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Effect of Green and Red Tea Extracts on Histological Changes of Kidney in Albino Rats Infected with *Escherichia coli*

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ABSTRACT

Antibiotic resistance is increasingly emerging and represented as a top healthcare challenge that necessitates seeking alternative antibiotic remedies including herbal resources. Tea widely used beverage and different effective actions have been documented for tea extracts, such as antioxidant, antibacterial, and anticancer effects. We aimed to compare the antibacterial action of green versus red tea extracts. To do so, a model was created by exposing rats to E Coli infecting their kidneys and then using green and red tea extract to tackle the infection, if any. The results of the study were documented through histopathological analysis of rat's kidney alongside measuring serum creatinine and urea of studied group using control negative and control positive group for comparisons. Additional ciprofloxacin was used, as a prototype antibiotic effective against urinary tract infection, for comparison with red and green tea extracts. Green tea extracts were found to have an effective role in reducing lesions green tea was superior to red tea and was similar to ciprofloxacin in decreasing the pathological effects of *E. coli* bacteria on rat kidney tissue.

1. Introduction

Clinical and experimental research have generated a lot of debate concerning the link between kidney disease and oxidative stress, which can be caused by hyperglycemia, reactive species toxicants, or bacterial infections [1]. The underlying mechanism is based on the generation of free radical inducing biomolecular damage leading to subsequent tissue and organ damage [2], [3].

In several investigations, free radical inhibitors and antioxidants have been demonstrated to protect against kidney injury [4]. *Escherichia coli* that produce Shiga toxin (STEC) This strain of E. coli is linked to food and waterborne E. coli contamination [3], [5], [6].

Urinary tract infections are the second most prevalent type of nosocomial infection, and Escherichia coli is

the most common type of bacteria that causes them, accounting for 90% of infections worldwide [7], [8]. Antibiotic resistance in bacteria is regarded as one of the world's most serious health and economic challenges, and researchers are increasingly interested in finding new antimicrobial medicines made from plant extracts [9].

Green tea (Camellia sinensis) has been revered for generations for its numerous health advantages [10], including degenerative disease, cardiovascular diseases, diabetes, and cancers [11]. Green tea's involvement in the direct and indirect prevention of chronic diseases must define, as it is already one of the most popular beverages in the world. [12]. Green tea's components neutralize free radicals thereby exerting its therapeutic effects [13], [14]. Green tea is high in polyphenols, particularly flavonoids, which have been proven to have several pharmacological benefits as well as a role in the prevention of degenerative diseases and cardiovascular diseases [15], [16]. Studies demonstrating the effect of green and red tea on urinary tract infection are limited, therefore, we aimed to investigate this effect in our current study.

2. Material and Methods

Preparation of bacterial strains: Ten strains of Escherichia coli were taken from the College of Science/Department of the Biology/University of Mosul and diagnosed using selective media and biochemical tests, "IMViC tests (Indole, methyl red, Voges-Proskauer, citrate use)", urease, triglycerides, iron (TSI), and then subcultured in nutrient broth incubated at 37 °C under aerobic conditions for 24 hours (pure gold) [17]. Two types of tea green tea and H. sabdariffa (red tea) bought from the local market for the preparation of green and red tea extracts, and the extract was made using a method described in one of the studies [12] for use in experimental animals.

Prepare the extracts: Leaves must be pure, free of impurities or excess elements (aroma, color, or taste), and there must be only one stock, not a mixture of other herbs or teas, in terms of species. Every day, water extracts of green and red tea are made, and the aqueous extract is made in a 1:10 ratio.

Preparation of extracts: Five grams of tea leaves with a diameter of less than 5 mm (according to FS-B22: 2002 and Eur.Ph.01 / 2002,2.1.4) 60 milliliters (ml) boiling water after having time for the extract to drain and cool, the resultant solution is filtered after 20 minutes.

Measurement of creatinine and urea: the serum creatinine and urea in mg/dl were measured by kits supplied by Elabscience (USA) using a spectrophotometer (FUJIFILM NX500 instrument, Japanese).

Experimental animals: White test rats were utilized in this experiment, with a total of 25 adult rats weighing between 100 and 150 grams and aged between 10 and 12 weeks, randomly divided into five groups (G1, G2, G3, G4, and G5), each with five rats (table 1)

Table 1. Distribution of rats in groups with treated agents.

Used agents	Group administered
Control group	G1
E Coli	G2, G3, G4, G5
Green tea Dose: 200mg/kg (two times per day for 5 days)	G3

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Red tea Dose: 250mg/kg (two times per day for 5 days)	G4
Ciprofloxacin Dose: 20mg/kg (two times per day for 5 days)	G5

After the experiment treatment ended, the rats were anesthetized with ether. Blood was extracted from the Orbital eye veins and the serum was collected and kept frozen until utilized in measuring creatinine and urea.

Then rats were sacrificed for post mortem and observation of macroscopic tissue changes, after which the organs were collected and preserved with 10% neutral formalin. After which samples were prepared in the tissue section for preparing tissue sections of the kidney and stained with hematoxylin and eosin dye. Finally, the tissue sections were examined under a light microscope [18].

Statistical analysis: The numerical results were presented as mean and standard error. One-way analysis of variance (ANOVA) was used to identify the differences between groups if any; followed by the LSD test in SPSS software. Significance considered at p-value less than 0.05.

3. Results

Compared with kidneys of the control group revealed normal architectures of renal tissue, the kidneys of *the E. coli* infected group showed pathological changes including the presence of inflammation (glomerulonephritis) represented by infiltration of inflammatory cells, renal cyst, shrinkage of glomeruli, enlargement of Bowman's space, cell bulging and necrosis of epithelial cells lining renal tubules and severe hemorrhage between renal tubules. Also, the kidneys of red tea extract with *E. coli* infected group G4 revealed the same lesions but less severe as atrophy of glomeruli, dilation of Bowman's space, cell swelling, and necrosis of epithelial cells lining renal tubules without improving comparing control group G1. Whereas the kidneys of green tea extract with *E. coli* infected group G3 and ciprofloxacin with *E. coli* infected group G5 revealed improving renal tissue lesions except there was dilation of Bowman's space in the glomerulus, slight cell swelling of epithelial cells lining renal tubules comparing and congestion of blood vessels control group G1 (Figure 1).

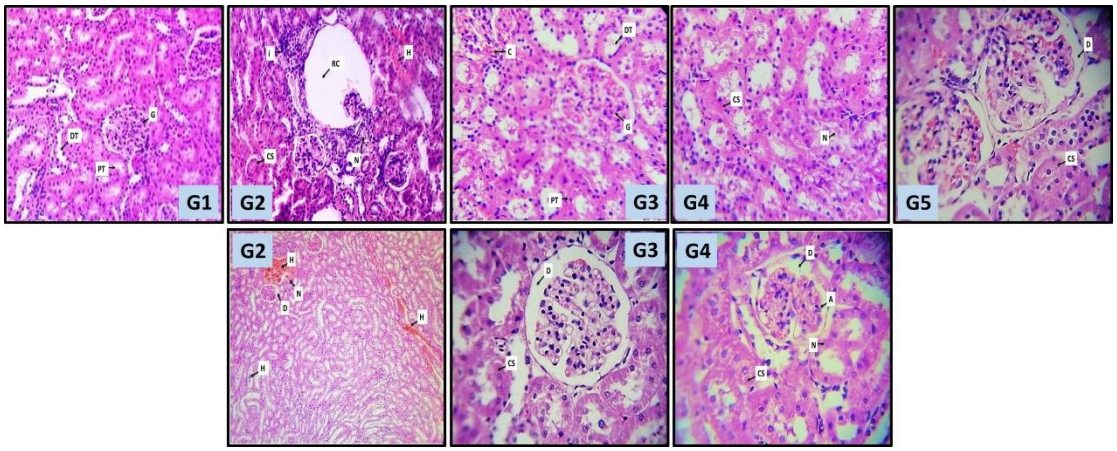

Figure 1. Histological study of renal tissue using eosin-hematoxylin staining technique. G1= control group, G2= E Coli treated group, G3 and G4= E Coli + Green Tea extracts, G4= E Coli + Red Tea extracts. the treated group, G5=Ciprofloxacin treated group.

E. coli-infected group had the greatest significant difference in creatinine values when compared to the control group and the other groups, the green tea and ciprofloxacin groups showed a significant reduction When compared to the *E. coli* group, whereas the red tea group was significantly different from the control group and the *Escherichia coli* group. While the urea concentration data in Table 2 showed that the G2 group had the greatest significant difference in urea levels when compared to the control group and the other groups. the green tea and ciprofloxacin groups showed a considerable decrease when compared to the *Escherichia coli* group and the red tea group. The red tea group outperformed the control and *E. coli* groups significantly (Figure 2).

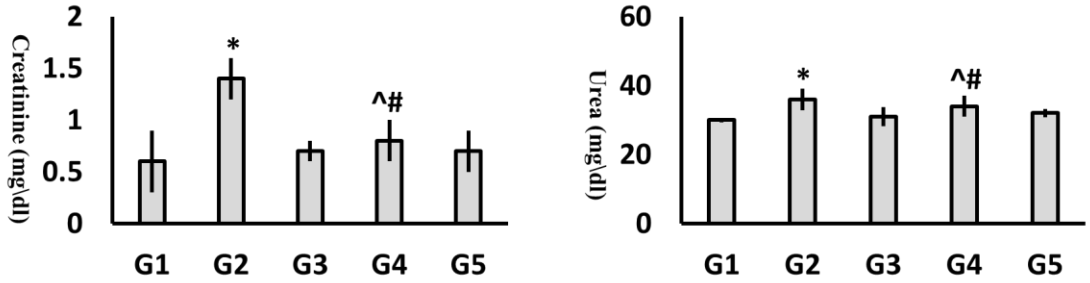

Figure 2. Rat renal function tests in studied groups. Creatinine and urea concentration (mg/dl) were measured in all treated groups. Data expressed as mean±SD. *p<0.05 as compared to all groups, ^p<0.05 compared to control group, # p<0.05 as compared to G3 and G5 group. G1= control group, G2= E Coli treated group, G3 and G4= E Coli + Green Tea extracts, G4= E Coli + Red Tea extracts treated group, G5=Ciprofloxacin treated group.

4. Discussion

The emergence of antibiotic resistance as a result of the widespread and unstudied use of antibiotics raised interest in natural alternatives derived from plant extracts, such as green. Our current findings demonstrated the ability of green tea water extract to treat and reduce pathological lesions caused by *Escherichia coli*, or rather the bacteria's toxins.

Necrosis of the epithelial cells lining the renal tubules, as well as severe hemorrhage between the renal tubules These pathogenic changes have been discovered in several studies, including the pathological effect of *E.coli* on Renal tissue [19]. Because Shiga toxin produced by *Escherichia coli* (STEC) is one of the pathogens associated with *E. coli* bacteria contamination in food and water, the period of proliferation of toxins in the kidneys begins about 4 days after the stage of hemorrhagic colitis, and this is the reason for choosing 5 days for this study. This *E. coli* is also known as Shiga toxin-producing *E. coli* (STEC) or verotoxin-producing *E. coli* (FER) (VTEC), Many other STEC compounds, unlike Shiga toxins, are thought to act locally in the intestine rather than systemically [3].

The pathological lesions in the green tea-treated group improved. This result is consistent with another study that found that administering green tea extract to rats at a dose of 25 mg/ml for 7 days suppressed Escherichia coli [20]. Green tea contains qualitatively essential components such as free amino acids, caffeine, and polyphenols, according to a study. Catechins, the major component of polyphenols, are well-known antioxidants, which has led to an investigation into their use in a variety of disorders linked to free radicals, including cancer, cardiovascular disease, and neurological diseases [13].

"Catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin" are the most common catechins found in tea leaves, antioxidants are related to catechins' characteristics primarily through the quantity and

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position of hydroxyl groups in molecules, which bind and neutralize free radicals [15]. In vitro, green tea antioxidants are a good electron donor and effective scavenger of physiologically relevant reactive oxygen species, such as superoxide anions [21].

Green tea contains a variety of polyphenols, with up to 90% of them being polyphenols. Reactive oxygen and nitrogen metabolites are thought to play a role in the etiology of kidney disease, and in vivo free radicals of NO have been implicated in cell damage and renal dysfunction [13]. The pathological consequences of peroxynitrite anion (ONOO) free radical and its breakdown product contribute to antioxidant depletion, tyrosine nitrate-induced changes in protein structure and function, and oxidative damage in human disease and animal models of disease [15].

Because of the kidney's ability to collect and accumulate toxic compounds through specialized cells, it is a particular target for toxic molecules and bacteria [22]. As a result, it is one of the devices targeted when exposed to Escherichia coli bacteria, and it has been linked to elevated levels of creatinine and urea in Escherichia coli-infected animals because of nephron damage [13]. There was a considerable improvement in the level of creatinine and urea in the group treated with green tea and ciprofloxacin, as green tea acts to maintain the integrity of the cell membrane and maintain its function, as well as minimize DNA damage by acting on Redox balance in the body because it contains many antioxidants [21].

The limitation of our study is the small sample size, use of locally purchased non-standardized tea product, one stain (E Coli) infection model, and the outcomes were based on laboratory test with confounding parameters which might interfere with test results; hence, larger sample size, more accurate tests should be used and confirmation should be obtained based on bacterial culture techniques.

5. Conclusion

We concluded from this study that green tea has a therapeutic ability superior to red tea and was comparable to ciprofloxacin in treating the pathological effects of E. coli at the dose specified earlier in the research text.

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Conflict of interest: the authors declare that there is no conflict of interest.

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