INTRODUCTION

Green tea (Camellia sinensis) consumed worldwide since ancient times, is considered beneficial to human health, it has been used as a medicinal beverage, particularly in Asia for almost 4,000 years (Balentine, 1997). It is only recently that the world has realized its full potential as a medicinal herb. Rich in vitamins, mineral and polyphenols, green tea is also brimming with goodness for whoever consumes it. Its antioxidant properties help scavenge the destructive free radicals in the body (Yoko and Santosh, 2005). Recent studies suggest that green tea has positive effects on several body organs in laboratory animals (Hisamura et al., 2006; Adhami and Mukhtar, 2007; Asfar et al., 2007; Safer et al., 2007; Khan et al., 2007; Al-Bloushi et al., 2009). The effects of which have been well-documented when used for the purpose of tissue damage recovery (Adhami and Mukhtar, 2007; Asfar et al., 2007; Safer et al., 2007; Al-Bloushi et al., 2009), or when synergistically used with cofactors like CoQ9 in rats in lowering LDL-Cholesterol (Upaganlawar et al., 2006; Afzal et al., 2007). Reserpine is an important antihypertensive drug, but with serious side effects, through oxidative stress. It is a naturally occurring potent alkaloid, derived from roots of several members of Rauwolfia genus (Doyle et al., 1955). Reserpine depletes biogenic amines such as noradrenalin, dopamine and serotonin and is a potent oxidant (Osubor and Nwanze, 1994; Metzger et al., 2002). It has deteriorating effects on rat liver structure and function...
(Al-Bloushi et al., 2009). We have undertaken to assess the revival of kidney cells as a result of green tea administration to rat and the status of thiobarbituric acid reactive substances (TBARS).

MATERIALS AND METHODS

Materials

Chemicals
Reserpine and thiobarbituric acid were obtained from Sigma-Aldrich, Inc. (St. Louis, Milwaukee, WI, USA). Green tea was purchased from the local market. Cholesterol kits were obtained from Randox (Crumlin, UK).

Methods

Green tea aqueous extract preparation
Dried tea leaves (100 g) were powdered in a Waring blender, and extracted with double distilled water (1 l), at 35°C for one hr. 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 was shaken with distilled water, warmed at 35°C, and centrifuged again. The clear supernatant was pooled, lyophilized and the resulting material was stored at −20°C in a screw-capped bottle.

Animals
Sixty male Sprague-Dawely (S.D.) 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/dark period, and treated gently. The rats were randomly distributed into six groups, with one control, and five experimental groups, each with ten animals. All rats had free access to tap water ad libitum, and pellet rodents chow (SDS, Witham, UK). The composition of the diet was 14.7% protein, 2.6% fat, and 5.3% cellulose.

Experimental design
The experiment was designed over a period of 48 days. The lyophilized tea extract was re-dissolved in double distilled water and fed to animals by gavage. Reserpine solution was made in 0.8% glacial acetic acid and given by intraperitoneal injections. Group I: Animals received standard diet and free access to water for 48 days. This group was treated as control. Group II: Animals received a daily dose of reserpine for 48 days. Group III: Animals were exposed to a daily dose of 50 mg/kg green tea extract, dissolved in double distilled water, for 48 days. Group IV: Animals were treated with a daily dose of reserpine and green tea extract simultaneously with the same dose, as for group II and III animals, for 48 days. Group V: Animals received green tea extract for 24 days, like group III, followed by reserpine treatment for 24 days, like group II animals. Group VI: Animals were given reserpine for 24 days, like group II animals, followed by green tea extract for 24 days, like group III animals.

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. Kidney specimens were divided into two parts, the first part was 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 ultramicrotome (Leica, 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, Peabody, MA, USA), operated at 80 kV.

Biochemical analyses
TBARS were measured in serum and kidney samples as malonaldehyde (MDA). Serum cholesterol was measured using commercially available Randox kits. 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
Reserpine induced oxidative kidney damage, as shown by highest level (55.2875 MDA) of lipid peroxidation,
measured by TBARS and calculated by the standard curve. Level of TBARS varied with the time of green tea administration (Fig. 1). Thus the lowest level of TBARS was observed in group IV animals where rats were given reserpine simultaneously with green tea extract.

Green tea extract is known to decrease LDL cholesterol in serum (Allain et al., 1974). Cholesterol was calculated by the standard curve. Reserpine increased LDL cholesterol level in the kidney and showed no effect on the serum cholesterol level (Fig. 2).

**Ultra-structural study**

Control group kidney (G I), showed normal cell. The nucleus, endoplasmic reticulum, and mitochondria, all appeared normal and healthy. The cells distribution, size, shape, and ultra-structure also appeared normal, along with normal plasma membrane, and a narrow slit (Fig. 3). Samples from G II, showed the effect of reserpine treatment on the rat kidney cells for 48 days. The cells exhibited clear damaged mitochondrial cristae or nearly absent, endoplasmic reticulum appeared fragmented, with stripped ribosomes where the later are roaming all over.

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**Fig. 1.** Changes in the level of TBARS in kidney and serum samples affected by reserpine and green tea extract. Results represent mean of triplicate experiment, G2 (n = 8), all other groups (n = 10). Bars marked with *, #, £ & § are significantly different from the control with a P < 0.001.

**Fig. 2.** Measurement of cholesterol levels in kidney and serum samples affected by reserpine and green tea extract. Error bar corresponds to ± S.E.M., results represent mean of triplicate experiment, G2 (n = 8), all groups (n = 10). Bars marked with *, #, £ & § are significantly different from the control with a P < 0.001.
the cytoplasm of the cell (Fig. 4).

Group III exhibited kidney cells from rats, fed with green tea for 48 days. The cells appeared normal, as in control group (G I). The nucleus, nucleolus, and nuclear envelope, all appeared near normal. The distribution, size, shape, ultrastructure of mitochondria and rough endoplasmic reticulum (rER) were also near normal (Fig. 5). Kidney cells from G IV showed the combined effect of intoxication with reserpine and damage repair with green tea treatment for 48 days. The cells appeared near normal, with hardly any moth-eaten appearance in the cytoplasm. Nucleus, mitochondria, and rER, were all normal in morphological appearance and distribution (Fig. 6).

In group G V, kidney cells from rats fed first with green tea for 24 days, followed by reserpine for the next 24 days, as post-dose, showed damage cell parts like in group II, where cell exhibited damaged mitochondrial cristae and nearly absent, endoplasmic reticulum appeared fragmented, with stripped ribosomes where the later are roaming all over the cytoplasm of the cell (Fig. 7). Some ribosomes are condensed on the rER surface in an unusual manner (Fig. 7A); in some occasions rER are half-way through stripping off its ribosomes (Fig. 7B). In group G VI, kidney cells from rats were first fed with reserpine for 24 days, followed by green tea treatment for the next 24 days, as post-dose, showed improvement in kidney damage recovery. The distribution of organelles and ultra-structure appeared healthier, size and shape are all normal. rER looked normal in its distribution and ultrastructure. Mitochondria showed normal cristae (Fig. 8).

**DISCUSSION**

Not very many studies have been done on the inhibiting effects of green tea on the kidney as compared to the liver after the exposure to the oxidative stress inducer - reserpine. Several studies with green tea have demonstrated a causal relationship between green tea exposure and recovery of kidney damage (Itoh, 2005; Yokozawa et al., 2005; Upaganlawar et al., 2006; Hisamura et al., 2006). Results obtained in the present study supported the data previously obtained on hepatic recovery by green tea. Indicating a remarkable degree of recovery from reserpine induced damage, with a significant diminution in oxidative stress (Al-Bloushi et al., 2009).

Green tea extract when simultaneously administered with reserpine, significantly lowered TBARS levels in serum up to 120% and up to 50% in kidney. This inhibition of oxidative stress by green tea extract was consistent with many reports published about green tea antioxidant activity (Yoko and Santosh, 2005; Hisamura et al., 2006; Adhami and Mukhtar, 2007; Asfar et al., 2007; Safer et al., 2007; Khan et al., 2007). Previous studies have demonstrated that green tea inhibits oxidative stress with dif-
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Fig. 5. Group III - Kidney cells from rats, fed with green tea for 48 days. The cells appeared normal, as in control (Group I). Mitochondria (M) and endoplasmic reticulum (rER) were normal. (See insert for more detail). Scale bar = 2 μm. insert 1 μm.

Fig. 6. Group IV - Kidney cells from rats, fed with both reserpine and green tea simultaneously for 48 days. The cells appeared normal, with hardly any abnormality appearance in cytoplasm. The nucleus (N), mitochondria (M), and endoplasmic reticulum (rER), all showed normal morphological appearance and distribution. Scale bar = 1 μm insert 1 μm.

Fig. 7. Group V - Kidney cells from rats, fed with green tea for 24 days, followed by reserpine for the next 24 days, as post-dose. The cytoplasm showed no shrinkage, nor moth-eaten pattern, and endoplasmic reticulum (rER) seen with stripped off ribosomes in many places (long arrows) (see both insets A and B). Many detached ribosomes are reaching the very end parts of the cell. However, some rER still retained the stacked ribosomes on them (arrow head), mitochondria (M) appeared with diminished cristae, nucleus. Scale bar = 1 μm. insert A. 500 nm, insert B. 500 nm.

Fig. 8. Group VI - Kidney cells from rats, fed with reserpine for 24 days, followed by green tea for the next 24 days, as post-dose. The cell appears very normal. As in groups I, III and IV. Scale bar = 1 μm, insert 500 nm.
ferent inducers in different organs (Hong et al., 2001; Das et al., 2002; Hiroyasu et al., 2002; Skrzydlewska et al., 2002; Zhang et al., 2002; Chen et al., 2004; Dobrzyńska et al., 2004; Augustyniak et al., 2005; El-Beshbishy, 2005; Erba et al., 2005; Mohamadin et al., 2005; Sadzuka et al., 2005; Skrzydlewska et al., 2005; Yamamoto et al., 2006). Green tea suppresses reactive oxygen species (ROS) formation through polyhydroxy phenolics such as catechins that impart strong antioxidant activity. Green tea catechins are known to reduce ROS through penetration of their hydrophobic fragments into the lipid bilayer (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). Catechin polar groups can interact with phospholipid polar head groups decreasing the membrane fluidity at polar surface of the phospholipid bilayer (Chen et al., 2002). Rats receiving green tea extract followed by reserpine as a post-dose, showed lower level of TBARS as compared to rats that received reserpine alone. Green tea decreased TBARS by 15% in the kidney and 41% in the serum. The administration of green tea extract before reserpine offered little protection but failed to stop extensive damage to the kidney. This indicates that oxidative damage needs to be in place before treatment with green tea extract. This was evident from the experiment where oxidative damage was induced in rats by reserpine and then green tea extract was administered to combat the oxidative damage. In this case green tea extract had the capacity to decrease 35% of TBAR values in the kidney and 73% in the serum when compared to rats receiving reserpine alone. Treatment with green tea extract prior to reserpine administration showed little reversal of oxidative damage caused by reserpine. However, if green tea was given for a longer period of time to the second group of rats, it could have repaired the kidney damage caused by reserpine.

Importance of plasma cholesterol and lipoproteins in atherosclerosis 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 (Napoli et al., 1995; Davi et al., 1997). Rat model has some cholesterol metabolism characteristics which are different from human, unlike other rodents, such as hamsters, which are closer to the human on this point. Moreover, rats exhibit a unique nutritional flexibility which is less noticeable with animals such as hamster, rabbit or guinea pig. The effect of reserpine on lipid metabolism has been studied in both rats and rabbits (Shafi et al., 2000a, 2000b, and 2002) and it is known that reserpine lowers serum cholesterol in these animals (Shafi et al., 2000a, 2000b). In our study, reserpine decreased an accumulation of cholesterol in serum which is supported by the previous studies. Serum clearance of cholesterol by liver 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 cholesterol from serum was due to an increased uptake of cholesterol by the liver. The mechanism of this increase was probably up-regulation of high affinity receptors in the liver. Shafi et al. (2000a, 2000b) have shown that in rabbits and rats, reserpine increased cholesterol receptor expression in liver leading to an increase in liver cholesterol. Green tea catechins have well-established anticholesterolemic properties that may in fact prevent the occurrence cardiovascular diseases (Kakuda, 2002). In the present study, hypcholesterolemic effect of green tea was evaluated. It was demonstrated that green tea effectively reduced cholesterol level elevated in response to reserpine when given simultaneously with tea extract, or as a post dose. It is known that cytochrome P450 is involved in the metabolism of cholesterol (Myant and Mitropoulos, 1977). Thus, administration of green tea to rats may be responsible for stimulation of cholesterol hydroxylation in liver, which is an early step in the conversion of cholesterol to bile acids. Löest et al. (2002) and Kobayashi et al. (2005) have reported that green tea has the ability to inhibit cholesterol absorption in the intestine. Juhel et al. (2000) have observed that green tea extract significantly inhibits the activities of gastric and pancreatic lipase, interfering with lipid emulsification inhibiting fat digestion by pancreatic lipase in the intestinal lumen. Zhang et al. (2002) have suggested that green tea may increases bile acid excretion in rats causing a reduction in hepatic cholesterol and therefore, a reduction in serum cholesterol.

In the present study, the ultra-structural changes accompanying cell damage in the kidney proximal tubule of male SD rats have been demonstrated in an oxidative stress-dependent manner. Accordingly, the distribution of cytoplasmic organelles noted in the present study may explain the latter findings of Clementi and Zocche (1963); Chen et al. (1969); Hagopian and Nunez (1972); Teichberg and Holtzman (1973); and Crivellato et al. (2006); and in particular the comparable finding of Safer et al. (2007) in hepatocytes, suggesting that reserpine has the ability to damage plus other organelles, the mitochondrial cristae and the breaking rER cisternae and its attached ribosomes.
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Ribosomal stripping off the rER and its disintegration, misplacement and condensation in and around other cell organelles was profound feature of reserpine-treated rats in this study. These cyto-pathological changes were considerably more pronounced and became more marked in group II (reserpine - treated) and group V (green tea followed by reserpine) than in group IV (reserpine and green tea simultaneous) and group VI (reserpine followed by green tea) when continued treatment of reserpine alone for 48 days. ROS generated by reserpine have the ability to react with macromolecules and other essential targets. ROS, formed during the metabolism of reserpine, had resulted in increased hepatic microsomal lipid peroxidation. However, the moth-eaten appearance is not pronounced in the kidney as it was a phenomenon of liver damage in our previous study. These findings are in agreement with other studies using different ROS generators, such as crude oil (Akbar, 1996) and butylated hydroxytoluene (Al-Nughamish, 1998).

Similar findings on alteration of rER by reserpine were reported by Clementi and Zocche (1963); Teichberg and Holtzman (1973). The rER is responsible for protein synthesis and its proliferation is a marker for an increase in total protein and DNA content. A diffuse appearance and autolysis of mitochondria was pronounced. The onset of transformation of mitochondria from normal state to wrecked cristae form was evident. Autolyzed mitochondria were clearly seen in our study not only in pure reserpine administration animals, but also in green tea followed by reserpine ones. The different mitochondrial shapes exhibited structural abnormalities, which indicated signs of cell injury. In accordance with Chen et al. (1969) and Hagopian and Nunez (1972), reserpine caused mitochondrial damage. In the present study, images of mitochondrial alteration were observed. Mitochondrial cristae were near absent, while the outer membrane appeared dissolved and damaged. Other authors have shown similar findings, following the administration of different cytotoxins, such as crude oil, H2O2, butylated hydroxytoluene, streptozotocin, alcohol, carbon tetrachloride, and acetalminophen (Akbar, 1996; Al-Nughamish, 1998; Saha et al., 2002; Coll et al., 2003; Junnila et al., 2000; Heinloth et al., 2004). T different mitochondrial shapes observed in this study showed that the structural abnormalities, that may affect mitochondrial function, mainly inhibited the electron flow through the respiratory chain. This would inhibit respiration, reduce amount of ATP for cellular activity, and result in death of the cell.

Green tea, as an antioxidant, has the ability to react with ROS, decreasing oxidative stress and lowering liver damage. Our observation suggests that green tea extract, when administered with reserpine, may result in kidney remedial, which, therefore, appears to be responsible for reversing reserpine cytotoxicity. A significant number of reports exist in literature, supporting the role of green tea in oxidative stress-damage recovery in animal models (Das et al., 2002; Baltaziak et al., 2004; Chen et al., 2004; Di Paola et al., 2005; Hisamura, et al., 2006; Itoh et al., 2005; Yokozawa et al., 2005; Upaganlawar et al., 2006). Green tea extract represents a rich source of natural polyphenols that has in vivo protective effects on liver and serum. The present study suggests that green tea extract may also protect kidney cells as in other organs like liver hepatocytes against oxidative stress caused by aging and reserpine intoxication. Flavonoids, the most polyphenol present in green tea, can penetrate the lipid bilayer and decrease ROS generation. They can chelate metal ions, especially iron and copper, which in turn inhibit the generation of hydroxyl radical, reducing the oxidative stress.

The discovery that green tea inhibits the damaging effects caused by the oxidative stress of reserpine on the kidney cells are very similar to those of liver cells suggests that green tea - because of its catechin epigallocatechin-gallate (EGCG), which is the main flavonoid compound putative beneficial health effects - has the capability of curing both the structure and function of cell parts to certain degree, which may lead to improved health, although it varies from organ to organ.

REFERENCES


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