Effect of phenolic compound extract of green tea to ameliorate the Cadmium Sulphate toxicity on the female rat kidneys

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Abstract
In the recent years, green tea has become asubject of widely studied for its beneficial effect in the treatment and prevention of human disease. The purpose of this study was to investigate the effects of daily oral consumption of phenolic compound extract of green tea for 30 days on kidney function in female rats that exposure to Cadmium Sulphate. Female Rats (6-8 weeks) age were divided in to four groups : control group , treatment group1(receives 50 mg/L of Cadmium Sulphate with drinking water) ,treatment group2(receives 50 mg/L of cadmium sulphate and 400 mg/kg of phenolic compound of green tea)and treatment group 3(receives 400 mg/kg of phenolic compound extract of green tea).Plasma uric acid and creatinine levels were significantly increased in T1 group compared with control group and significantly decreased in T2 group compared with T1 group.The study continued for 30 days and the kidneys were collected for histopathological studies. The phenolic compound extract of green tea protected kidney tissues against toxicity effect of cadmium sulphate as evidenced from amelioration of histopathological alterations and normalization of kidney biochemical parameters.

Introduction
Cadmium is widely distributed in the environment because of its many industrial application. The health risk to humans from acute and chronic cadmium exposure has been well documented. Cadmium is considered one of the toxic trace elements in the environment (Dunnick & Fowler, 1988). Cadmium enters the food chain when cadmium waste from the industrial production is disposed in the sewages and the sewages sludge is used as agricultural fertilizer. The crops bioaccumulate cadmium in roots, tubers, leaves and other parts of the plant that used as food. Hence cadmium poisoning in human body is evident. Ingested cadmium is stored in the renal and sex organs and cause renal dysfunction and testicular necrosis (May & McKinney, 1981). Cadmium is considered one of the most active and abundant catechin in green tea (Camellia Sinensis), green tea is a non-fermented tea and contains more catechins than black tea. Its content of certain minerals and vitamins increases the antioxidant potential of this type of tea. Green tea contains nearly 4000 bioactive compounds of which one third is contributed by polyphenols (Tariq et al., 2010). Other compounds are alkaloids (Caffeine, theophylline, chlorophyll, fluoride, aluminum, minerals and trace elements (Cabrera et al., 2003). Polyphenols found in green tea are mostly flavonoids the polyphenols large group of plant chemicals that include the catechins together are responsible for the health benefits that have traditionally been attributed to tea especially green tea (Babut et al., 2006). Major catechins are epicatechingallate (ECG), epicatechin (EC), epigallocatechin (EGC) and epigallocatechingallate (EGCG). The most active and abundant catechin in green tea is epigallocatechin-3-gallate (EGCG) (Wu and Yu, 2006). Green tea has been considered a medicine and healthful beverage since ancient times, the traditional chinese medicine has recommended this plant for headaches, body aches and pains, digestion, depression, detoxification, as an energizer and ingeneral to prolong life, its contain three main components which act up on human health.
Effect of phenolic compound extract of green tea to ameliorate the Cadmium Sulphate toxicity on the kidney function in female rats that exposure to cadmium sulphate.

Materials and methods

1-Extraction of phenolic compound
The leaf of green tea was obtained from local market of AL-Qadisyia university. Then dried and powdered, according to Gayon method (1972). 200 g ram of plant powder was weight and added to 800 ml of 2% acetic acid and extract, the mixture was left for 24 hours in an incubator at 50°C, then filtered through filter paper to remove all the residual materials. The clear extracted solution treated with the same volumes of N-propanol and then saturated with NaCl. The upper layer was separated by funnel, then dried at 45°C using an incubator.

2-Model:
Twenty four (24) healthy Albinio female rats of age (6-8) weeks experiment animals were housed in the animals house of college of veterinary medicine /AL-Qadisyia university. The experiment period was one months (from 25 February 2013 to 25 March 2013). Animals were divided in to 4 groups as 6 animals in each group as the following:

1-Control group (C) receives distilled water.
2-Treatment group 1 (T1): receives Cadmium Sulphate with drinking water at a dose (50 mg/L).
3-Treatment group 2 (T2): receives Cadmium Sulphate with drinking water at a dose (50 mg/L) of and orally gavage with phenolic compound extract of green tea at a dose (400 mg/kg of body weight).
4-Treatment group 3 (T3): orally gavage with phenolic compound extract of green tea at a dose (400 mg/kg of body weight).

3- Blood collection
Blood samples were collected from the animals after 24 hours from experimented and the blood samples were obtained by the heart puncture. The blood was centrifuge in atest tube at 5000 rpm for 15 minutes, serum was carefully separated and stored with frozen condition. Uric acid and creatinine were estimate using an automatic analyzer (Reflotron® plus system, Roche, Germany).

4- Tissue preparation
The animals were scarified by heart puncture and kidney specimens collected for histological examination. Tissues samples were kept in 10% neutral buffered formalin and the histopathological preparations was carried out according to (Luna,1968).

Statistical analysis
Result were expressed as mean± S.E, the differences between mean values were analyzed by using F-test and LSD, significance was considered when P<0.05 (Schefler,1980).

Results and Discussion
The results point out a significant increase (P<0.05) in uric acid (2.121±0.061) and creatinine (0.66±0.019) levels in T1 group (a group which receives 50 mg/L of Cadmium Sulphate with drinking water) compared with control group (1.651±0.048) and (0.531±0.021), this is consisted with the earlier works of (Harstand&Klaassen,2000; Aprioku,&Obianime,2008) in which they showed that cadmium induced increase in ALP and urea, suggest hepatic and renal toxicities respectively. Moreover, (Obianimeetal., 2011) shown that increased in creatinine and urea levels in guinea pigs that exposure to cadmium chloride because of renal toxicity and this is agreed with previous studies of (Harstand&Klaassen ,2000; Aprioku&Obianime,2008;Obianime&Robert,2009). While the results recorded a significant decrease uric acid (1.886±0.008) and creatinine (0.565±0.013) levels in T2 group (a group which receives 50 mg/L cadmium sulphate and 400 mg/Kg of phenolic compound extract of green tea) compared with T1 group, because the...
catechins in green tea is clearly effective in reducing oxidative stress and inflammatory reactions in kidney tissue (Choi et al., 2004). Another interpretation made by (Kwag et al., 2001) was reported that green tea catechin has been found to improve the ratio of prostaglandin I2/thromboxane A2 by controlling the cyclooxygenase way of the arachidonic acid in the microsome and glomeruli of diabetic rat kidney. (Yokozawa et al., 2005) have recently shown that the combination of green tea polyphenols (GTP) and partially hydrolysed guar gum (PHGG) reduced kidney weight, level of blood urea and serum creatinine in diabetic animals. Moreover, (Silan et al., 2007) have shown that green tea could help kidneys process toxins and waste products more efficiently. As well as (Byrav et al., 2010) showed the powerful antioxidant capacity and anti-inflammatory effect of green tea polyphenols.

Table (1): changes in uric acid and creatinine levels in different studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uric acid (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.651±0.048</td>
<td>0.531±0.021</td>
</tr>
<tr>
<td>T1</td>
<td>2.121±0.061</td>
<td>0.66±0.019</td>
</tr>
<tr>
<td>T2</td>
<td>1.886±0.088</td>
<td>0.56±0.013</td>
</tr>
<tr>
<td>T3</td>
<td>1.713±0.027</td>
<td>0.47±0.028</td>
</tr>
<tr>
<td>LSD</td>
<td>0.126</td>
<td>0.06</td>
</tr>
</tbody>
</table>

- Results expressed as mean ±S.E.
- Different letters refer to significant differentiations between groups at (P<0.05).
- Similar letters refer to significant differentiations between groups at (P<0.05).
- C: Control group
- T1: receives Cadmium Sulphate with drinking water at a dose (50 mg/L).
- T2: receives Cadmium Sulphate with drinking water at a dose (50 mg/L) and orally gavage with phenolic compound extract of green tea at a dose (400mg/kg of body weight).
- T3: orally gavage with phenolic compound extract of green tea at a dose (400mg/kg of body weight).

The histopathological results of kidney sections showed that kidney section of the control group revealed high–cellularity normal renal convoluted tubules with normal glomeruli as in (Fig1 and Fig 2). While kidney sections of T1 group (a group which receives Cadmium Sulphate with drinking water at a dose 50 mg/L) revealed dilation of renal convoluted tubules with sever hemorrhage in the renal tissue, severe degeneration in the endothelial cells which lining the renal convoluted tubules as in (Fig 3 and Fig 4), as well as deposition of hyaline material in the renal tissue (hyaline degeneration), this is belonged to cadmium compounds that cause kidney damage (Habeebu et al., 1998; Jones & Cherian, 1988) showed that cadmium caused liver, kidney and testicular damage.

Histopathological sections of kidney in T2 group (a group which receives Cadmium Sulphate with drinking water at a dose 50 mg/L) and orally gavage with phenolic compound extract of green tea as a dose (400mg/kg of body weight), showed normal glomeruli and few dilation of renal convoluted tubules which lining with normal endothelial cells, there is tubular basophilia (tubular regeneration), no congestion and no hyaline material as in (Fig 5 and Fig 6), In the study of (Silan et al., 2007) shown the protective effect of green tea extract (300mg/Kg) against histopathological alternations induced by gentamicin in the kidney tissues. GTE blocked cellular inflammatory process as indicated from alleviation of perivascular edema and reduction in mononuclear leucocytes inflammatory cells infiltration and scavenging of free oxygen radicals prevent irreversible renal cell injury, necrosis and degeneration. While, the kidney section in the T3 group (a group which orally gavage with phenolic compound extract of green tea at a dose 400mg/kg of body weight), showed high cellularity glomeruli and normal renal convoluted tubules which lining with normal endothelial cells as in (Fig 7 and Fig 8), this group appears like control group.
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**Fig 1:** Control kidney: Normal renal tissue characterized with high cellularity normal glomeruli (red arrows) with normal renal convoluted tubules (yellow arrows). 20X H&E.

**Fig 2:** Control kidney: Higher magnification. High cellularity normal glomeruli (red arrows) with normal renal convoluted tubules (yellow arrows). 200X H&E.

**Fig 3:** T1 kidney: There is dilation of renal convoluted tubules with severe hemorrhage in the renal tissue (red arrow), deposition of hyaline material in the renal tissue (hyaline degeneration) (yellow arrows). 20X H&E.

**Fig 4:** T1 kidney: There is dilation of renal convoluted tubules (red arrows) with severe hemorrhage in the renal tissue (yellow arrows), severe degeneration in the endothelial cell which lining the renal convoluted tubules (blue arrows). 50X H&E.
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Fig 5: T2 kidney: there is normal glomeruli, few dilation of renal convoluted tubules (red arrows). There is no congestion and no hyaline material. 20X H&E.

Fig 6: T2 kidney: there is normal glomeruli, few dilation of renal convoluted tubules which lining with normal endothelial cells. Also there is tubular basophilia (tubular regeneration) (red arrows). There is no congestion and no hyaline material. 50X H&E.

Fig 7: T3 kidney: there is high cellularity glomeruli (red arrows), normal renal convoluted tubules which lining with normal endothelial cells. 20X H&E.

Fig 8: T3 kidney: Higher magnification. There is high cellularity glomeruli (red arrows), normal renal convoluted tubules which lining with normal endothelial cells. 50X H&E.
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