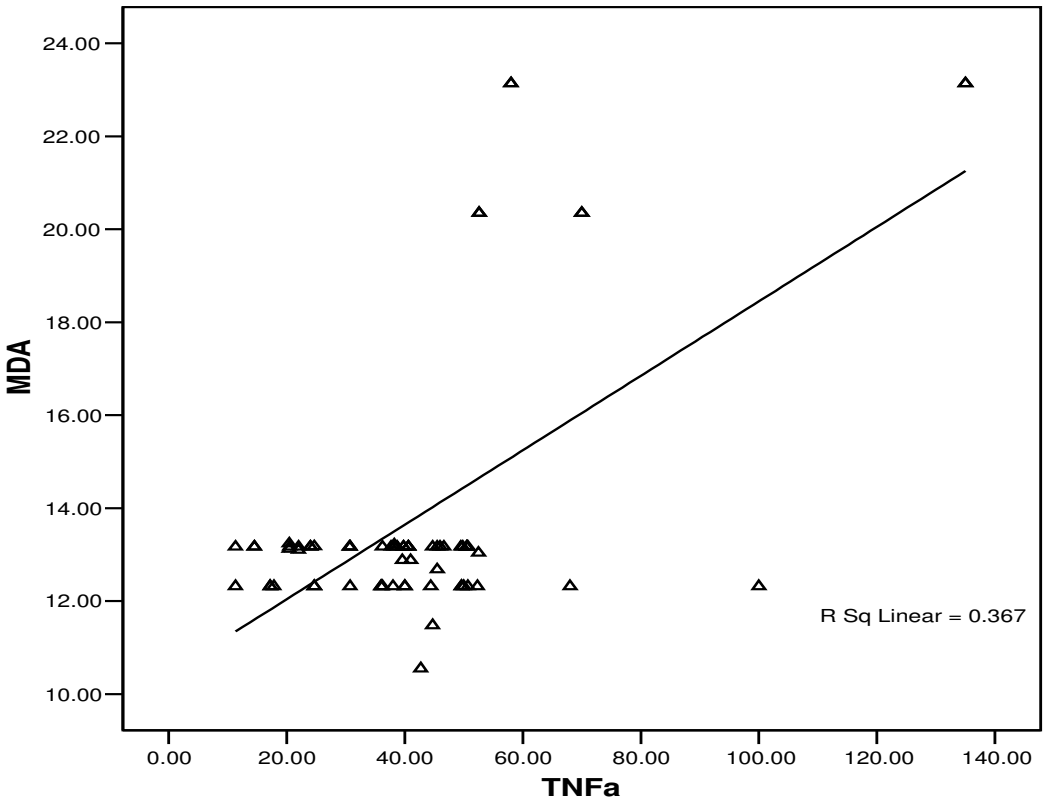

Figure 8. Correlation between TNF-α and MDA in different studied groups.

from both morphological and functional points of view (Najmi et al., 2005). It is thought to induce hepatotoxicity by inhibiting the synthesis of RNA and protein through a decrease in cellular uridine tri-phosphate (UTP) concentration, which finally leads to necrosis of liver cells. Also, it has great liver specificity because hepatocytes have high levels of galactokinase and galactose-1-uridyltransferase; so it does not affect other organs (Lee et al., 2000).It provokes a state of oxidative stress by influencing free radical generation and antioxidant status (Najmi et al., 2005).

Food derived antioxidants have a strong potential for long-term use as chemo-preventive agents in disease states involving oxidative stress, such as hepatitis and alcoholic liver diseases because they are safe (McKim et al., 2002).

Tea and cocoa are food sources rich in phenolic compounds and have several biological activities, such as the ability to scavenge superoxide and hydroxyl radicals, reduce lipid peroxyl radicals and inhibit lipid peroxidation (Othman et al., 2007)

In this study DGa1N / LPS significantly increased serum liver enzymes levels which could be taken as an index value of liver damage, that is may be due to the fact that DGa1N administration disturps plasma membrane permeability causing leakage of liver enzymes from the cell, which leads to elevation in serum enzymes levels (Mitra et al., 2000).

Oxidative stress has been reported as one of the major causes of DGa1N-induced liver damage, excessive production of free radicals resulting from oxidative stress can damage macromolecules as lipids (Najmi et al., 2005). In the current study DGa1N / LPS injection increased the mean value of liver TBARS and decreased liver GSH, SOD and CAT, these results were in agreement with Najmi et al. (2005) who indicated that DGa1N-intoxicated rats showed an increased TBARS level, a typical parameter of lipid peroxidation. Also, Zhou et al. (2008) indicated that treatment with DGa1N decreased antioxidative enzyme activities as glutatihione reductase (GR), catalase and SOD.

In this study, DGa1N / LPS increased the mean value of serum TNF-α, this result was in agreement with Han et al. (2006) who reported that liver injury induced by DGa1N is associated with an elevation of serum TNF-α. In the same line, Bradham et al. (1998) stated that LPS is known to activate macrophages, including Kupffer cells and stimulate the synthesis and release of inflammatory cytokines such as TNF-α, interleukin-1ß and interleukin-6 Moreover, the present results appeared a positive correlation between TNF-α and liver enzymes and oxidative stress products. In the same line there was a negative correlation between TNF-α and antioxidant parameters. These results agree with Bradham et al. (1998) who indicated that TNF-α plays a critical role in the pathogenesis of LPS-induced hepatitis.

The current results showed that, all used extracts prevented liver enzymes from elevation, also decreased oxidative stress which appeared in decreasing the mean value of liver TBARS and increasing liver antioxidant parameters compared to DGa1N / LPS group. It also decreased the mean value of serum TNF-α in prophylactic groups compared to DGa1N / LPS group indicating the decrease of liver inflammation.

The potential activities of these beverages may be related to the presence of catechins which possess the ability to prevent free oxygen radicals formation through inhibiting the activity of enzymes participating in their generation, and to scavenge the free radicals as well as to chelate transition metal ions which enhanced radical reactions (Luczaj and Skrzydlewska, 2004).The present results indicated that green tea extract showed the maximum improvement in liver enzymes and antioxidant parameters while cocoa extract showed the maximum improvement in TNF-α level compared to green tea and black tea.

Leunge et al. (2001) suggested that black tea contains smaller amounts of catechins in comparison to green tea; its antioxidant properties are also significantly lower. However, some examinations had proved that multimeric polyphenols of black tea-theaflavins and thearubigins generated during tea leaves fermentation possess even stronger antioxidant abilities than precursors-catechins.

Majchrzak et al. (2004) found that green tea extracts have higher antioxidant capacity than black tea solutions and the total antioxidant potential correlates strongly with the total phenolic contents of tea of all types. Cocoa is a good source of flavonoids, which contains epicatechin and its oligomers rather than the epigallocatechin gallate found in green tea (Mckim et al., 2002). Also, cocoa contains mainly flavonoids such as (-)-epicatechin, (+)-catechin, and polymers derived from these monomers called procyanidins including theobromine and theophylline which are analogues of caffeine (Ramiro-Puig et al., 2007). Also, Cocoa contains high amounts of epicatechin and epicatichin-based oligomeric procyandins. Where, a daily dose of 400 mg/kg not only dramatically protected the liver from oxidative stress and necrosis but also blunted steatosis and inflammation (McKim et al., 2002). In addition to caffeine, theobromine, one of the characteristic constituents of cocoa, was found to suppress LPS-induced hepatitis, suggesting that cocoa might also have a hepatoprotective effect (He et al., 2001).

Conclusion

DGa1N/LPS administration induced liver injury due to oxidative stress and increasing the secretion of proinflammatory cytokines such as TNF-α. Administration of polyphenolic extracts such as black tea, green tea and cocoa significantly suppress the increase of serum TNF-α and oxidative stress. All used extracts have a beneficial and prophylactic effect against DGa1N/LPS induced liver injury in rats although, differences in their activities which

is related to the difference in their chemical structures.

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