



Title	GENO PROTECTIVE AND ANTI-APOPTOTIC EFFECT OF GREEN TEA AGAINST PERINATAL LIPOPOLYSACCHARIDE-EXPOSURE INDUCED LIVER TOXICITY IN RAT NEWBORNS
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Abstract

Background: This study aims to examine the protective effect of green tea on the disturbances in oxidative stress and apoptosis related factors, mostly produced due to perinatal lipopolysaccharide (LPS) exposure, that subsequently induces liver cell damage.

Materials and Methods: Anti-free radical, Antioxidant, scavenging, geno-protective, and antiapoptotic activity of aqueous green tea extract (AGTE) were assessed against LPS-induced hepatic dysfunction in newborn-rats. AGTE at doses of 100 & 200 mg/kg was orally administered daily to rat dams, during gestation and lactation.

Results: AGTE was observed to exhibit protective effects by significantly attenuating LPS-induced alterations in serum AST, ALT, bilirubin, and albumin levels. Significant increase in the total antioxidant capacity (TAC), DNA contents, and reduction in nitric oxide (NO) levels were observed in AGTE treated rats comparing LPS-toxicated ones. Additionally, AGTE treatment significantly down-regulated apoptotic markers and this effect was directly correlated to the degree of hepatic fibrosis. The possible mechanisms of the potential therapeutic-liver protective effect of AGTE could be due to free radical scavenging potential and antiapoptotic properties caused by the presence of antioxidant polyphenolic components in AGTE.

Conclusion: We thereby propose, based on our findings, that the anti-free radical and anti-apoptotic inducing properties of AGTE active constituents attribute to its functional efficacy as anti-fibrotic agent.

Key words: *green tea, lipopolysaccharide, liver dysfunction, apoptosis, newborns*

Introduction

Endotoxemia resulting from infection with gram-negative bacteria are one of the major reasons of mortality and morbidity in many hospitals especially the units of intensive care. Whereas, injury, damage, and sepsis of various organs, such as liver was attributed to the endotoxicity of lipopolysaccharide (LPS) that is present as a major glycolipid component of the outer cell wall of gram-negative bacteria (Baranova et al., 2016). It has been reported previously that, LPS-induced injury in the liver and systemic circulation was linked to the release of toxic mediators such as cytokines, interleukins, as well as increases in oxidative stress parameters as superoxide radicals and nitric oxide (NO) (Ojiako et al., 2015). The toxicity induced by LPS is characterized by changes in metabolic responses, leukocytosis, and redox status impairment, consequently leading to DNA and protein damage along with cell apoptosis (Baranova et al., 2016). This association of free radical oxidative stress and cell apoptosis in liver tissue injury argues that dietary antioxidants of plant origin may enhance the potential efficiency of treatment protocols designed to mitigate LPS-induced endotoxemia. It has been reported that active phytochemicals present in vegetables, fruits, teas and spices possess protective biological properties, including anti-apoptotic, anti-inflammatory, antioxidant and other beneficial effects (Li et al., 2013).

Several studies have previously reported that green tea extract possesses antioxidant, antibacterial, antiviral, anti-carcinogenic, anti-apoptotic, and anti-mutagenic functions (Bitu Pinto et al., 2015). These healthy effects of green tea are thought to stem from the polyphenols with its antioxidant properties. The extracts of green tea include polyphenols such as flavandriols, flavanols, and phenolic acids. Catechins are the most important type flavonoids which are present at about 10% of the dry weight. Catechins have been shown to display biological activity as oxy-radicals scavengers (Ide et al., 2016). A number of studies have also shown that green tea contains high levels of vitamin C with considerable beneficial effects to human health (Bitu Pinto et al., 2015). Polyphenols isolated from water extract of green tea have been shown in several animal models to prevent chemically-induced carcinogenesis in organs including liver (Stagos et al., 2012). For example, it has been shown that liver injury induced by GalN alone could be effectively

protected by dietary green tea intake potentially due to the flavonoid, glycosides and soluble dietary fiber that are present in the green tea (He *et al.*, 2001).

The current study has been designed to evaluate genoprotective, antioxidant, and antiapoptotic activities of aqueous green tea extract (AGTE) against LPS -induced hepatic dysfunction in rats newborns.

Materials and Methods

AGTE preparation

Green tea has been purchased from the Othaim Market in Riyadh, Saudi Arabia. The green tea leaves extracts have been prepared daily by addition of 10 g of leaves to 750 ml boiled distilled water and the mixture has been preserved at 90 °C for 3 min, subsequently filtered and cooled to be administered to the pregnant and lactating female rats (Mustata *et al.*, 2005).

Screening of phenolic compounds in AGTE

The total phenolic compounds in 100 mg of AGTE have been analyzed and performed with a liquid chromatography "HP1050" according to the reported method by Waskmundzka *et al.* (2007).

Total phenolic content estimation in AGTE

Total polyphenols have been determined spectrophotometrically according to Folin-Ciocalteu procedure as mentioned previously by Lamien-Meda *et al.* (2008). The results were obtained from gallic acid calibrated curve and showed as mg of Gallic Acid Equivalents/100 mg of fractions. The estimation of total flavonoids have been done spectrophotometrically by the Dowd method as modified by Lamien-Meda *et al.* (2008). The content of flavonoids is expressed in mg rutin equivalent (RE) per g.

Estimation of Antioxidant Activity of AGTE

The DPPH free radical scavenging assay

The radical scavenging ability of green tea extract against DPPH has been determined spectrophotometrically as mentioned by Brand *et al.* (1995). Various concentrations from the extracted green tea have been prepared in methanol. The concentrations have been subjected to the inhibition rate of DPPH radical and the decrease in absorbance has been measured at $\lambda = 517$ nm. The activity of radical scavenging has been calculated by the following equation:

$$\% \text{ of radical scavenging activity} = (Abs_{control} - Abs_{sample} / Abs_{control}) \times 100,$$

Abs_{sample} and $Abs_{control}$ are samples and control absorbance values, respectively.

β -Carotene-linoleic acid assay

The antioxidant activities of AGTE have been measured spectrophotometrically as previously reported (Mothana, 2011). The antioxidant activity has been determined by measuring the inhibition rate of peroxidation in linoleic acid system and has been calculated by the following equation:

$$\% \text{ of antioxidant activity} = (Abs_0 - Abs_t) / (Abs_0 - Abs^*t) \times 100,$$

Abs_0 and Abs^*0 are the absorbance values measured at 0 time of incubation for sample and control, respectively. Abs_t and Abs^*t are sample and control absorbance values, respectively, at $t = 120$ min.

Total Antioxidant Capacity (TAC)

Plasma TAC has been measured by Colorimetric Assay Kit (BioVision Incorporated, CA, USA). The concentrations of antioxidant have been measured at 570 nm as a function of Trolox concentration according to the manufacturer's instructions equivalent.

$$Sa / Sv = \text{nmol} / \mu\text{l or mM Trolox equivalent},$$

Sa = sample amount (in nmol) which has been read from the standard curve.

Sv = the undiluted sample volume added to the wells.

Plasma NO free radical concentrations

NO concentration in plasma has been estimated as nitrate and nitrite using HPLC as previously reported (Tsuchiya *et al.*, 2002) at 540 nm wave length.

Experimental Design

Virgin wistar albino rats *Rattus Norvegicus* (30 females and 10 males), weighing 120 -150 g have been recruited in this study. The animals have been housed as single rat per cage under pathogen-free, healthy conditions and subjected to normal feeding, drinking, and health care mechanism according to the guidelines of the experimental animal care, college of science, King Saud University, Riyadh, Saudi Arabia. The experimental procedures were approved by the Ethics Committee of the Experimental Animal Care Society at King Saud University (Permit Number: PT 1012). Male and female rats have been maintained at 22-25 °C on a light/dark cycle (12:12 h). Mating has been done between pro-estrous females and males overnight by housing one female with one male in special cages used for mating (stainless steel wire cages). Vaginal plug deposition at morning determined the zero day of gestation (Allam *et al.*, 2011). AGTE has been orally administered daily to non-anesthetized treated groups by gastric intubation at a dose of 100 mg/kg/day and 200 mg/kg/day from day zero of gestation till postnatal day 30. Totally, 1 g/kg of LPS (*Escherichia coli* 055 B5; Difco, MI, USA) has been dissolved in 0.5 ml of sterile, pyrogen-free 0.9% sodium chloride and injected intraperitoneally to partially anesthetized LPS-treated female groups on three stages; at day 7 of gestation, postnatal day 1 and Postnatal day 15 (5 ml/kg every time).

The pregnant and lactating female rats have been labeled and divided into 4 groups as follows:

Control: female rats inoculated with normal saline and fed on normal diet plus a solution.

LPS: female rats injected with LPS.

100 mg/kg AGTE + LPS: female rats treated with AGTE (100 mg/kg/day) and injected with LPS.

200 /kg AGTE + LPS: female rats treated with AGTE (200 mg/kg/day) and injected with LPS.

Histological and biochemical analysis of the newborns livers

Six male pups from each group have been anesthetized by light ether and sacrificed by decapitation at postnatal day 30. Blood samples have been collected from pups at time of sacrifice. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities, bilirubin, and albumin have been determined in the serum according to methodology as previously described (Thapa and Anuj, 2007). Liver tissues have been immediately dissected and cut into small pieces of 3 mm³. Few of the parts have been fixed in 10% neutral buffered formalin for 24 hours and the others have been preserved under -80°C for further studies. Histological and pathological examinations of liver tissue samples have been performed according to routine methods as previously described in literature (Bancroft and Gamble, 2002).

Determination of single-stranded DNA (ssDNA) and DNA content in the new-born rat liver

DNA samples were extracted from liver tissues using diphenylamine organic solvent. The amount of DNA has been measured calorimetrically as shown previously in literature (Dische and Schwartz, 1937). ssDNA has been estimated as an early marker for liver cell apoptosis using immunoassay technique (ssDNA ELISA kit, Chemicon, Germany).

Determination of soluble fas antigen (sFas) as apoptotic marker

sFas (ng/ml) concentrations in plasma were determined using commercial quantitative enzyme immunoassays (Quantikine®, R&D Systems Inc., MN, USA) according to manufacturer's protocol.

Statistical analysis

The data of this study have been analyzed using SPSS version 17. All data have been tabulated as mean ± SD. The statistical correlation, among the parameters in treated and control groups, has been performed by Student's t-test. P < 0.05 considered to be statistically significant.

Results

Measurements of the total Phenolic and flavonoids in AGTE

The total phenolic and flavonoids constituents comprise 35.6 mg and 12.4 mg per 100g of AGTE extract, respectively (Table1). The active biological constituents of AGTE were further estimated using liquid chromatography. From Table 2, our data confirmed that catechin content is the highest (31.8 mg/g) followed by pyrogallol (21.8 mg/g), naringenin (11.9 mg/g), catechol (10.9 mg/g), epicatechin (10.5 mg/g), and small amounts of other phenolic compounds.

Table 1: Biological activities, total phenolic and flavonoids constituents of aqueous green tea extract (AGTE; mg/100 g).

Contents / biological activity	AGTE (100 mg)
Phenolic content (mg/100 g)	35.6
Flavonoids content (mg/100 g)	12.4
Radical scavenging activity (BCLA; %)	
At cons. of 500 µg/mL	88.3
At cons. 1000 µg/mL	97.6
Total antioxidant activity (DPPH; %)	92.62

Table 2: The phenolic compounds content (mg/g) of AGTE using liquid chromatography analysis (HPLC).

Component	Phenolic content (mg/100g)	Component	Phenolic content (mg /100g)
Gallic Acid	1.6	Ferulic	0.58
Pyrogallol	21.8	Iso-ferulic	0.29
Catechein	31.8	Alpha-coumaric	1.2
Chlorogenic	8.6	Benzoic	0.89
Catechol	10.9	3,4,5-methoxy-cinnamic	3.8
Epicatechein	10.5	Coumarin	1.9
Caffeine	2.7	Salycilic	1.9
p-OH benzoic	2.3	p- coumaric	0.16
Caffeic	6.9	Cinnamic	2.8
vanillic	8.4	Naringenin	11.9
tannin	3.8	synergic	2.1

Antioxidant activity and free radical scavenging of AGTE

The biological antioxidant activity of green tea was measured *in vitro* and calculated in relation to the inhibition of linoleic acid oxidation and DPPH radical scavenging activity. AGTE recorded free radical scavenging activity of 88.3% and 97.6% at concentrations of 500 and 1000 µg/mL respectively, while the same extract reported antioxidant activity with mean of 92.62% according to the β-carotene bleaching rate of green tea extract (Table 1).

Effects of AGTE on body and liver weights

No mortality was recorded in mothers and new-born rats during the experimental procedure. The litter size at birth was recorded as given in Table 3. Significant reductions in litter size and newborns body weights at day 0 and postnatal day 30 were observed in LPS treated group as compared to control group while this reduction was absent in AGTE+LPS treated group. At postnatal day 30, the newborn-liver weights exhibited a highly significant ($P < 0.01$) increase in LPS treated group compared to control group, while this increase was slightly attenuated in AGTE+LPS treated group (significance, $P < 0.05$ compared to control group). Compared to the LPS treated group, it is observed that the two doses of AGTE (100 and 200 mg/kg) were able to significantly ($P < 0.01$ compared to LPS treated group) restore the total body-weight and liver weight towards control weights.

Table 3: Effect of aqueous green tea extract (AGTE) on litter size, body and liver weights in the newborn-rats.

Group	Litter size at day 0(units?)	Body weights at day 0(g)	Body weights at day 30 (g)	Liver weight
Control	9.2±1.2	6.83±0.66	35.4±3.6	0.51±0.03
LPS	6.2±2.3*	4.89±0.80*	28.2±1.8*	0.88± 0.12**
100 mg/kg AGTE+LPS	7.3±2.2	5.98±0.82	31.6±2.8	0.76± 0.12*
200 mg/kg AGTE+LPS	8.2±1.3	6.01±.56	33.8±3.5	0.59± 0.18*

All values represent mean ± SD. * $P < 0.05$; ** $P < 0.01$ compared to control; Student's t-test.

Histological examination

The protective effect of AGTE against LPS toxicity was evaluated in the newborn rat-liver tissues using histological examinations as shown in Figure 1. Heamatoxylin and Eosin staining of liver sections revealed normal liver parenchyma, and glomeruli medulla control rats (Figure 1A), while LPS treatment showed congestion of blood vessels, focal replacement of the hepatic parenchyma, and liver cell apoptosis (Figure 1B). AGTE treatment at 100 mg/kg (Figure 1C) and 200 mg/kg (Figure 1D) showed regeneration and restoration of hepatic parenchyma; mild congestion and lower leukocytes; minimal coagulation of periportal necrosis and lower apoptosis of the hepatocytes. The data obtained on histological level were in agreement with the improvement in liver functional markers given below and significantly support the protective effect of AGTE against LPS-induced liver toxicity.

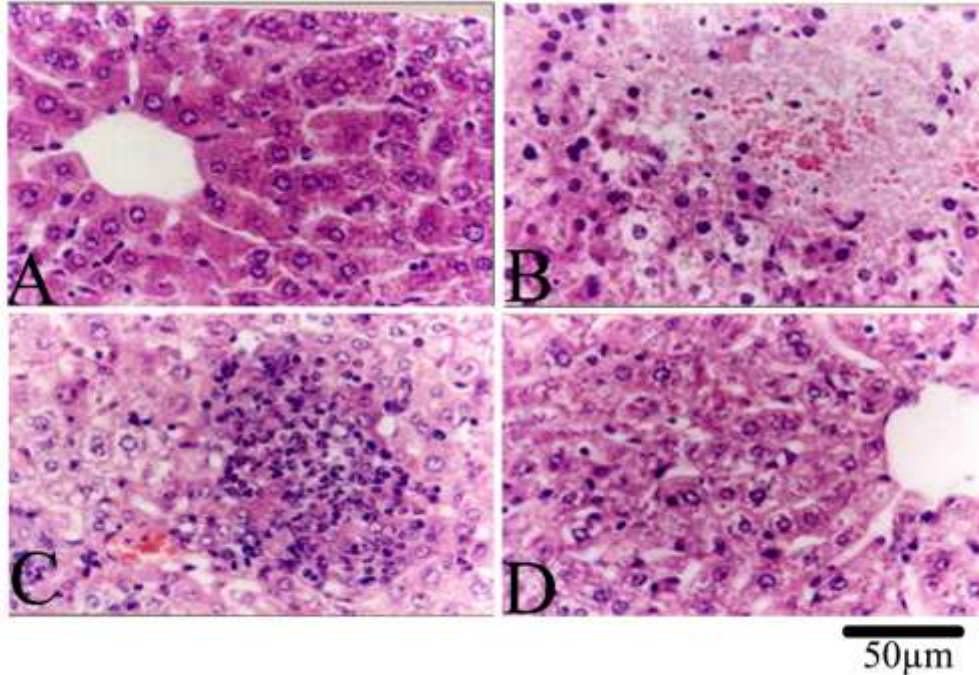


Figure 1: H and E stained sections of newborns liver (A) Show the liver of control newborns. (B) Show degeneration in the hepatocytes and apoptosis in the liver tissues of LPS treated group. (C) Show mild degeneration of the hepatocytes and aggregation of kupffer cells in 100 mg/kg AGTE+LPS treated group. (D) Show minimal focal lesion of the hepatic parenchyma with lower leukocytes in 200 mg/kg AGTE+LPS treated group.

Effect of AGTE on liver functions

LPS- toxicity resulted in significant increase in the levels of serum ALT (86.35 ± 11.6 vs. 22.5 ± 2.5 IU/l), AST (120.6 ± 9.3 vs. 31.7 ± 1.3 IU/l), Bilirubin (5.8 ± 2.6 vs. 0.46 ± 0.15 mg/dl), and decrease in Albumin (2.5 ± 0.12 vs. 4.2 ± 0.8 mg/dl) of LPS- treated group compared to control rats as shown in figure 2. AGTE treatment at 100 mg/kg recovered the levels of serum ALT (69.2 ± 5.8 vs. 86.35 ± 11.6 IU/l), AST (82.7 ± 4.7 vs. 120.6 ± 9.3 IU/l), Bilirubin (3.4 ± 0.56 vs. 5.8 ± 2.6 mg/dl) and Albumin (2.78 ± 0.6 vs. 2.5 ± 0.12 mg/dl) from LPS-toxicity to some extent while still significantly different from control group. AGTE treatment at 200 mg/kg altered the levels of serum ALT (42.7 ± 3.6 vs. 86.35 ± 11.6 IU/l), AST (38.5 ± 4.6 vs. 120.6 ± 9.3 IU/l), Bilirubin (2.85 ± 0.94 vs. 5.8 ± 2.6 mg/dl) and Albumin (3.91 ± 1.54 vs. 2.5 ± 0.12 mg/dl), thereby recovering the effect of LPS-toxicity to a greater extent but significantly different from control group as well (Fig. 2).

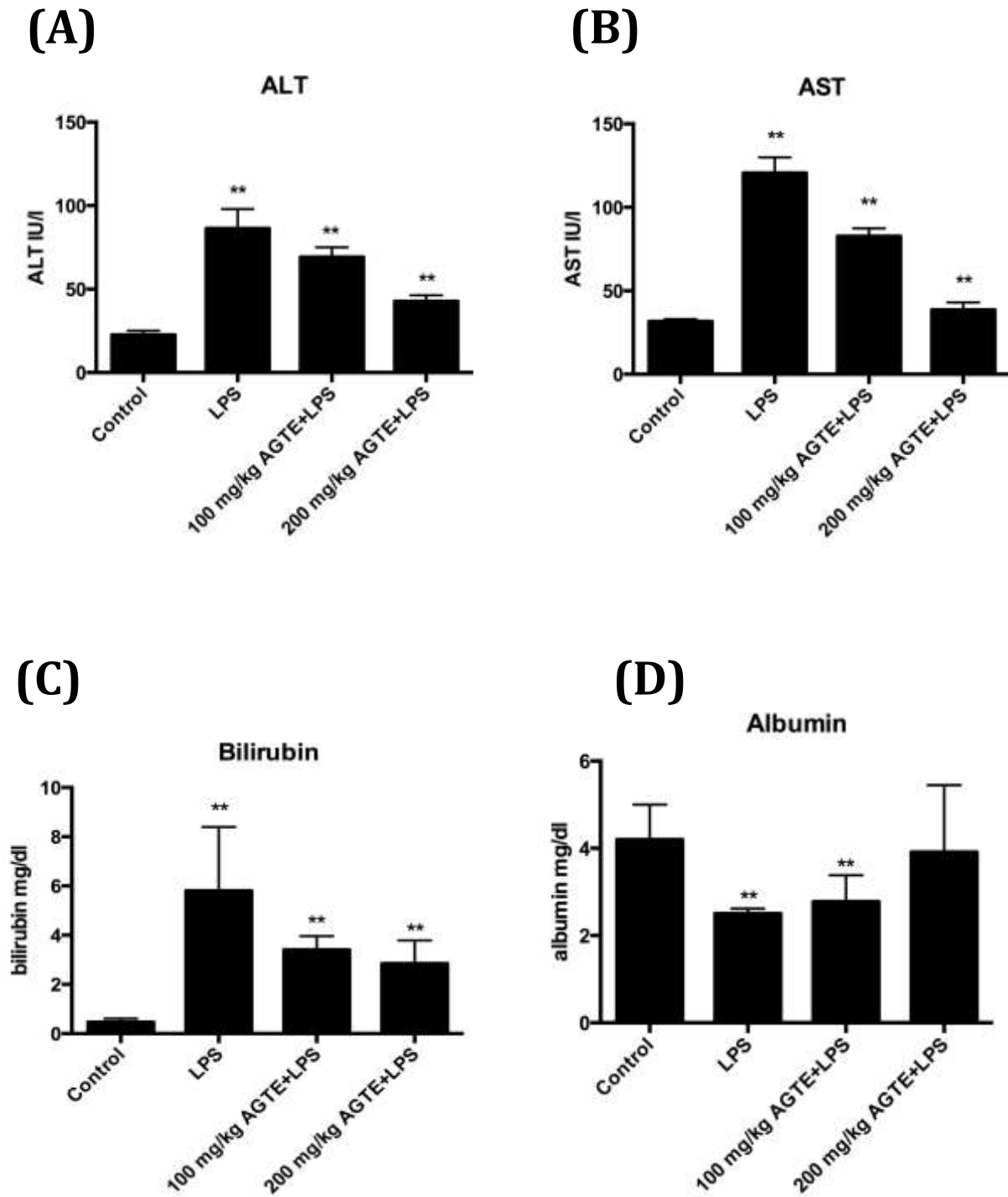


Figure 2: Effect of aqueous green tea extract (AGTE) on the levels of liver Function biomarkers in the experimental rat's newborns. All values represent mean \pm SD. * $P < 0.05$; ** $P < 0.01$ compared to control; Student's t-test.

Effect of AGTE on oxidative stress markers and DNA content

The oxidative free radical stimulation by LPS induced liver toxicity was indicated through measurement of NO and TAC, that are conversely related markers of oxidative-antioxidant status respectively. LPS treatment significantly increased the plasma NO content compared to control rats (36.5 ± 5.2 vs. 11.3 ± 3.4 nmol/dl) while daily supplementation of 100 mg/kg and 200 mg/kg AGTE recovered the NO levels to 16.2 ± 3.1 and 9.4 ± 1.5 nmol/dl (Fig. 3A), thereby bringing about a significant recovery in the oxidative damage caused by LPS. Similarly, when compared to control rats LPS treatment significantly reduced the antioxidant status by bringing down the TAC levels from $12.4 \pm$

3.6 to 4.2 ± 2.5 nmol/mM Trolox eq., which was recovered by daily intake of 100 mg/kg (8.7 ± 3.5 nmol/mM Trolox eq.) and 200 mg/kg (12.9 ± 4.2 nmol/mM Trolox eq.) as shown in figure 3B.

Additionally, DNA content of liver cells was estimated as molecular marker of oxidative liver tissue damage induced by LPS-toxicity on molecular level. Significant decrease in DNA content was observed in LPS-treated rats compared to control group (0.31 ± 0.18 vs. 0.82 ± 0.15 $\mu\text{g}/106$ cells). Supplementation of AGTE caused significant improvement of liver tissue cells by increasing DNA content as compared with intoxicated group (0.56 ± 0.16 $\mu\text{g}/106$ cells at 100 mg/kg and 0.78 ± 0.23 $\mu\text{g}/106$ cells at 200 mg/kg of AGTE) (Fig. 3C). The improvement of DNA content on molecular level confirms the preventive action of green tea against LPS-liver toxicity.

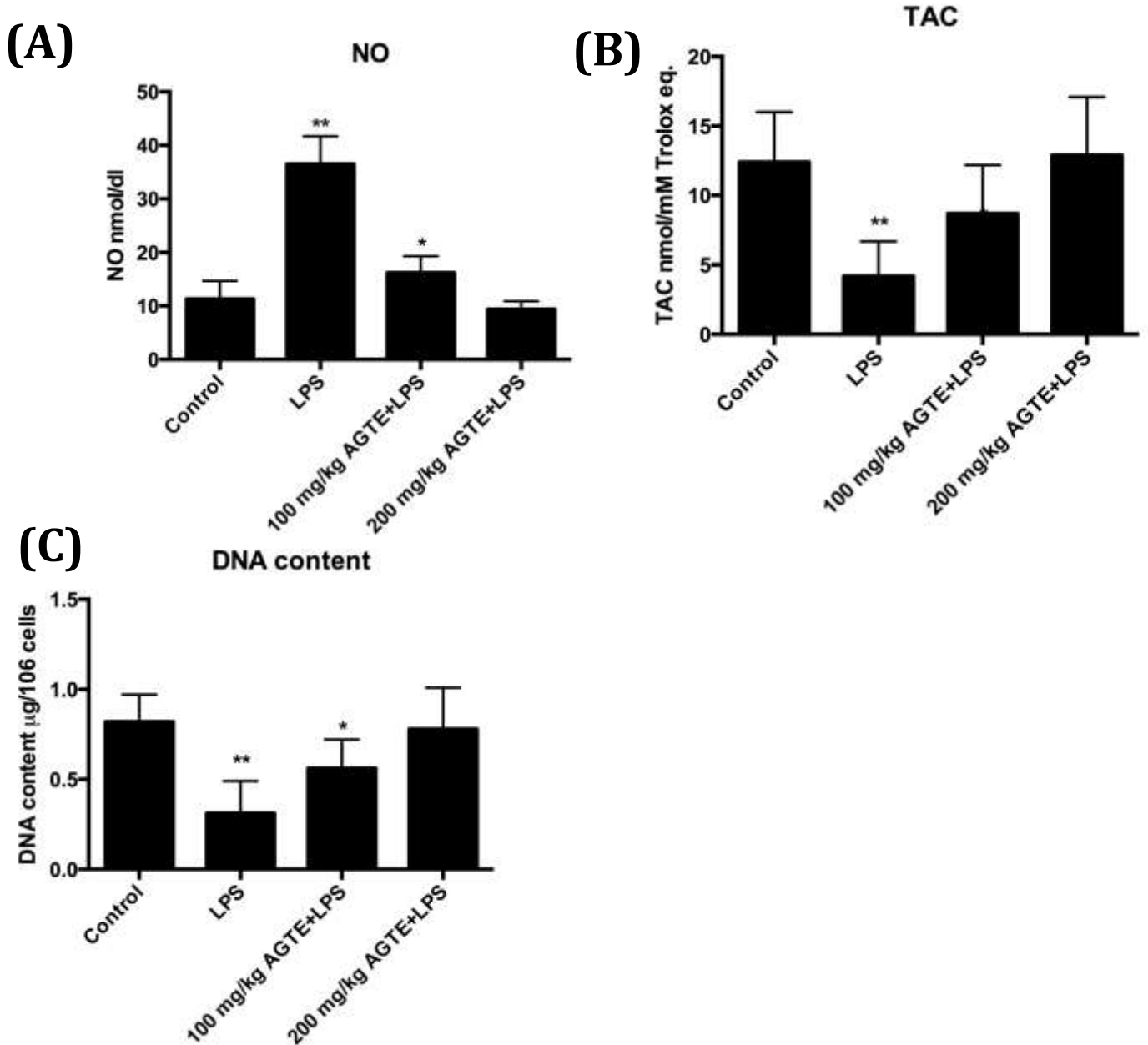


Figure 3 Effect of AGTE on liver lipid peroxidation (A), Total antioxidant capacity (B), and DNA content (C) as genotoxic marker in the experimental rat's newborns. All values represent mean \pm SD. * $P < 0.05$; ** $P < 0.01$; compared to control. Student's t-test. NO: nitric oxide; TAC: total antioxidant capacity; DNA: deoxyribonucleic acid.

Effect of AGTE on sFas and ss DNA concentrations in liver tissues.

sFas and ssDNA were estimated as apoptotic markers in plasma and liver tissue samples with varying fibrotic scores of LPS-toxicated rats. Rats treated with AGTE at 100 and 200 mg/kg brought about significant decrease in the level of Fas antigen and increase in ssDNA concentrations in all fibrotic stages (scores: 0-1, $P < 0.01$; 2-3, $P < 0.001$) as

compared with LPS intoxicated group as shown in figure 4 and the recovery was directly correlated with the increasing fibrotic score. The results thereby indicate the antiapoptotic activity of AGTE and its significant association with the score of liver fibrogenesis.

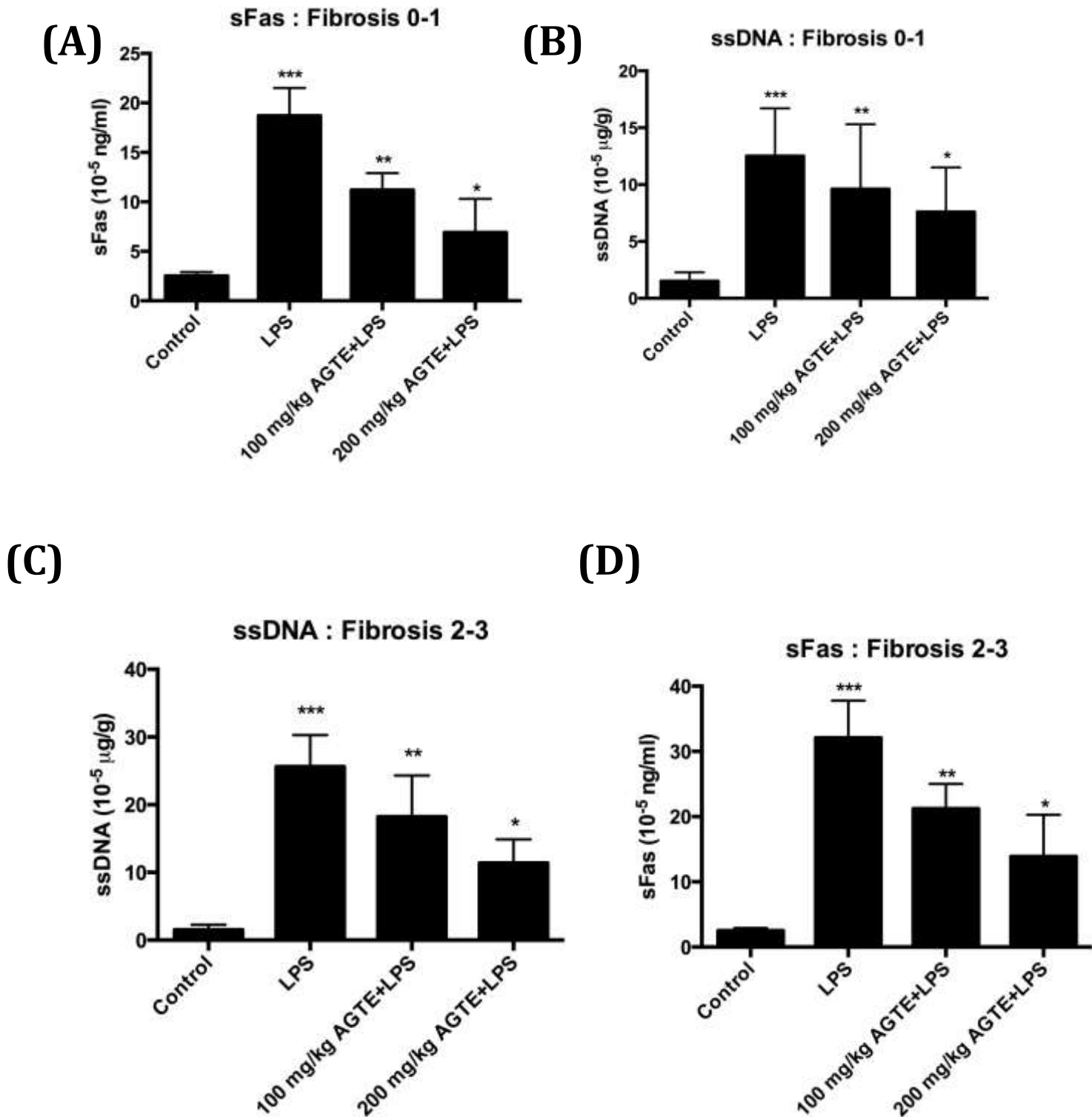


Figure 4 Effect of green tea extract (AGTE) on single-stranded DNA (ssDNA) (A,B) and of soluble fas antigen (sFas) (C,D) as markers of liver cell apoptosis induced by LPS –toxicity in experimental rats newborns. All values represent mean ± SD. * P < 0.05; **P < 0.01; *** P < 0.001 compared to control. Student’s t-test.

Discussion

The present study provides evidence that dietary supplementation of AGTE to female, during gestation and lactation period, protects against LPS-induced hepatotoxicity in newborn rats. An i.p. injection of LPS to pregnant female rats at gestation day 7 was sufficient to induce hepatotoxicity in new-born rats and AGTE, supplemented daily at 100 mg/kg and 200 mg/kg, clearly ameliorated the damaging effects of LPS in liver. Increases in liver weights and reduction in total body weight due to LPS stimulation was significantly recovered by AGTE intake. Restoration of liver function by AGTE, as seen by the correction of ALT, AST, Bilirubin and Albumin serum levels to normalcy substantiates the above finding. Additionally, AGTE supplementation also significantly attenuated oxidative damage in liver tissue and recovered the hepatic anti-oxidant levels. This was further confirmed by histopathological analysis of liver tissue. Most importantly our results showed that AGTE extract could attenuate increasing levels of LPS induced-apoptotic liver cells induced by LPS at increasing fibrotic scores. Based on our results we further propose that the protective effect of AGTE in hepatic fibrosis stems from its anti-oxidative and anti-apoptotic properties.

To the best of our knowledge, this study is the first to examine the protective effect of AGTE on hepato-toxicity induced by LPS during pregnancy and lactation in newborn rats. However, the mechanistic insights underlying the protective effect of AGTE against hepatotoxicity are yet to be clearly defined. Based on the evidences available from our current work and work from other groups, several possibilities could be drawn. Strong evidences exists that food products of plant origin, specifically tea, contains high amounts of phenolic and polyphenolic constituents that were reported to reduce the susceptibility to certain human diseases including cancer (He *et al.*, 2001). In the present study, high levels of polyphenolic (35.6 mg) and flavonoid (12.4 mg) were found in 100 g of AGTE. The detailed polyphenol content of AGTE, measured using liquid chromatography, revealed that catechin was the highest amount (31.8 mg/g) followed by pyrogallol (21.8 mg/g), naringenin (11.9 mg/g), catechol (10.9 mg/g), and epicatechin (10.5 mg/g). This was in addition to small amounts of other phenolic compounds such as vanillic, synergic, salicylic, benzoic, ferulic, coumarin, and cinnamic. The results were different from previously reported studies showing that catechins comprise 30 to 36% of AGTE dry weight, whereas epigallocatechin-3-gallate (EGCG) constitutes up to 63% of the extract (Roomi *et al.*, 2016). However, it is also reported that polyphenol content varies according to brewing time, type of tea, commercial brand and producing country (Nash and Ward, 2016) and this could explain the different polyphenolic content of AGTE in our study.

In vivo supplementation of AGTE to female rats during gestation recovered the LPS induced hepatic injury in new-born by recovering the reductions on total body weight and increase in liver weight. Several earlier reports have shown that LPS induced hepatic damage leads to subsequent increases in the level of serum aminotransferases (Amat *et al.*, 2010). Consistently, LPS resulted in an elevation in all circulating markers of hepatocyte injury such as serum ALT, AST, bilirubin, and reduction in albumin level and AGTE intake corrected these levels to normalcy. The presence of flavonoids and polyphenols in green tea could be the causal reason for protective effect of AGTE observed in our study. These constituents have been shown to have the ability to stabilize and preserve the integrity of the hepatocyte membrane, stimulating hepatocyte regeneration, and hepatocellular protein synthesis, to repair damaged hepatic tissues (Safer *et al.*, 2015).

The protective effect of tea has been linked with the presence of polyphenolic contents such as catechins and tannic acid (Tomaszewska *et al.*, 2015) and these compounds were shown to have a considerable antioxidant capacity against oxidative free radical (Gramza *et al.*, 2005). We therefore estimated the free radical scavenging activity of AGTE by both DPPH (92.62%) and β -carotene (88.3% and 97.6% at 500 and 1000 μ g/mL respectively) tests. The obtained results suggested that the phenolic constituents of AGTE have the capability to suppress the chain reactions of lipid peroxidation via free radical scavenging mechanism. These results were in accordance with other authors, who reported free radicals scavenging ability of AGTE (Panat *et al.*, 2016). Consistent with the *in vitro* observations, AGTE was able to reverse *in vivo* lipid peroxidation by LPS in liver tissue.

Previous studies reported the presence of lipid peroxidation in many tissues such as liver, heart or brain of rats exposed to LPS-endotoxicity as an indicator of free radical oxidative damage (Baranova *et al.*, 2016). The impairment of liver tissues has been shown to be due to oxidative stress caused by LPS via initiation of highly reactive free radicals (Poli, 2000). Most studies reported that the hepatic and systemic toxicities of LPS is attributed to the release of chemical mediators such as superoxide, NO and proinflammatory cytokines which ultimately induce liver cell damage (Ojiako *et al.*, 2015). In this study, LPS treated rats showed significant increase in NO and decrease in TAC indicating lipid peroxidation damage of liver tissue. Under LPS toxicity NO free radical was initiated and secreted from activated Kupffer cells and reacts with $O_2^{\cdot-}$ to form peroxynitrite which is a potent cytotoxic, oxidative agent that can elicit lipid peroxidation (Poli, 2000). The presence of NO as a free radical molecule in higher concentrations plays an essential part in hepatocellular necrosis following LPS administration (Shuto *et al.*, 2004). The increase in oxidative free radicals produce redox imbalance, change in antioxidant enzymatic proteins and lipids of biological membranes, and induction of lipid peroxidation which in turn produce tissue cell and DNA damage (Baranova *et al.*, 2016). Significant decreases in DNA content were also observed in our study, as a molecular marker of oxidative liver tissue damage, in LPS-treated rats compared to control group. Administration of AGTE showed significant improvement in TAC and level of DNA of liver tissues together with depletion of NO level compared to LPS-toxicated rats. This improvement of damaged hepatic cells might be due to anti-radical scavenging activity of green tea constituents against harmful oxidative free radicals (Safer *et al.*, 2015). Some polyphenol-rich plant extracts have been reported to ameliorate LPS-

induced hepatic injury (Amat *et al.*, 2010). Green tea extracts have been shown to have a positive effect on hepatocytes via antiradical activity against oxidative stress radicals initiated from LPS toxicity or toxic metals. Previous reports have shown that age-related diseases are associated with oxidation-induced damage to protein, lipid and DNA and that the antioxidant activities of plant-rich diets lower the risk of age-related diseases via reduction in oxidation-induced damage to protein, lipid and DNA. Green tea was reported as one of the most popular tea containing higher amounts of polyphenolic antioxidants with many reputed health benefits (Bitu Pinto *et al.*, 2015).

Significant increase in liver apoptosis have been reported in rats treated with LPS and other injurious agents (He *et al.*, 2001). In this study too, liver cell apoptosis was significantly augmented in LPS-treated rats as evidenced by the levels of sFas antigen and ssDNA in plasma and liver tissue samples. There was a significant increase in the level of sFas antigen and decrease in ssDNA in LPS-toxicated rats with varying fibrotic scores. Liver apoptosis has been reported to occur in LPS-treated animals via cytokines extrinsic signaling pathways (Baranova *et al.*, 2016). It was further reported that LPS induced liver damage through both initiation of pro-inflammatory cytokines and liver apoptosis (Zhong *et al.*, 2006). Our finding that LPS regulated pro-apoptotic markers (sFas and ssDNA) to a significantly increasing extent in increasing liver fibrotic scores, were in accordance with other similar reports (Baranova *et al.*, 2016). Importantly, our results showed that administration of AGTE showed significant anti-apoptotic activity in correlation with score of liver fibrogenesis induced by LPS. Recent evidences have shown that AGTE diminishes hepatic fibrosis induced by carbon tetrachloride and ethanol (Safer *et al.*, 2015). The mechanisms behind this therapeutic effect of AGTE have not been clearly studied yet. From our study, we further propose that the anti-fibrosis activity of AGTE could originate from its antioxidant and anti-apoptotic functional aspects.

Previous research works on human, animal, and cell culture reported that regular intake of tea correlated positively with health improvement (Kager *et al.*, 2009). The preserved activity of green tea depends mainly on antioxidant capacity of its total polyphenolic content and the antioxidant content of human plasma has been shown to increase significantly within 30 minutes following ingestion of single dose of green tea (He *et al.*, 2001). In previous study, it was reported that tea has much stronger antioxidant property than vitamins E or C due to the presence of catechins in large amounts (Lee *et al.*, 2002). These properties provided green tea to be promising chemopreventive agent against toxic chemicals and carcinogens.

The mechanism(s) of protection by AGTE could be due to direct antioxidant effects of absorbed polyphenols of AGTE, inducing up-regulation of endogenous cellular defenses and increased DNA repair. Previously reported *In vitro* finding supported that EGCG, the polyphenolic compound of green tea, has various downstream cytoprotective effects and can induce significant redox-sensitive antioxidant response (Nash and Ward, 2016). The protective activity is modulated with increase in cellular antioxidant mediators such as haeme oxygenase-1, glutathione, and antioxidant and DNA repair enzymes (Soares and Bach, 2009), which decrease basal DNA damage and ultimately cytoprotective pro-oxidant change. Also, it was reported that green tea polyphenols provide significant cytoprotection against most hepatotoxicants via antiapoptotic mechanism (He *et al.*, 2001).

Hepatotoxicants and biological agents including viral hepatitis and bilharzia infestations were shown to be the major causes of acute and chronic liver pathology worldwide (Gabr *et al.*, 2014). Although, many drugs of chemotherapeutic origin were applied in clinical trials for the treatment of liver damage, significant difficulties still existed to achieve satisfactory therapeutic effects. Therefore, the use of traditional medicine of plant origin has a very important significance in both theoretical and clinical trials to prevent liver damage (He *et al.*, 2001). Tea was reported as naturally healthy source of polyphenols that could be used as medicine for human health specifically for its antiallergic action and antimicrobial properties, anticancer, psoriasis, and rheumatoid arthritis (Gabr *et al.*, 2014; Ide *et al.*, 2016). Green tea was reported as one of the most popular tea containing higher amounts of polyphenolic antioxidants with many reputed health benefits (Bitu Pinto *et al.*, 2015).

In conclusion, the data from the current study revealed that green tea can reverse the fibrotic damage in liver tissue caused by LPS intoxication through inhibition of lipid per oxidation, improvement of DNA content, and suppression of sFas and ssDNA apoptotic related markers. We further put forward that the anti-free radical and anti-apoptotic properties of green tea active constituents are the main mechanistic source of the therapeutic effect of green tea as an anti-fibrotic agent.

Declarations

A. Ethics approval and consent to participate

All the experimental protocols and investigations were approved and complied with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at King Saud University (Permit Number: PT 1010).

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