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Comparison of the Antimicrobial Efficacy of Green and Black Tea Extracts Against E.Coli: In Vitro and In Vivo Study

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ABSTRACT
Green and black tea is one of the most popular beverages consumed worldwide. Moreover, during the last two decades it has received much attention in regard to its beneficial effects on various human health problems .This study aimed to investigate the antibacterial effect of hot and cold extracts of green tea and black tea separately against *E. coli* and after infecting the burned skin rat with them, also to screen the effect of both types of tea on rat liver and kidney. Sixteen male rats divided into four Groups (4 animals each), Group 1: untreated rat (control). Group 2: burn rats infected with *E.coli*(1.5×10^8 bacteria /ml). Group 3: burn treated by Green tea aqueous extract (1000 µg/ml) for *E.coli* bacteria. Group 4: burn treated by black tea extract (800 µg/ml) for *E.coli* bacteria. The tea extracts antimicrobial activity was analyzed by agar diffusion inhibition assay and minimum inhibitory concentration. Results exhibited that *E. coli* in particular, have high levels of resistance against 13 antimicrobial and response only to Imepeneme and Meropenem . Hot watery green tea extract is more effective against bacterial type than cold once. Based on assessment of inhibition zones. *E. coli* was more sensitive black tea extraction, according to the inhibitions zone. Depending on MIC, we concluded that hot extract from two types of teas have the best antibacterial effect than cold extract demonstrated that the best MIC against *E.coli* is hot green tea extract (400 µg/ml) then hot black tea extract (600 µg/ml) followed by cold green tea extract (800 µg/ml) and less activity is cold black tea extract (1000 µg/ml). Infected rats showed inflammation and hemorrhage. While treating rats with tea extracts dermally approximately restored kidney normal histological architecture and liver became nearly normal in histological structure. Black tea extract showed greater effectiveness in tissue recovery than green once.

Keywords: *E. coli*, multiresistance, Green tea, Black tea, Liver, Kidney, Rat.

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INTRODUCTION

The emergence of multi drug resistance in bacteria against major antibiotic groups has played havoc in recent years and has posed serious challenge to clinicians in the prevention, control & management of infectious diseases. The emergence & spread of multi drug resistant bacteria in community as well as in hospital settings is a major public health threat with most of antibiotics being rendered ineffective, clinicians are left helpless with very few alternatives left which are also slipping off their hands. To tackle this menace there is an increasing need to develop newer antimicrobial agents particularly from medicinal plant extracts (Reygaert, 2014) [1]. Green tea is derived from non-fermented leaves of the *Camellia sinensis* plant. Oolong and black tea are made from fermented leaves of the same plant. Green tea has been a favored drink, traditionally, in Asian countries. Because of studies that have shown the potential health benefits of green tea, it is now gaining worldwide popularity as a drink that is important in preventative medicine. Studies using green tea have shown it to have potential benefits, most notably in: cardiovascular disease, cancer, diabetes, obesity, oral health, bone health, and cognitive function (Chacko *et al*., 2010)[(2).] In addition, green tea has been shown to have antimicrobial effects (Song and Seong, 2007)[(3).] Even though scientists are constantly working to develop new and improved antimicrobials, almost as soon as a new drug is released, the bacteria show resistance to it. These isolates are also showing resistance to drug combinations such as amoxicillin/clavulanic acid, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole (Oliveira *et al,* 2011)[(4).] Anti-bacterial effects of tea and GTCs have been demonstrated against Gram-positive and Gram-negative bacteria including *Staphylococcus aureus*, *Vibrio cholerae, Escherichia coli, Shigella spp., Salmonella spp., Bacillus spp., Klebsiella spp. and Pseudomonas aeruginosa* (Ech , 2012)[(5).] Since ancient times, green tea has been considered by the traditional Chinese medicine as a healthful beverage. Recent human studies suggest that green tea may contribute to other physiological functions such as anti-hypertensive effect, body weight control, antibacterial and antivirusic activity, ultraviolet protection, bone mineral density increase, anti-fibrotic properties and neuro-protective power. Increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties (Cabrera *et al*., 2006)[(6).] *Camellia sinensis* (Tea) has a wide range of effects on animal and human health. It is anti-inflammatory and has been reported to have beneficial effects in conditions such as collagen-induced arthritis (Haqqi *et al*.,1999)[(7)] , inflammatory bowel disease (Varilek *et al*.,2001)[(8)] and carrageenan-induced paw oedema (Das *et al*., 2002)[(9)] An increased consumption of green tea may reduce the risk of liver disease (Zheng and Li, 2008)[(10)] . Green tea polyphenol prevents oxygen free radical-induced hepatocyte lethality, prevent lipopolysaccharide-induced liver injury through inhibition of inducible nitric oxide synthase and tumor necrosis factor-α expression and inhibits carcinogen or toxin-induced liver oxidative DNA damage (Cai *et al*.,2002) [(11).] Epigallocatechin gallate, isolated from green tea, has antioxidant properties and is thought to act as an antioxidant in biological systems (Valcic *et al*.,1999)[(12)] .This study aimed to investigate the antibacterial effect of hot and cold extracts of green tea and black tea separately against *E. coli*., after infecting the burned skin rat with *E. coli* we screen the effect of both types of tea on the liver and kidney of rats

through the skin, to see if it has a beneficial effect and side effects at any hazard doses.

MATERIAL AND METHODS

1-Collection and preparation of plant samples

Dried leaves of green and black tea (*Camellia sinensis*) were purchased from the market of Erbil city, Kurdistan Region-Iraq, then they were classified by Asst. Prof. Dr. Abdullah Sh. Sardar/Department of Biology, College of Education, Salahaddin University-Erbil-Iraq. The plants are processed into powder and packed for further storage in polyethylene bags at 4 ° C in the refrigerator.

2-Cold and Hot Watery extract Preparation

Ten grams of plant leaves were added separately to 100 ml of cold and hot water, heated to boil and filtered with muslin cloth, then filtered with filter paper (Whatman No. 1). To obtain the stock extracts, the extracts were dried in the oven (40 ° C) (Hernandez, *et al*., 1994)[13] and kept at 5 ° C for use in the refrigerator.

3-Preparation of inoculums

Bacterial suspensions of *E.coli* which isolated from wound samples in Rizgary Hospital were designed to the standard of McFarland. A 24-hour culture has been used for bacterial suspension preparation. Organism suspension was made in a standard sterile saline and the turbidity was set to contain around 1 * 106 CFU / ml. It was achieved by adapting the bacterial suspension's optical density to 0.5 McFarland turbidity standards (MacFaddin, 2002) [14].

4-Antibacterial susceptibility

Different doses are provided for each black tea and green tea by diluting the water stock extract to obtain the concentrations (100%, 75%, 50%, 25% and 12.5%). To estimate the inhibitory effect of increasing plant concentration, an agar diffusion inhibition assay was conducted. *E. coli* isolates were grown on nutrient agar media, the inoculated plates were left for half an hour, then the cork-borer bored four wells with a diameter of (6) mm on the plate. Plant extract (0.1) ml was appended to each cultivated plate wells which were incubated for 18-24 hrs at 37 ° C. The inhibition zones were then assessed (Gandhiraja *et al*., 2009) [15].

5-Minimum inhibitory concentration (MIC) Minimum and bactericidal concentration (MBC)

Nutrient broth was incorporated with control tube contained only *E. coli* and media at different concentrations of plant extracts (1000, 500, 250 and 125 µg / ml). All tubes have been incubated for 24 hours at 37 °C. The extract MIC is the lowest extract concentration that has not shown bacterial growth and turbidity. Minimum inhibitory concentrations (MICs) were determined after 24 hours as the lowest extract concentration inhibiting each organism's visible growth on the agar plate. There was no regard for the presence of one or two colonies. All experiments in triplicates were applied. Spread plate method was used to determine minimum bactericidal concentration (MBC) from the MIC range. In Petri dishes, Mueller Hinton agar was sub-cultivated from tubes without growth and incubated for 24 hours at 37 ° C. Macroscopically, the petri dishes are observed. The highest dilution on a solid medium that did not produce a bacterial colony was taken as MBC (Umachigi *et al*., 2008) [16].

6-Sensitivity to antimicrobial agents

The isolates are checked aginst 15 commonly antibiotics used using the Kirby-Bauer standardized single-disk approach (NCCLS, 2002) [17]

7-Design Experience

Sixteen male Albino rats (*Rattus norvegicus*) (150-180 g) aged between 10 and 12 weeks were used for all in vivo experiments. This experiment performed in May 2018 in Department of Biology, College of Education, Salahaddin University- Erbil city. Rats were kept in a light, diet and normal temperature room and all rats were acclimatized for more than 1 week prior to beginning the experiments. The dorsal back skins of the rat were shaved and also ethanol (70%) was used as antiseptic for the shaved region and then burned by using inflamed knife and then contaminated with bacteria *E. coli* (1.5×10^8 bacteria /ml), After two days of injury the inflammation, redness and suppuration region were observed.

8-Division of groups

The experimental rat were randomly divided into the following groups (n = 4):-

Group 1: untreated rat (control).

Group 2: burn rats infected with *E.coli.* at the shaved region.

Group 3: burn treated by ointment with Green tea aqueous extract (concentration 1000 µg/ml) for E. coli bacteria

Group 4: burn treated by ointment with Black tea aqueous extract (concentration 2000 µg/ml) for E.coli bacteria.

The treatment continued twice each day during 15 consecutive days and the numbers of bacteria was calculated (Umachigi,*et al*., 2008) [16].

9- Isolation of organs (Anesthesia and Dissection of animals)

All animals were ketamine anesthetized (35mg/kg B.W.) and xylazine (5mg/kg B.W.) (Laird *et al*., 1996) [18], sacrificed at the end of experiment. Liver and kidney divided into small pieces (less than 0.5cm3 thickness) then kept in definitive fixative.

10-Histological study

Preparation of histological sections:

Liver and kidney specimens fixed mainly 10% formaldehyde. Then processed for paraffin method by dehydrating through serial dilutions of alcohol (80% for 1/2 hour, 96% two changes each for 2 hours, cleared in xylene for 2 hours, dried and infiltrated in paraffin wax at 60°C, then embedded in paraffin wax. Paraffin blocks were made for portions at 4 µm thickness section. The gained tissue sections were provided on glass slides, deparaffinized by xylol and rehydrated by descending serial of ethanol, then stained by gill hematoxylin for 20 minutes, washed by tap water for 2 minutes then

stained by eosin for 3 minutes followed by dehydration via ascending serial of ethanol. Finally, cleared by xylol, mounted with Canada balsam (Al-Kinani, 2013) [19].

RESULTS AND DISCUSSION

Antibiotics Susceptibility of E. coli

The emergence of *Escherichia coli* isolates with multiple-antibiotic-resistant phenotypes, involving coresistance to four or more unrelated families of antibiotics, has been previously reported and is considered a serious health concern (Maynard *et al.*, 2003)[20]. Transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons (Carattoli,,2001) [21] are important factors that can contribute to the increase in multiresistant bacteria.

Our research finding *E.coli* are more resistance to the commonly used antimicrobial agents (Aztreonam ATM (81%), Cefazolin CZ (82%), cefotaxime CTX (91%), Ceftazidime CAZ (73%), trimethoprim TMP (91%), ,piperacillin/tazobactam T—ZP (54.55%), amoxicillin/clavulanate AMC (81.82%) Ciprofloxacin CIP (45.45%), FEP (90.91%), FOX (63.64%), Gentamicin CM (63.64%), and Tetracycline TC (63.64%)). But appears low resistance to carbapenem antibiotic (Imipenem IMP (10%) and Meropenem MEM (18%)) as in table 1. Currently research observed the most antibiotic are in active against *E. coli* (except carbapenem antibiotic (Imipenem IMP (90%) and Meropenem MEM (82%) appears high effect against *E. coli*). The genes encoding antibiotic resistance enzymes are particularly plasmid mediated, are often co-transferred on the same plasmid (Canton and Coque, 2006) [22]. Furthermore, many these expression cross-resistance of antimicrobials. Consequently, both clinicians and clinical microbiologists face a significant therapeutic challenge (El Bouamria *et al*., 2015) [23].For example, the resistance of *E. coli* to amoxicillin/clavulanate in this study (81.82%) was similar to the resistance rate reported in Morroco (85%) (Tlamcani *et al*., 2009) [24] and higher than the one reported in Tunisia (67.5%) (Khalifa and Khedher, 2012) [25]. The clinical relevance that has been numerous published reports on clinical failure with the use of commonly used antimicrobial agents have been well documented. The choice of antimicrobial agents that are effective against organisms is therefore very limited at present (Othman *et al*., 2019) [26].

Table 1: Resistance patterns of E. coli

Antibiotics		*E. coli*		
		R	I	S
Ceftazidim(CD)	%	45.00	0.00	55.00
Aztreonam (ATM)	%	81.00	9.00	10.00
Cefazolin (CZ)	%	82.00	0.00	18.00
Cefotaxime (CTX)	%	91.00	0.00	9.00
Imipenem (IMP)	%	10.00	0.00	90.00
Meropenem (MEM)	%	18.00	0.00	82.00
Ceftazidime (CAZ)	%	73.00	10.00	17.00
Trimethoprim(TMP)	%	91.00	0.00	9.00
Piperacillin-Tazobactam (T---ZP)	%	54.55	9.09	36.36
Amoxicillin (AMC)	%	81.82	9.09	9.09
Ciprofloxacin (CIP)	%	45.45	9.09	45.45
Cefepime (FEP)	%	90.91	0.00	9.09
Foxitin (FOX)	%	63.64	9.09	27.27
Gentamicin (GM)	%	63.64	0.00	36.36
Tetracycline (TC)	%	63.64	0.00	36.36

R:resistant, I:intermediaye, S:sensitive

In this study, we examined two herbal teas ' antimicrobial activities. Black and green teas have been found to be effective against the studied microorganisms according to these experiments .The flow table (2) of the investigation is summarizes the effects of cold and hot watery green tea extract concentrations on the bacterial growth *E. coli*, exhibited that the hot watery green tea extract is more effective against bacterial growth than cold watery green tea extract, based on assessment of inhibition zones. The *E. coli* growth is sensitive to both extraction of green tea, the inhibition zone (2mm) begin from the lowest concentration (12.5 µg/ ml).

Table 2: Antibacterial effect of cold and hot watery green tea extract

Bacteria isolate	Cold Watery green tea extract Inhibition zone in (mm)	Hot Watery green tea extract Inhibition zone in (mm)		Antibiotic VAN.

	Concentration of extract											(Control)
	12.5	25	50	75	100	12.5	25	50	75	100		
E. coli	2	4	10	12	20	2	5	11	15	23		22

The minimum inhibitory concentration of cold and hot green tea watery extracts has shown good results against *E. Coli*, as an antibacterial (Table 2). The antibacterial activity of different green tea extracts was tested against *E. coli* exhibited activity at different extract concentrations. These extracts are rich in alkaloids, phenolic compounds, flavonoids, and tannins. Identical results were found in previous study, (Al-Matani *et al*., 2015)[27], there results showed that extracts effect on *E. coli* growth with inhibition diameters of 0 to 9mm. And the MIC results of the water extract green tea against *E. coli,* 2000 and 8000 µg/ml, respectively (Shohayeb and Halawani, 2012) [28]. The lowest MIC of boiled water of green tea showed that extracts have a stronger antimicrobial ability and may be due to increased catechin presence (Tiwari *et al*., 2005) [29]. Studies with *E. coli and Pseudomonas aeruginosa* have shown that green tea catechins binding to the bacterial cell membrane can result in generation of H2O2 which is involved in damage to the cell membrane(Xiong *et al*.,2017) [30]

Green tea is one of the most popular drinks consumed worldwide. Produced mainly in Asian countries from the leaves of the *Camellia sinensis* plant, the potential health benefits have been widely studied. Recently, researchers have studied the ability of green tea to eradicate infectious agents and the ability to actually prevent infections(Reygaert *et al*.,2018)[31] Green tea has been shown to have ant carcinogenic, anti-inflammatory, antimicrobial, and antioxidant properties and is beneficial in cardiovascular disease (CVD), diabetes and obesity, and neurologic and oral health. The ant carcinogenic properties include controlling cell proliferation, apoptosis and angiogenesis in tumor cells (Crew *et al*.,2015) [32] Oxidative stress results from the damaging effects of reactive oxygen species (ROS). The antioxidant properties of green tea include the ability to limit the amount of free radicals by binding to ROS, up regulating basal levels of antioxidant enzymes, and increasing the activity of these antioxidant enzymes (Newsome *et al*.,2014) [33]

Table (3) illustrated the effects of Black Tea Extraction (Cold Watery and Hot Watery) concentrations effect on the bacterial growth (*E.coli*), presented that the *E. coli* growth, appeared the hot black tea extract cause the inhibition zone (5mm) begin from the lowest concentration (12.5 µg/ ml), and the cold black tea extract cause the inhibition zone (4) began from third extract concentration (50 µg/ ml).

Table 3: Antibacterial effect of cold and hot watery black tea extract Bacteria isolate

	Cold Watery Black Tea Extract Inhibition zone in (mm)					Hot Watery Black Tea Extract Inhibition zone in (mm)					Antibiotic VAN. (Control)
	Concentration of extract										
	12.5	25	50	75	100	12.5	25	50	75	100	
E. coli	-	-	4	6	9	5	7	12	14	19	22

Results of current study showed that hot black tea had greater potential activity than cold black tea extraction. Active substances in different concentration extracts cause inhibition zones in *E. coli* growths. Water extract of black tea showed effect on these bacterial growth (table 3). Highest concentration extract exhibit the higher efficiency of antimicrobial as compared a few previous studies that mentioned that water extract exhibits the superior antimicrobial activity (Kaur *et al*., 2015) [34].Identical results have been obtained by other researchers (Novy *et al*., 2013) [35]. These findings confirmed that herbal teas could be a prophylactic or first-aid source for bacterial infections (Hacioglu *et al*., 2017) [36].The aqueous, black tea extract had a powerful antibacterial activity against E. coli with 0.31 and 0.15 mg / ml of best MIC, respectively (Al-Bayati and Al-Mola, 2008) [37]. Black tea improves oral health by inhibiting the growth of bacteria and reducing the incidence of dental cavities. Thus, it can be used as a natural treatment for periodontal disease (Sen and Bera, 2013) [38]. In addition to the direct antimicrobial effects of catechins in tea (damage to the bacterial cell membrane, inhibition of fatty acid synthesis, inhibition of enzyme activity, etc.), there are also some effects that may contribute to the total antimicrobial effect in infected individuals. These effects include inhibition of inflammation (particularly inflammation caused by oxidative stress, such as vascular), more specifically, by increasing the synthesis of nitric oxide (Yamakuchi *et al*., 2008) [39].

Although Minimum inhibitory concentration (MIC) is still the gold standard for determining the antimicrobial activities of agents (Hacioglu *et al*., 2017) [36]. Black, and green teas (hot and cold extracts) are effective against bacterial growth *E.coli*. We demonstrated that the best MIC against *E.coli* is hot green tea extract (400 µg/ml) then hot black tea extract (600 µg/ml) followed by cold green tea extract (800 µg/ml) and less activity is cold black tea extract (1000 µg/ml) (Table 4).

When compare the effectiveness of hot and cold of black and green teas extracts on depending on MIC, and we concluded that hot extract from two types of teas have the best antibacterial effect than the cold extract of black and green extracts.

Table 4: MIC for cold and hot watery extract for both green and black teas

Bacteria isolate	MIC µg/ml			
	Cold Black Tea Extract	Hot Black Tea Extract	Cold Green Tea Extract	Hot Green Tea Extract
E. coli	1000	600	800	400

Table 4 summarizes the MIC values of black and green teas active in cold and hot activities against clinical and standard bacterial strains. These assumptions can be traced to green tea and black tea containing polyphenols and catechin compounds. It has been found that these compounds have antibacterial activity (Othman *et al*., 2019) [(26).] *E. coli*, were found to be sensitive to green tea and black tea extracts (hot and cold) .Teas of *C. sinensis* undergo different manufacturing processes. Green tea is produced by steaming (Japan) or panning (China) to prevent catechin oxidation by polyphenol oxidase (Graham 1999) [(40)]With no fermentation, green tea leaves retain their green color and almost all of their original polyphenol content while black tea is fully fermente.The different processes of manufacturing give the various teas their characteristic colors and flavors. Oolong tea has an excellent characteristic combining the freshness of green tea and the fragrance of black tea(Wan *et al* ., 2008) [(41).]Similar findings have been reported earlier. Several studies have shown *that* catechins from green and black teas, particularly EGCG and ECG, inhibited the growth of many bacterialspecies (Hamilton-Miller *et al*.,1995) [(42)]Contrary to findings from this study, Extracts of green tea have been reported to be more effective in inhibiting bacterial growth than black tea (Tiwari *et al*., 2005) [(43)]In general, antibacterial activity decreased when the extent of tea fermentation increased (Almajano et al.,2006) [(44)] (Gramza and Korczak, 2005) [(49)]hypothesized that antimicrobial activity of tea extracts could be due to the fact that the negatively charged EGCG binds strongly to the positively charged lipid bilayer of bacteria. Catechins partitioning in the lipid bilayer membrane result in loss of cell structure and function and finally result in cell death. Bacterial cell membrane damage inhibits the ability of the bacteria to bind to host cells and to each other to form biofilms. Bacterial cell membrane damage also results in an inability of the bacteria to secrete toxins. Other researchers have also pointed out that the antibacterial effect of catechins could be due to their inhibitory effect on certain proteins (Okamoto *et al*.,2003) [(45)] or it may also be due to iron deprivation or hydrogen binding with vital proteins such as microbial enzymes (Cushnie *et al*., 2011) [(46)]These supports the fact that polyphenols are responsible for the antimicrobial activities of the tea extracts.

These our results were found to be contravene with earlier results, In a study, all extracts and fractions of green, black, and herbal teas showed no antibacterial activity against Gram-negative *E. coli*, *S. typhi*, and *P. aeruginosa*. The inhibition of tea extracts against *P. aeruginosa* and *E. coli* has been reported (Almajano *et al*.,2008)(44)although an earlier study has explicitly reported that tea extracts are not effective against *P. aeruginosa* and *E. coli* (Toda *et al*.,1889) [(47)]The disparity in findings could be due to different strains of bacteria used, and to the different concentrations and types of extracts investigated. Gram-negative bacteria are less susceptible to antibiotics as their outer membrane of lipoproteins and lipopolysaccharides is able to regulate the access of antibacterial agents into the underlying structures.

Effect of green tea extract and black tea extract on rat liver and kidney tissue

Green Tea Extract and Black Tea Extract were seen in rat kidney and liver treatment-related changes. The reality because both the kidney and the liver, two organs rich in metabolic activity, were the main target tissues in the current study leads us to believe that metabolism and activation of green tea extract and black tea extract, at least in part, are responsible for the adverse effects observed (Chen *et al*., 2010) [(48).] In our existing research, rats were more hardly affected after infected with *E. coli* bacteria, but when treated with green tea extract and black tea extract lead to improve histopathological lesions in the liver, and kidney.

Effect of green tea and black tea extract on kidney and liver tissue of rat infected with pathogenic bacteria

Escherichia coli are bacteria that live in the guts of animals and people, and can be shed in feces, causing serious disease (Osman *et al*., 2016) [(26).] Acute inflammation was indicated by the inflammatory reactions, which consisted mainly of mononuclear cells. *E. coli* non-specific exotoxin Intracellular signaling disturbance leading to cell death As the number of bacteria that damage the integral barrier increases, these colonizing bacteria invade and skin and soft tissues develop (Ki and Rotstein, 2008).[(49)] It has been reported that 46%, 25%, and 21% of *E. coli* isolates that cause wound infections are resistant to ampicillin, tetracycline, and fluoroquinolones, respectively. *E. coli* isolates have many virulence factors that include outer membrane protease, cytotoxic necrotizing factor 1, drug resistance, and hemolysin (Welch, 2016) [(50).]

The most common caused infection, *E. coli* is a urinary tract infection that normally spreads from the intestine to the urinary tract. *E. coli* is also the most common cause of cystitis and may cause pyelonephritis in a minority of the infection. (Cristea *et al*., 2017) [(51).] Also in rats infected with *E. coli*, was distortion of the renal Bowman`s capsule and the glomerulus, which is responsible for the filtration of plasma (Osmana *et al*., 2016[(26).], also severe breakage of the basement membrane of Bowman`s capsule, and fatty degradation in kidney. These are as a result of *E. coli* being able to produce cytotoxic toxin which has a cytotoxic effects on cells of the kidney or due to immune response to the production of toxin by *E. coli* (Abin, *et al*., 2010) [(52),] same changes detected in the current research.In rats infected with *E. coli*, separation in the sinusoid of the liver tissue, as a result of production of

cytotoxic toxins by *E. coli* which have cytotoxic effects on the sinusoid in the tissues and the endothelial cells of the central veins thereby leading to their breaking down (Anibijuwon *et al*., 2017) [53]. On the other hand, *E. coli* caused necrosis of the hepatocytes around the central vein, which appears associated with inflammatory lymphocytic infiltration (Osmana *et al*., 2016) [26], as a result of immune response to the production of toxins by *E. coli.* There is an evidence of hemorrhage and lysis of cells (Anibijuwon *et al*., 2017) [53]. Necrosis and apoptosis of the hepatocytes; and hemorrhagic, which may be caused by intravascular hemolysis by toxin produced from *E. coli* causing vascular damage (Al Zamely and Zfalh, 2011) [54], this is also proved by our results.

Effect of green tea extract and black tea extract on rat kidney tissue

Histologically histological structure of kidney was normal in healthy control cohort of rats (first group), of kidney Bowman's capsule, proximal and distal convoluted tubules (Figure 1).the second cohort of rats that infected with *E. coli* bacteria, the kidney appears sever hemorrhage in glomeruli and tubules, some glomeruli nearly completely and firmly destructed, dilated tubular (figure 2) and show sever hemorrhage in glomeruli and tubules, destructed of endothelial cells of Bowman's capsule with inflammatory cell infiltration, highly dilated tubules (figure 3). The third cohort of rats that infected with *E. coli* bacteria, when treated with black tea extract the kidney shows restoration of approximately normal features of glomeruli mild dilatation and hemorrhage proximal and distal convoluted tubules as compared to *E. coli* infected cohort (figure 4). The fourth cohort of rat which infected with *E. coli* bacteria, after treated with green tea extract, the kidney shows somewhat restoration of approximately normal histological feature of glomeruli, proximal and distal convoluted tubules, and hemorrhage level less than control rat infected with *E. coli* bacteria (Figure5).

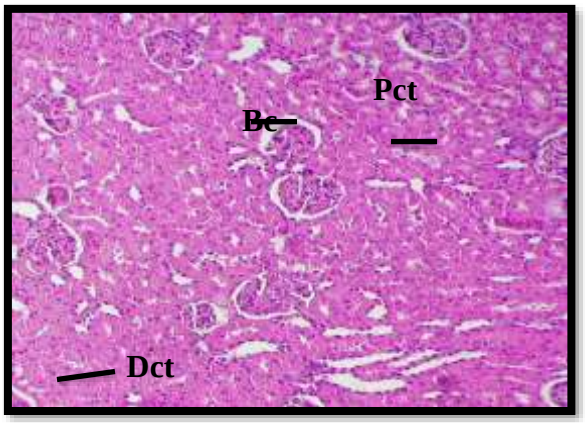

Figure 1: Photomicrograph from control rat shows the normal histological structure of kidney include: Bowman's capsule (Bc), proximal (P ct), distal convoluted tubules (Dct), (100x. H and E).

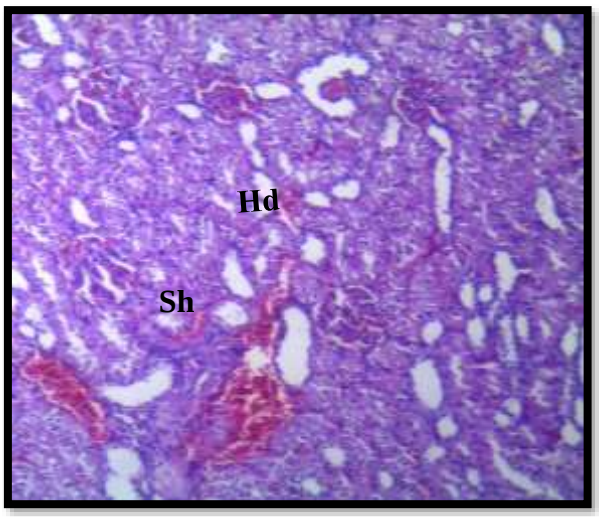

Figure 2: Photomicrograph from rat kidney infected with E. coli bacteria shows sever hemorrhage (Sh) in glomeruli and tubules, some glomeruli nearly completely and firmly destructed (G dis) , dilated tubules (Hd) (100X. H and E).

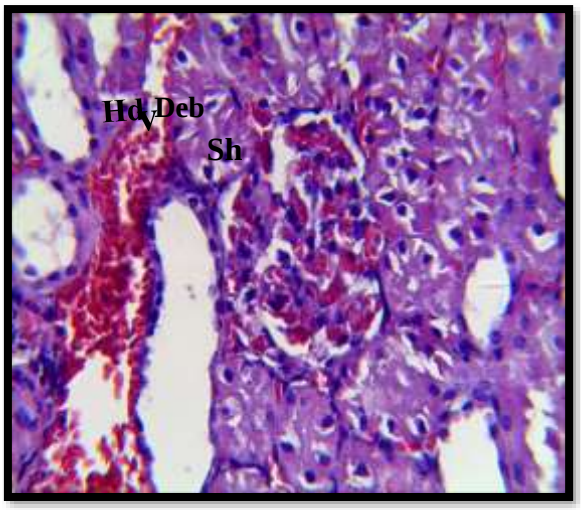

Figure 3: Photomicrograph from rat kidney infected with E. coli bacteria shows sever hemorrhage (Sh) in glomeruli and tubules, destructed of endothelial cells of Bowman's capsule (Deb) with inflammatory cell infiltration(I), highly dilated tubules (Hd) (400X. H and E).

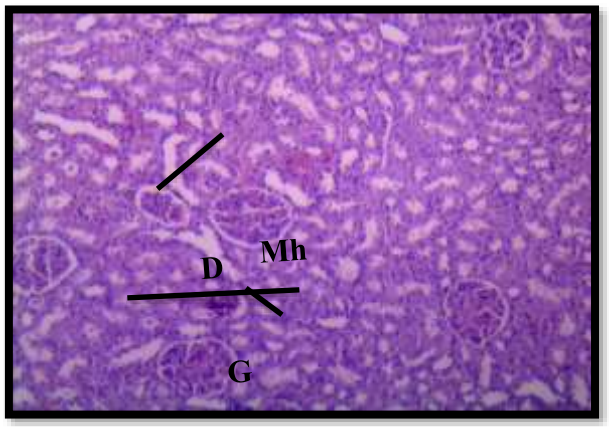

Figure 4: Photomicrograph from rat kidney infected with E. coli bacteria and treated with black tea extract shows restoration of approximately

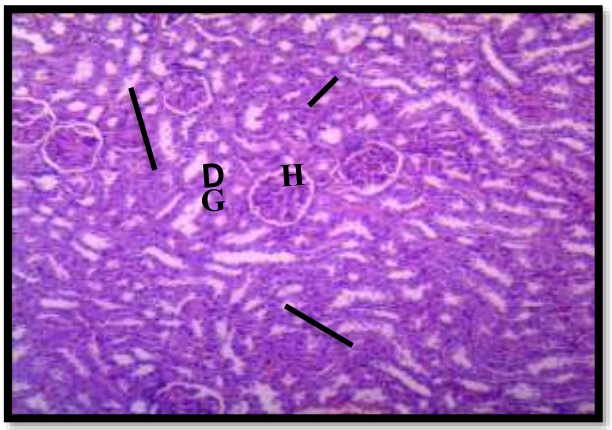

Figure 5: Photomicrograph from rat kidney infected with E. coli bacteria and treated with green tea extract shows somewhat restoration of approximately normal histological feature of glomeruli (G), dilated proximal and distal convoluted tubules (D), hemorrhage level (H) less

The cohorts of ras that infected with *E. coli* bacteria, when treated with green tea extract and black tea extract, the kidney shows restoration of approximately normal features of glomeruli proximal and distal convoluted tubules, and decreased hemorrhage level. In line with this our findings, In a study by (Mohamadin *et al*. 2005) [55] investigating the

renoprotective effect of green tea extract following Cyclosporine A (CsA) induced nephrotoxicity, it was found that administration of green tea extract was associated with renoprotective effects . Other studies have shown beneficial effects on kidney function including reduction in diabetes-induced hypertrophy and nephropathy of kidneys (Juśkiewicz et al.,2008) [56].

Phytochemical EGCG of green tea has been demonstrated to have a protective role against acute kidney injury regardless of the etiology. Bao and colleagues addressed the beneficial role of EGCG in renal damage induced by iron overload. They demonstrated that EGCG could act as an iron chelator (siderophore) by forming a stable complex with iron and neutrophil gelatinase associated lipocalin , resulting in reduction of chemical reactivity of iron-reactive oxygen species (ROS) and, subsequently, protection against renal injury (Bao *et al*.,2013) [57]. Two other studies have identified renoprotective effects of EGCG in acute kidney injury induced by cardiopulmonary bypass operation . EGCG was also shown to improve renal function and reduce hypoxic damage and nitrosative stress . The antioxidative and antiapoptotic properties of EGCG were suggested to be responsible for reversing such adverse effects of cardiopulmonary bypass (Twal *et al*.,2013 [58]. Similarly, another study demonstrated that pretreatment with EGCG could ameliorate cardiopulmonary bypass induced acute kidney injury in diabetic rats (Funamoto *et al*., 2016) [59]. EGCG prevented renal tubular damage and reduced amounts of kidney injury.

Effect of green tea extract and black tea extract on rat liver tissue

When made photomicrograph to control cohortof rats (first cohort) the liver appearance the normal histological structure of hepatic lobule, normal central vein sinusoids (figure 6).

Histopathologically, liver of infected rat with *E. coli* bacteria (second cohort), the changes incudes sever hemorrhage, highly tissue destruction, and hepatic drainage, reduced cellularity as compared to normal control rat liver (figure 7). Also the infected liver appears sever hemorrhage and vascular dilatation, highly tissue destruction and hepatic drainage, reduced cellularity and necrotic inflammation (figure 8). But when the infected liver of rat with *E. coli* bacteria (sixth cohort), when treated with black tea extract shows restoration of approximately normal features of hepatocytes, increase cellularity mild hemorrhage in central vein and sinusoids as compared to positive control (figure 9). Finally, in seventh cohort, the pathologically liver of infected rat with *E. coli* bacteria, that treated with green tea extract appears mild sinusoidal dilatations, increase cellularity hemorrhage in central vein and sinusoids (figure 10).

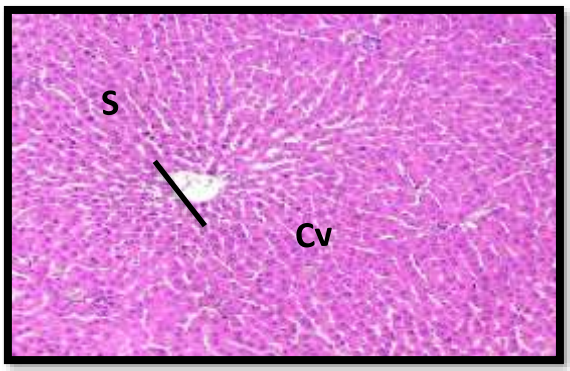

Fig.6: Photomicrograph from control rat liver shows the normal histological structure of hepatic lobule, normal central vein (Cv) sinusoids (S) (100x. H and E).

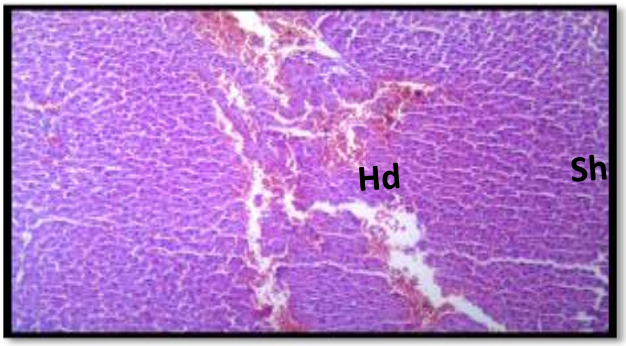

Figure 7: Photomicrograph from control rat liver infected with E. coli bacteria shows sever hemorrhage (Sh), highly tissue destruction and hepatic drainage (Hd), reduced cellularity (100x. H and E).

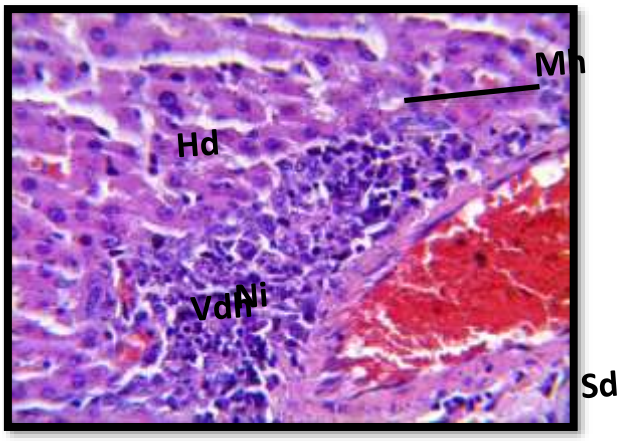

Figure 8: Photomicrograph from control rat liver infected with E. coli bacteria shows sever hemorrhage and vascular dilatation (VdH), highly tissue destruction and hepatic drainage (Hd), reduced cellularity and necrotic inflammation (Ni) (100x. H and E).

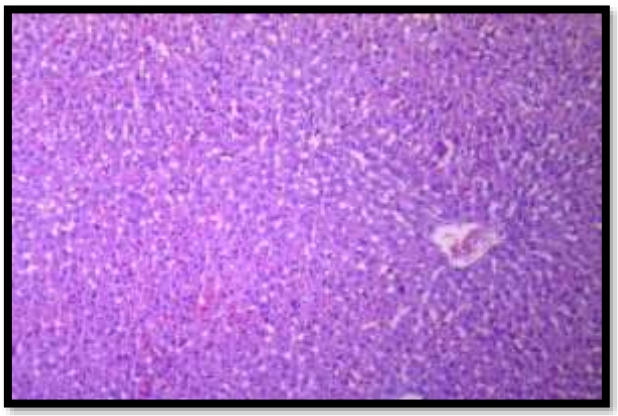

Figure 9: Photomicrograph from rat liver infected with E. coli bacteria and treated with black tea extract shows restoration of approximately normal features of hepatocytes, increase cellularity, mild hemorrhage (Mh) in central vein (Cv) and mild sinusoidal dilatations (Sd) as compared to positive control, (100x. H and E).

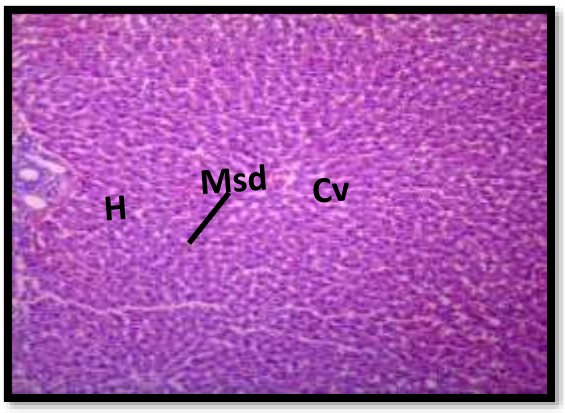

Figure 10: Photomicrograph from rat liver infected with E. coli bacteria and treated with green tea extract shows mild sinusoidal dilatations (Msd), increase cellularity, hemorrhage (H) in central vein and sinusoids (100x. H and E).

The effect of different doses from water extract prepared from green tea, and black tea for treatment the rats infected with *E. coli* bacteria, appears restoration of nearly normal histological structure of hepatocytes and increase cellularity and reduced hemorrhage as well as hepatic drainage. This result is congruent with the observations published by (Ramesh *et al*., 2007) (60), finding is congruent with our findings, data demonstrated that the therapy of green tea extract significantly improves the histopathological status of liver function rats. The protective effects of tea extracts or tea polyphenol against liver fibrosis and liver cirrhosis in rats have been reported (Li *et al*.,2004) (61), and confirmed when

a study on the hepatotoxicity of high concentration of the tea on Wistar rats was found to be safe. Green tea has also been found useful in the treatment of other body ailments. The polyphenols contained in the tea are antimutagenic and anticarcinogenic by inhibiting cancer cell proliferation and induction of apoptosis (Fujiki *et al*., 1999) (62)

Also, these our result is consistent with several previous studies have reported a protective effect of green tea on rats ' Results of numerous studies suggest the protective effects of green tea against various poisons and medicines. Mantal et al. studied male albino rats and found that green tea extract could significantly reduce ALT and AST in rats that received the insecticide Fenitrothion (Chen *et al*., 2004) (48) and his research team concluded that taking green tea extract could significantly reduce aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP)levels in rats with liver failure induced by leflunomide (Khorsandi *et al*., 2010) (63)Green tea extract has antioxidant and free-radical-scavenging properties (Crespy *et al*.,2004)(64) Reduced levels of the mentioned enzymes following green tea extract administration may result from the antioxidant properties of the extract and from the resultant prevention of intracellular enzymes from leaking out of cells due to cellular stabilization or regeneration (Manal *et al*., 2008) (65) In a study conducted by on immature male Balb/C rats, it was found that injecting these rats with green tea extract could improve liver failure caused by taking acetaminophen due to the strong antioxidant effects of the extract (Sai *et al*.,1998) (66-)

A source of active and efficient medicinal agents has been provided in nature. Based on the usage of natural products in traditional medicine, a large number of modern medicines have been extracted from natural sources (Cragg and Newman, 2002) (67). The general safety of green tea and its catechins have been demonstrated by both human and animal studies]. Consistent with this finding, histochemical observations revealed that the degree of hepatic injury was suppressed by green tea in the galactosamine-treated group. Suppression of these factors may also be involved in the mechanism by which green tea and catechins exhibit their preventive effect on other diseases including cancer and neurodegenerative diseases (Abe *et al*., 2005) (68). Green tea (Camellia sinensis) which is a product of dried leaves has been consumed by East Asian people for health promotion since 3000 B.C. (Kim *et al*., 2008) (69)Abundantly found in Asia, green tea is also one of the most prevalent drinks worldwide. sample evidence indicates that this plant, with anti-oxidant,anti-cancer, anti-aging, and anti-inflammatory effects, could also prevent exaggerate collagen production and accumulation and induce changes in immune responses, as well (Park *et al*., 2008) (70)

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