Original Article

Green tea modulates reserpine toxicity in animal models

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ABSTRACT — Reserpine, a natural product extracted from Rauwolfia serpentina or Rauwolfia vomitoria, is a known dopamine depleter that inhibits several neurotransmitters. Reserpine has been used clinically to control hypertension, schizophrenia, insomnia and insanity. The use of this drug, however, has been limited because of its side effects which include oxidative damage to organs, including the liver. Green tea catechins are potent antioxidants that have the potential to counteract reserpine induced oxidative stress. This study investigated the merits of administering green tea concurrently with reserpine to prevent oxidative hepatic damage in Sprague-Dawely (SD) rats. Reserpine was found to cause hepatic damage, with elevated levels of oxidative stress markers, such as Thiobarbituric Acid Reactive Substances (TBARS), transaminases and cholesterol. Reserpine also induced hepatic ultra-structural damage in the cytoplasmic membrane, nuclear envelope, endoplasmic reticulum (rER), ribosomal stripping and mitochondria. Electron microscopy examination showed revival of liver cells as a result of green tea extract administration to experimental rats.

Key words: Green tea, Antioxidant, Oxidative stress, Reserpine, Hepatic damage

INTRODUCTION

Camellia sinensis is an evergreen shrub (Brown, 1999) cultivated in many parts of the world. Green tea made from the leaves of Camellia sinensis has been used as a medicinal beverage in Asia for almost 4,000 years (Balentine, 1997). It is only recently that the world has realized the full potential of green tea as a medicinal herb. Green tea contains a number of antioxidant molecules that can act to scavenge destructive free radicals in the body (Yoko and Santosh, 2005).

Polyphenols account for 30-40% of the green tea dry weight (Thiagarajan et al., 2001). Tea polyphenols are known to have high affinity for metals, alkaloids and biological macromolecules such as lipids, carbohydrates, proteins and nucleic acids (Yang and Landau, 2000). Most of the polyphenols present in green tea are flavonoids, flavanols, flavandiols and phenolic acids (Brown, 1999). (-) Epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC) and (-) epigallocatechin gallate (EGCG) are the main catechins that can be found in green tea (Brown, 1999) See Fig. 1. Catechins have diverse activities and are known to reduce the mobility of reactive oxygen species (ROS) in lipid bilayer (Luper, 1999). Reports have shown that catechin hydrophobic fragments can penetrate lipid bilayers influencing antioxidant capabilities in bio-membranes (Yang and Landau, 2000). Catechins can enter the hydrophobic core of the membrane where they exert a membrane-stabilizing effect by modifying the lipid packing order (Arora et al., 2000). Catechins also maintain intracellular protein thiol levels that maintain the intracellular oxidation-reduction balance (Luper, 1999).

Reserpine is a potent, naturally-occurring alkaloid, derived from roots of several members of Rauwolfia genus (Doyle et al., 1955) see Fig. 2. Reserpine has the capability to deplete biogenic amines such as noradrenalin, dopamine and serotonin and is a powerful oxidant (Metzger et al., 2002). Clinically reserpine is an important antihypertensive drug, but its use is limited by its side-effect including organ damaging oxidative stress.

MATERIALS AND METHODS

Materials

Reserpine and thiobarbituric acid were obtained from...
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**Fig. 1.** Source of reserpine and its chemical structure

**Fig. 2.** Structure of EGCG (main ingredient in green tea extract)

Sigma-Aldrich, Inc. (St Louis, MO, USA). Green tea was purchased from the local market. Glutamic-oxaloacetic transaminase (GOT), Glutamic-pyruvic transaminase (GPT) and Cholesterol kits were obtained from Randox (Crumlin, UK). All solvents and other chemicals were obtained from Fluka (Buchs, Switzerland) and were of analytical grade.

**Methods**

**Green tea aqueous extract preparation**

Dried green tea leaves (100 g) were powdered in a Waring blender and extracted with double-distilled water (1 l) at 35°C for one hour by stirring on a magnetic stirrer plate. The extract was filtered through a nylon filter, and the filtrate was centrifuged at 3,000 rpm for 15 min. The clear supernatant was taken and the residual pellet shaken with distilled water, warmed at 35°C, and centrifuged again. The clear supernatants were pooled and lyophilized to give water soluble material (7.30 g) that was stored in a screw-cap bottle at −20°C.

**Animals**

Sixty male Sprague-Dawely (SD) rats (160-260 g body weight (BW)) were obtained and housed in the animal resources center, Health Sciences Centre, University of Kuwait. The rats were kept in plastic cages in a controlled environment of 40% humidity at 22°C with 12-hr light/
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dark period, and were treated gently. The rats were randomly distributed into six groups, each with ten animals. All rats had free access to tap water ad libitum, and pelleted rodent’s chow (SDS, UK). The composition of the diet was 14.7% protein, 2.6% fat and 5.3% cellulose.

Experimental design

The experiment was carried out over a 48-day period. The lyophilized tea extract was re-dissolved in double distilled water and fed to the animals by gavage. Reserpine solution was made in 0.8% glacial acetic acid and administered by intraperitoneal injections.

Group (G1): Animals received standard diet and free access to water for 48 days. This group of animals was treated as control.

Group (G2): Animals received a daily intraperitoneal dose of 100 μg/kg BW reserpine solution for 48 days, with free access to diet and water.

Group (G3): Animals were given a daily dose of 50 mg/kg BW, green tea extract for 48 days.

Group (G4): Animals were treated with a daily dose of reserpine and green tea extract simultaneously with the same dose for groups 2 and 3 animals for 48 days.

Group (G5): Animals received green tea extract for 24 days, such as group 3, followed by reserpine treatment for 24 days for group 2 animals.

Group (G6): Animals were given reserpine for 24 days, such as group 2 animals, followed by green tea extract for 24 days for group 3 animals.

There was no animal mortality during the experimental period.

On day 49, rats were anaesthetized with 0.6 ml/100 g BW of 25% urethane via intraperitoneal injection and sacrificed. Blood samples were collected in plane tubes through a cardiac puncture, and centrifuged at 3,000 rpm for 10 min. The resultant serum was collected and stored at −80°C until used. Liver specimens were divided into two parts, the first part kept in aluminum foil stored at −80°C, for use in biochemical analyses, and the second part used for electron microscopy.

Tissue preparation for transmission electron microscopy (TEM)

A tissue processor was used for fixation in 3% glutaraldehyde/phosphate buffer pH 7.2 dehydrated in degraded series of ethanol and infiltrated in propylene oxide. Each specimen was embedded in a mould (capsule/plate) filled with epon resin and kept in an oven at 70°C for 3 days. After complete polymerization, samples were ready for ultra-microtomy. Semi-thin sections (1.0 μm) were cut using an LKB ultra-microtome (Leica microsystems, Wetzlar, Germany) and stained with toluidine blue for light microscopic survey. Thereafter, ultrathin sections were double-stained with uranyl acetate/lead citrate and were examined under a Jeol 1200 EXII electron microscope (JEOL Ltd., Tokyo, Japan), operated at 80 kV.

Biochemical analyses

Thiobarbituric Acid Reactive Substances (TBARS) were measured in serum samples as malonaldehyde (MDA). Tetramethoxypropane was used to generate MDA at different concentrations and a standard curve was prepared. Sample results were expressed as nM/ml MDA. GPT, GOT and serum cholesterol were measured using commercially available Randox kits.

Statistical analyses: Statistical analysis was performed by one-way ANOVA and comparing the mean ± S.D. from each experimental group with its respective control group. Statistical significance was defined as P < 0.05.

RESULTS

Biochemical analysis

Measurement of serum TBARS in animals treated with reserpine showed 64.55 nM/ml of MDA indicating acute oxidative damage. Serum TBARS levels were reduced in groups feed green tea extract. The lowest levels of TBARS for groups receiving reserpine were observed in group 4 animals where reserpine and green tea extract were given simultaneously (Fig. 3).

Serum Glutamic-pyruvic transaminase (SGPT) and Serum Glutamic-oxaloacetic transaminase (SGOT) levels were significantly elevated in G2, G4, G5 and G6, indicating hepatic damage through reserpine-induced oxidative stress. A comparative study of these enzymes in G4, G5 and G6 animals, with G2 animals, showed a significant decrease in SGOT when animals were treated with green tea extract. However, SGPT decreased significantly in group 4 animals as compared to G2 animals (Fig. 4).

Hepatic low density lipoprotein (LDL) cholesterol level increased in response to reserpine. However, there was little effect on the serum cholesterol level (Fig. 5).

Ultra-structural study TEM

The livers of the control group animals showed normal cell size, shape and cell-to-cell spacing. The nucleus, endoplasmic reticulum, and mitochondria of the hepatocytes all appeared normal and healthy. The cell distribution, size, shape and ultra-structure also appeared normal, along with normal plasma membrane (Figs. 6G1, 7G1 and 8G1). Samples from G2 showed an effect of reserpine on the rat liver cells after treatment for 48 days.
These cells demonstrated clear injury, with abnormal cytoplasm showing moth-eaten phenomena, large empty spaces and damaged organelles forming patchy structures that were present in all cells. The nuclei appeared abnormal, with chromatin material mislaid and condensed in areas and apparent damage to the nucleoli, nuclear envelope and nuclear fenestrae. The endoplasmic reticulum appeared fragmented, with intermingled patches scattered in cytoplasm in an irregular manner. Additionally, there appeared to be glycogen deficiencies in cytoplasm. The cell membranes showed defined spacing, with increased fibrous structures, resulting in a thickened, wider space. Mitochondria lacked cristae and outer mitochondrial membrane appeared dissolved and damaged (Figs. 6G2, 7G2 and 8G2).

Liver cells taken from G3 appeared normal and similar to the control group (G1). The nucleus, nucleolus and nuclear envelope all appeared near normal, including cell-to-cell spacing. The distribution, size, shape, mitochondrial ultra-structure and rough endoplasmic reticulum were also close to normal (Figs. 6G3, 7G3 and 8G3). Liver cells from G4 showed the combined effect of intoxication with reserpine and damage repair with green tea treatment for 48 days. The cells appeared to be nearly normal, with little moth-eaten appearance in the cytoplasm. Nucleus, mitochondria, and rough endoplasmic reticulum were all typical in morphological appearance and distribution (Figs. 6G4, 7G4 and 8G4).
In group G5, liver cells showed recovery in liver damage. Here, the cytoplasm of the hepatocytes showed no shrinkage, or moth-eaten pattern. Rough endoplasmic reticulum looked normal in its distribution in some cells, while in others it appeared gauche, with some moth-eaten appearance. Mitochondria were electron-opaque in appearance. The overall magnitude of damage in this group was observed to be relatively more than that observed in G4 and G6 animals (Figs. 6G5, 7G5 and 8G5).

Liver cells in Group G6 animals showed recuperation in liver damage. The distribution of organelles and ultra-structure appeared healthier. Additionally, the size and shape of mitochondria appeared normal. Rough endoplasmic reticulum, nuclear structures and cell-to-cell spacing were all close to normal (Figs. 6G6, 7G6 and 8G6).

**DISCUSSION**

Several studies with green tea have demonstrated a causal relationship between green tea exposure and recovery of liver damage caused by oxidants other than reserpine (De Oliveira *et al.*, 2006; Singal *et al.*, 2006; Yamamoto *et al.*, 2006). Results obtained in the present study report for the first time that green tea extract is a potent antioxidant against oxidative damage caused by reserpine.

**TBARS**

TBARS were significantly elevated in reserpine-treated rats in G2 (364% in serum and 236% in liver) as compared to the control G1 rats. These results were in agreement with the previously reported studies by Burger *et al.* (2004), and Naidu *et al.* (2006). These results suggested that ROS formed by reserpine administration could result in lipid peroxidation leading to reserpine cytotoxicity. Green tea extract when simultaneously administered with reserpine (G4), significantly lowered TBARS levels in the serum (120%) and liver (53%). The potent antioxidant activity of green tea extract was consistent with many reports published in recent literature (Yamamoto *et al.*, 2006). Green tea suppresses ROS formation through its polyphenolic contents such as catechins imparting a strong antioxidant activity. Green tea catechins also reduce the oxidative capacity of cellular membranes by inserting their hydrophobic fragments into the lipid bilayers (Chen *et al.*, 2002). This helps the hydrophobic core of membrane through modification of the lipid packing order resulting in membrane stabilization (Arora *et al.*, 2000). In addition, catechin polar groups could interact with phospholipid polar head groups influencing the membrane fluidity of the phospholipid bilayer (Chen *et al.*, 2002).

Rats receiving green tea extract followed by reserpine as a post-dose (G5), showed lower levels of TBARS as compared to rats receiving reserpine alone. Green tea significantly decreased TBARS in the liver and serum. Administration of green tea extract before reserpine treatment offered little protection and failed to prevent hepatic damage. The green tea extract was more effective at combating hepatic damage when administered concurrently or after reserpine. This was evident from the experiments (G6) where oxidative damage was induced in rats by reserpine and then green tea extract was administered to counteract the oxidative damage. In this case, green tea extract had the capacity to decrease 40% of TBARS val-
Fig. 6. Group 1: Control liver with normal size cells, shape, and cell-to-cell spacing. The nucleus (N), endoplasmic reticulum (rER) and mitochondria (M) appear normal and healthy in distribution, with normal size, shape and ultrastructure. The plasma membrane (arrows) appear normal with a narrow slit. Scale bar = 4 μm

Group 2: The cytoplasm is abnormal, and shows moth-eaten phenomena (stars), and damaged organelles forming a erratic structure in all the cells. The nucleus (N) appear abnormal, with mislaid chromatin material. There are no glycogen granules in cytoplasm, and cell membranes show well-defined spacing, with more fibrous structures, giving it a thickened and wider space (arrows). Scale bar = 4 μm

Group 3: Cells appear normal, as in control (Group 1). The nucleus (N) and nuclear envelope are all normal, even mitochondria (M) and endoplasmic reticulum (rER) are normal, with typical cell-to-cell spacing (arrows). Scale bar = 4 μm

Group 4: Cells appear normal, with hardly any moth-eaten appearance in cytoplasm. The nucleus (N), mitochondria (M), and endoplasmic reticulum (rER), all show normal morphological appearance and distribution. Scale bar = 4 μm

Group 5: The cytoplasm shows no reduction, nor moth-eaten pattern, and endoplasmic reticulum (rER) looks normal in its distribution and ultrastructure. However, some rER, mitochondria (M), nucleus (N), and cytoplasm reveal damage. Cell membranes show well-defined spacing, with more fibrous structures, giving it a thickened and wider space (arrows). Overall, the level of damage in this group is relatively more than in group 4. Scale bar = 4 μm

Group 6: The distribution of endoplasmic reticulum (rER) and mitochondria (M) is near normal, with reduced cell-to-cell spacing (arrows) as observed in normal cells (Group 1). Scale bar = 4 μm
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Fig. 7. **Group 1:** Control liver shows part of the nucleus (N), with normal mitochondria (M) and other cell components. Scale bar = 400 nm

**Group 2:** Cells show part of the nucleus (N), cell cytoplasm, with empty spaces, mitochondria (M) appeared without cristae, and outer membrane dissolved and damaged, while endoplasmic reticulum (rER) is dissolved and show condensed patches. Scale bar = 400 nm

**Group 3:** Cells show normal cells, as observed in control group of animals. The distribution of the organelles is similar to that observed for control (Group 1), and endoplasmic reticulum (rER) in particular was of normal shape. Scale bar = 400 nm

**Group 4:** Cells appear nearly normal, with little moth-eaten appearance in the cytoplasm, the nucleus (N) and mitochondria (M) appear normal, and endoplasmic reticulum (rER) distribution is normal. Scale bar = 400 nm

**Group 5:** Cells show no contraction, or moth-eaten pattern. Mitochondria (M), nucleus (N), and endoplasmic reticulum (rER) appear healthy, while rER are fragmented. The overall magnitude of damage in this group is relatively more than that observed in group 4 animals. Scale bar = 400 nm

**Group 6:** These cells show distribution, size and shape of mitochondria (M), endoplasmic reticulum (rER) and nucleus (N) close to normal, with some moth-eaten phenomena. Scale bar = 400 nm
Fig. 8. **Group 1:** Control cells show a magnified portion, the endoplasmic reticulum (rER) and mitochondria (M), their distribution, size, shape and ultrastructure, all appear normal and healthy. Scale bar = 400 nm

**Group 2:** Cells show a magnified portion of cytoplasm, which appear abnormal, with moth-eaten phenomenon (stars) and damaged organelles, forming inept structure. Mitochondria (M) are visible with less pronounced cristae. The endoplasmic reticulum (rER) lack normal pattern and appear fragmented and scattered, with some intermingled patches irregularly scattered in cytoplasm. No glycogen granules are visible in the cytoplasm. Scale bar = 400 nm

**Group 3:** It shows a magnified portion of cell cytoplasm. The endoplasmic reticulum (rER) and mitochondria (M), their distribution, size, shape and ultrastructure, all appear nearly normal. Scale bar = 400 nm

**Group 4:** It shows a magnified portion of cell cytoplasm, with some moth-eaten appearance (stars). Scale bar = 400 nm

**Group 5:** It shows a magnified portion of cell cytoplasm. The endoplasmic reticulum (rER) appear gawky, with some moth-eaten appearance (stars). Mitochondria (M) are electron-opaque in appearance. Some moth-eaten appearance (stars) is retained. Scale bar = 400 nm

**Group 6:** It shows a magnified portion of cell cytoplasm. The endoplasmic reticulum (rER) appear nearly normal but mitochondria (M) are electron-opaque in appearance. Some moth-eaten appearance (stars) is retained. Scale bar = 400 nm
ues in the liver and 73% in the serum when compared to rats receiving reserpine alone, G2, or animals in G5.

Transaminases

In addition to TBARS, liver damage and its recovery could also be assessed by measuring hepatic SGOT and SGPT levels. These transaminases are cytoplasmic markers of liver damage and changes in their level in serum are used as warning of liver dysfunction/damage. Any disturbance in membrane permeability, structure or fluidity causes the translocation of liver transaminases to the blood. Thus, hepatic necrosis is strongly associated with elevated transaminase levels in the serum (Bradham et al., 1998). SGOT and SGPT were elevated to 374% and SGPT 210% in rats treated with reserpine alone (G2) indicating a serious hepatic damage. Reserpine administration is known to disrupt permeability of plasma membrane causing leakage of these enzymes from the liver into serum (Ahmed et al., 1997). Our data suggests that lipid peroxidation could be averted by treatment with green tea extract. This was supported by the fact that green tea extract when given simultaneously with reserpine (G4) significantly reduced SGOT (176%) and SGPT (101%) levels. Oxidative hepatic damage and its reversal by green tea have been reported by many workers (De Oliveira et al., 2006; Singal et al., 2006).

SGOT and SGPT levels, in G5 animals were lower than in rats of (G2) but were higher in G4 animals. Best liver recovery could be achieved where oxidative stress with reserpine was administered before treatment with green tea. This substantiated our findings that oxidative damage had to be there before it is treated with green tea extract.

Cholesterol measurement

Importance of plasma cholesterol and lipoproteins in atherosclerosis has been reported and cellular cholesterol homeostasis is known for the prevention of cardiovascular diseases. Thus an increased level of plasma cholesterol and LDL are associated with increased risk of developing coronary diseases (Fredrickson et al., 1967). Hypercholesterolemia is associated with increased production of oxygen radicals and increased oxidation of LDL cholesterol (Davi et al., 1997). The effect of reserpine on lipid metabolism has been studied in both rats and rabbits, and it is known that reserpine lowers serum cholesterol in animals (Shafi et al., 2002). Our results support the previous studies and show that reserpine decreases cholesterol buildup in serum. Serum clearance of cholesterol was accelerated by reserpine administration, although reserpine increased cholesterol level in liver. It was likely that under the influence of reserpine, the accelerated clearance of serum cholesterol was due to an increased uptake of cholesterol by the liver. The mechanism of hepatic cholesterol increase may be due to an up-regulation of high affinity receptors in the liver. Thus Shafi et al. (2000) have shown that in rabbits and rats, reserpine increased cholesterol receptor expression in liver leading to an increase in liver cholesterol.

It is well-established that green tea catechins can help to reduce cholesterol, thus aiding in the prevention of cardiovascular diseases (Kakuda, 2002). In the present study, hypo-cholesterolemic effect of green tea was demonstrated by a reduced cholesterol level in G4 animals. Administration of green tea extract could stimulate cholesterol hydroxylation through cytochrome P450 in liver (Myant and Mitropoulos, 1977), which is an early step in the synthesis of bile acids. Kobayashi et al. (2005) have reported an inhibition of intestinal cholesterol absorption by green tea while Juhel et al. (2000) have observed that green tea extract significantly inhibits gastric and pancreatic lipase, impeding lipid emulsification. Zhang et al. (2002) have suggested that green tea may act to reduce hepatic and serum cholesterol by increasing bile acid excretion.

Ultra-structural studies

In the present study, the reserpine-induced histological and ultra-structural hepatic changes were examined. The cytoplasmic distribution, morphology and ultra-structural changes in the organelles, support the earlier observations reported by Crivellato et al. (2006). These researchers have suggested that reserpine has the ability to damage cell membranes, organelles and cytoplasm. Cytoplasmic organelle disintegration was found to be a unique feature of reserpine-treated animals.

The cyto-pathological changes were considerably more pronounced, and became more marked in G2, treatment of reserpine alone for 48 days. The interaction of ROS, generated by reserpine, with macromolecules and other essential targets resulted in an increased hepatic microsomal lipid peroxidation. Thus moth-eaten appearance of hepatic cells was prominent in animals treated with reserpine. These findings are in agreement with other studies in which different ROS generators, such as crude oil (Akbar, 1996) and butylated hydroxytoluene (Al-Nughamish, 1998) have been used. These ROS generators caused liver cells to lose their nuclei leading to nuclear degeneration. In the present study, the nuclei appeared abnormal with mislaid chromatin material and condensed in an area with damaged nucleolus and nuclear envelope. Many profiles of altered rER were also noted, showing broken cisternae with stripped off ribosomes that are scattered throughout
the cytoplasm as dense granules and patches. Reserpine assisted alteration of rER has been reported by Clementi and Zocchi (1963).

The hepatic cells of animals in G2 showed diffusion and autolysis of mitochondria indicated by structural abnormalities and cell injury. Mitochondrial cristae were nearly absent, while the outer membrane appeared dissolved and damaged. Mitochondrial damage by reserpine had previously been reported by Hagopian and Nunez (1972). An administration of different cytotoxins, such as crude oil, H2O2, butylated hydroxytoluene, streptozotocin, alcohol, carbon tetrachloride, and acetoniphen has similar effects on mitochondria (Akbar, 1996; Al-Nughjamish, 1998; Heinloth et al., 2004; Xu et al., 2004).

Role of green tea in combating oxidative stress has been reported by many researchers (Di Paola et al., 2005). Our observation of the G4 animals suggests that green tea has a potent ability to quench ROS, thereby, diminishing oxidative stress and preventing liver damage. It is hypothesized that the polyphenols present in green tea, such as catechins, may be responsible for these benefits. Catechins penetrate lipid bilayers and decrease ROS generation. Catechins are known metal chelators, especially iron and copper, which play a pivotal role in the inhibition of hydroxyl radical (Guo et al., 1996). Our results suggest that green tea extract is an excellent antioxidant tonic against reserpine, making this drug less injurious to hypertensive patients.

REFERENCES


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