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Israa M. Mohammed<sup>1</sup>;

Sawsan M. Abdulla Surchee<sup>1\*</sup>;

Wala'a T. Yousif<sup>2</sup>

<sup>1</sup> Department of Biology, College of Education, Salahaddin University

<sup>2</sup> College of Health and Medical Technology/Baghdad, Middle Technical University

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## In vivo, Antibacterial Effect of Green and Black Tea Extracts on Infected Liver and Kidney of Rats

### ABSTRACT

The aim of this study is to investigate the antibacterial effect of hot and cold aqueous extracts of green and black tea separately against *Klebsiella pneumoniae* after infecting the burned rat's skin with it, also to screen the effect of both types of tea on rat's liver and kidney. Sixteen male rats divided into four Groups (4 animals each), Group 1: untreated rat (control). Group 2: burn rats infected with *K. pneumoniae* ( $1.5 \times 10^8$  bacteria/ml). Group 3: burn treated by Green tea aqueous extract (1000 µg/ml) for *K. pneumoniae* bacteria. Group 4: burn treated by black tea extract (2000 µg/ml) for *K. pneumoniae* bacteria. The tea extracts antimicrobial activity was analyzed by agar diffusion inhibition assay and minimum inhibitory concentration. Results exhibited that *K. pneumoniae* in particular, have high levels of antimicrobial resistance to widely used agents. In conclusion hot extract from both types of tea have better antibacterial effect than cold extract. Additionally, infected rats showed inflammation and sever hemorrhage in the liver and kidney of rats, but when treated with tea extracts dermally approximately restored kidney normal histological architecture of glomeruli and tubules, also liver became nearly normal in histological structure of hepatic foci and central vein. Black tea extract showed greater effectiveness in tissue recovery than green once.

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## INTRODUCTION

Today, in pharmacology and medicine fields, green pharmacy plays a significant role. About 80% of the world's population is expected to rely on botanical preparations as medication to satisfy their health requirements (Sureshkumar *et al.*, 2013). In fact, the growing concentration of drugs to botanical pharmacy may also be due to the advent of bacteria of antibiotic resistance, side effects and financial concern. The alarming situation regarding the continuous rise in microorganisms of antibiotic resistance worldwide, resulting in enhanced disease followed by death (Ponmurugan *et al.*, 2016) and illustrating the search for a new antimicrobial agent (Stepanović *et al.*, 2003).

There are three main types of tea: depends entirely on the rate of tea sold abroad worldwide, where 78% is black, 20% green and 2% oolong. The black tea main aflavins are theaflavin, 3-gallate aflavin, 3-gallate aflavin, and 3-gallate aflavin. The aflavins are orange-red compounds that are responsible for black tea astringent flavor and copper color. Tea polyphenols are known for their antibacterial activity in addition to their antioxidant properties. Overall, when the extent of tea fermentation is prolonged, antibacterial activity is minimized, implying stronger activity in green tea than black tea. Green tea catechins, particularly epigallocatechin gallate EGCG and ECG, have antibacterial activity for both Gram-positive and Gram-negative bacteria. Through inhibiting oral

\* Corresponding author: E-mail: [sawsan\\_sorchee@yahoo.com](mailto:sawsan_sorchee@yahoo.com)

bacteria, green tea will prevent tooth decay. Black tea antibacterial activity was also reported (Chan *et al.*, 2011).

Additionally, tea is produced from *Camellia sinensis* leaves and is one of the world's most frequently consumed drinks. Green tea contains gallic acid, quercetin, kaempferol, myricetin and chlorogenic acid along with some amount of caffeine although half of that found in coffee (Singhal *et al.*, 2017). Green tea is an un-fermented / un-oxidized tea (produced to inactivate polyphenol oxidase by drying and steaming the fresh leaves). While oolong tea is a moderately-fermented tea (obtained when the fresh leaves are exposed to a partial fermenting phase before drying); and black and red tea (Pu-Erh) are fermented (which are subjected to a post-harvest fermentation phase before drying and steaming, although the fermentation of black tea is due to an oxidation catalyzed by polyphenol oxidase and that of Pu-Erh tea is gained by using microorganisms) (Cabrera *et al.*, 2006). The histopathological analysis is a great tool for assessing the state of the infection and provides the basis for its care. Many infections that are feasible in the cell originate from bacteria, fungi and viruses, the most popular and easiest to cure are bacterial infections (Alzoreky, 2003). The aim of this study is to investigate the antibacterial effect of hot and cold extracts of green and black tea separately against *K. pneumoniae*, after infecting the burned skin rat with this bacteria 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 aqueous extract preparation:**

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

### **3-Preparation of inoculums:**

Bacterial suspensions 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  $1 \times 10^6$  CFU / ml. It was achieved by adapting the bacterial suspension's optical density to 0.5 McFarland turbidity standards (MacFaddin, 2000).

### **4-Antibacterial susceptibility:**

Different doses are provided for each plant extract 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. The bacterial 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).

### **5-Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

Nutrient broth was incorporated with control tube contained only bacteria 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).

#### 6-Sensitivity to antimicrobial agents:

The isolates are checked using the Kirby-Bauer standardized single-disk approach against various antimicrobials (Bauer, 1966 ; NCCLS, 2002)

#### 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 ethanol (70 %) was used as antiseptic for the shaved region and then burned by using inflamed knife and then contaminated with *K. pneumoniae* and ( $1.5 \times 10^8$  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 *K. pneumoniae* at the shaved region.

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

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

The treatment continued twice each day during 15 consecutive days and the numbers of bacteria was calculated (Khayyal, *et al.*, 1993 ; Umachigi, *et al.*, 2008).

#### 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), sacrificed at the end of experiment. Liver and kidney divided into small pieces (less than 0.5cm<sup>3</sup> 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).

## RESULTS AND DISCUSSION

### Antibiotics Susceptibility of *K. pneumoniae*

There has been extensive use of wide-spectrum antimicrobial agent over the past several decades to meet the growing problem of treatment. Such microbes, however, have evolved several antimicrobial resistance mechanisms, including increased drug efflux, drug target shifts, and the development of plasmid-mediated β-lactamases (Tenover, 2006). The standard feature of

antimicrobial resistance is because there are always large temporary and local discrepancies (El Bouamria *et al.*, 2015).

Antimicrobial susceptibility testing has shown variable levels of resistance to the tested antibiotics, our research finding, *K. pneumoniae* in general, have high rates of resistance to the commonly used antimicrobial agents (Ancef AN (48%), Aztreonam ATM (77%), Cefazolin CZ (80%), Cefotaxime CTX (77%), Ceftazidime CAZ (80%), Trimethoprim TMP (70%), piperacillin/tazobactam T—ZP(70.45%), amoxicillin/clavulanate AMC (84.09%), Cefepime FEP (77.27%), Foxitin FOX (84.09%) , Gentamicin GM (77.27%), and Tetracycline TC (81.82%)). And show low resistance to Ciprofloxacin CIP (20.45%) and carbapenem antibiotic (Imipenem IMP (12%) and Meropenem MEM (14 %)).

**Table 1.** Antibiotics Susceptibility of *K. pneumoniae*

Antibiotic		<i>K. pneumoniae</i>		
		R	I	S
Ancef (AN)	%	48.00	9.00	43.00
Aztreonam (ATM)	%	77.00	3.00	20.00
Cefazolin (CZ)	%	80.00	2.00	18.00
Cefotaxime (CTX)	%	77.00	5.00	18.00
Imipenem (IMP)	%	12.00	2.00	86.00
Meropenem (MEM)	%	14.00	2.00	84.00
Ceftazidime (CAZ)	%	80.00	2.00	18.00
Trimethoprim(TMP)	%	70.00	10.00	20.00
Piperacillin-Tazobactam (T---ZP)	%	70.45	0.00	29.55
Amoxicillin (AMC)	%	84.09	0.00	15.91
Ciprofloxacin (CIP)	%	20.45	11.36	68.18
Cefepime (FEP)	%	77.27	0.00	22.73
Foxitin (FOX)	%	84.09	0.00	15.91
Gentamicin (GM)	%	77.27	0.00	22.73
Tetracycline (TC)	%	81.82	0.00	18.18

Green and black tea were made from the same leaves of the Chinese tea tree is the *Camellia sinensis* plant (Singhal *et al.*, 2017), and can be made into a beverage or an extract. Green tea made from unfermented leaves which aids to protect its antioxidant polyphenolic catechols, while black tea made from fermented leaves. Green tea may have beneficial effects on the liver, kidney, spleen, etc. although it isn't a good idea to drink large amounts of it (Hacioglu *et al.*, 2017).

Green tea has long been known to have antioxidant capacity to restore health properties and its ingredients. For this reason, green tea extracts were used alone as a herbal medicine and in conjunction with other herbals and dietary supplements marketed to improve health, prevent cancer and heart disease, minimize serum lipid levels and encourage weight loss, Reduce periodontitis or even treat diarrhea by *Clostridium*. Green tea extract (GTE) is included in over 100 herbal products over - the-counter, but is not licensed for any genuine medical use and is not monitored in terms of effectiveness and health. Multiple polyphenols in green tea are thought to be the active ingredients accountable for their alleged chemical, anti-proliferative and antioxidant assets (Othman *et al.*, 2019).

Although resistance to antibiotics production has been listed in a majority of gram-negative rods, most likely *K. pneumoniae* (El Bouamria *et al.*, 2015). Antimicrobial susceptibility testing of *K. pneumoniae* isolated showed a high resistance to commonly used antimicrobial agents. These our result are (table 1), consistent with previous findings reported by El Bouamria *et al.*, (2015), the bacterial resistance rates of *K. pneumoniae* isolates were as follow: trimethoprim–sulfamethoxazol (61%), amoxicillin/clavulanic acid (51%), ciprofloxacin (32 %), and Gentamicin (89%). And Sarathbabu *et al.*, (2012), found the *K. pneumoniae* resistance to Cefotaxime (46.73%), Gentamicin (38.56%), and Tetracycline (57.09%). Similarly, Abreu *et al.*, (2012), documented *K. pneumoniae* is

in charge for resistance against tetracyclines. Inhibitors of efflux pumps would retain the susceptibility to antibiotics (Othman *et al.*, 2019).

All *K. pneumoniae* isolates are extremely resistant to amoxicillin and ampicillin due to A  $\beta$ -lactamase (El Bouamria *et al.*, 2015). Beta-lactam resistance occurs in the cell walls of the bacteria by targeting penicillin-binding protein (PBP) enzymes. A significant therapeutic result would be the inhibition of penicillin-binding protein (PBP) (Abreu *et al.*, 2012). This is achieved by some agents either alone or by synergy, by blocking targets along the metabolic pathway, thus initiating cell death. Other antibiotics also increased PBP activity, such as cephalosporins and carbapenems (Shin *et al.*, 2018). Inhibiting bacterial enzymes which really deactivate antibiotics: beta-lactamases that entangle methicillin and related penicillins are the most common example of antibiotic inactivation. For Gram negative bacteria, this type of resistance is also used (Othman *et al.*, 2019).

The acquisition *K. pneumoniae* of Amoxicillin – clavulanic acid (AMC) resistance is a worldwide issue with varying rates of incidence. In our research exciting, it has been revealed that antimicrobial resistance to AMC is similar to that documented in Rabat (Morocco) (Tlamcani *et al.*, 2009) and Marrakech region (El Bouamria *et al.*, 2015), and in Algeria (50 %) (Bouzenoune *et al.*, 2009). In Tunisia, non-susceptibility to AMC was twice almost lower (23.7%), as reported by Ben Haj Khalifa and Khedher, (2012). Also agree with previous study in Uganda (Najjuka *et al.*, 2016).

In Marrakech, Benouda *et al.*, (2010), noticed that carbapenem-resistant *K. pneumoniae* appeared. *K. pneumoniae* strains demonstrates cross-resistance to both ertapenem and imipenem, implying highly carbapenem-resistant clonal spread. While a high susceptibility to carbapenem has been demonstrated worldwide, it is noteworthy that the efficacy of carbapenem therapy is speculated to become more limited since the emergence of carbapenem-resistant organisms. However, Barguigua *et al.* (2011) demonstrated the patterns in the growth of carbapenem-resistant Enterobacteriaceae with resistance gene carriage.

In this study, *K. pneumoniae* possessed an extreme level of resistance (84.09%) to amoxicillin/clavulanate, gentamicin and T/S. 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).

The flow table (2) of the investigation is summarizes the effects of cold and hot aqueous green tea extract concentrations on the bacterial growth *K. pneumoniae* exhibited that the hot aqueous green tea extract is more effective against bacterial growth than cold aqueous green tea extract, based on assessment of inhibition zones. The growth of *K. pneumoniae* has sensitivity to both aqueous green tea extract (cold and hot), the inhibition zone (5mm and 6mm respectively) began from the third concentration (50  $\mu$ g/ ml).

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

Bacteria isolate	Cold aqueous green tea extract Inhibition zone in (mm)					Hot aqueous green tea extract Inhibition zone in (mm)					Antibiotic VAN. (Control)
	Concentration of extract										
	12.5	25	50	75	100	12.5	25	50	75	100	
<i>K. pneumoniae</i>	-	-	5	11	13	-	-	6	13	14	16

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. Teas, particularly those containing catechin, are known to have many health-promoting properties, such as antibacterial, antifungal and antiviral (Bansal *et al.*, 2013). The catechin's antimicrobial activity has already been described against such a number of species, including multi-resistant medical isolates

of gram-negative and gram-positive bacteria and also yeasts (Gordon and Wareham, 2010; Park *et al.*, 2011).

Bacterial growth inhibition without harming the host cells is the qualities required for a safe and powerful therapeutic agent. The plants (e.g. green and black teas) have historically been used in folk medicine in many Arab countries to treat various pathogens and diseases for their medicinal function (Al Garni and Hafez, 2015).

Tea as well as its ingredients have many wellbeing-promoting capabilities, such as cardiovascular disease safety, obesity and diabetes regulation, and anti-aging, anti-arthritis effects (Hacioglu *et al.*, 2017). Tea also is beneficial in delaying cardiovascular disorders; decreasing postprandial plasma cholesterol and triglycerides levels (Obaid *et al.*, 2011), improve respiration, heart muscle (Alipoor and Rad, 2012) and endothelial function (Obaid *et al.*, 2011). The main bioactive (non-microbiological activities) compounds of teas types like catechin and polyphenol effect on inhibiting pathogenic growth (Cabrera *et al.*, 2006), reducing inflammatory response and preserving viable tissue (Takaki *et al.*, 2008). Many of the physiological activities documented for tea extracts have been found to be due to their compounds. Some of the more interesting of these include activation of leukocytes function in various ways, antioxidant (Jazani *et al.*, 2007) and enhanced angiogenesis and deposition of collagen leading to expanded local circulation and tissue formation in granulation (Obaid *et al.*, 2011). Anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, antimutagenic and anti-carcinogenic activities (Tedeschi *et al.*, 2004). Increase in sympathetic activity (Taylor and Samson, 2005). Tea can play role in treatment of maldigestion, dental carries and neurodegenerative diseases like Parkinson and Alzheimer, also and protection from the effects of radiation (Alipoor and Rad, 2012). Tea has properties for detoxifying and reduction of alcohol and toxins, to improve blood and urine, and to improve resistance to diseases (Al-Rejaie, 2009).

The minimum inhibitory concentration of cold and hot green tea aqueous extracts has shown good results against *K. pneumoniae* growth in (Table 2). And the MIC results of the aqueous extract of green tea against *K. pneumoniae*, 8000 µg/ml, (Shohayeb and Halawani, 2012). MIC values of aqueous extract of green tea from *K. pneumoniae* varied from 9.7 to 19 µg / ml. The antibacterial activity may be correlated with their primary alkaloid (Çoban *et al.*, 2017).

Extracts of the native Syrian green tea demonstrated antimicrobial activity against *K. pneumoniae* strains. These extracts showed activity against *K. pneumoniae* with MICs 12.5 mg/ml (Haroun and Al-Kayali, 2016). 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).

Table (3) illustrated the effects of black tea extraction (cold and hot aqueous) concentrations effect on the bacterial growth *K. pneumoniae*, presented the both extraction have the same effect on the *K. pneumoniae* growth, the third extract concentration (50 µg/ ml) led to the inhibition zone (3mm) in bacterial growth.

**Table 3:** Antibacterial effect of cold and hot aqueous black tea extract

Bacteria isolate	Cold aqueous black tea extract inhibition zone in (mm)					Hot aqueous 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	
<i>K. pneumoniae</i>	-	-	3	7	11	-	-	3	7	12	16

Results of current study showed that hot black tea had greater potential activity than cold black tea extraction. Aqueous extract of black tea showed effect on this bacterial growth (table 3). Highest concentration extract exhibits the higher efficiency of antimicrobial as compared a few previous studies that mentioned that aqueous extract exhibits the superior antimicrobial activity (Malini *et al.*, 2014; Kaur *et al.*, 2015)

Identical results have been obtained by other researchers (Novy *et al.*, 2013). These findings confirmed that herbal tea could be a prophylactic or first-aid source for bacterial infections (Hacioglu *et al.*, 2017).

The aqueous, black tea extract had a powerful antibacterial activity against *K. pneumoniae*, 0.15 mg/ml of best MIC, (Al-Bayati and Al-Mola, 2008). Reciprocation, all such extracts appeared to have a considerable antibacterial activity with inhibition zone diameters ranging from 14.3 to 28.2 mm (Kianbakht and Jahaniani, 2003). Microbiologically active molecules in black tea extracts are reported to be the polyphenolic compounds including several catechins, mainly epigallocatechin gallate, and theaflavins (Mandal *et al.*, 2011).

Our result disagreed with finding reported previously, where aqueous extracts of black tea has shown little antibacterial activity against six bacteria isolated. (Kumar *et al.*, 2012). And Mughal *et al.*, (2010), recorded the aqueous extract of black tea was least bactericidal as compared to other solvent extracts and showed medium activity.

We hypothesized that the differences in the different teas' antimicrobial activities would depend on either the microbial strain type or the tea. Comparable, results have been gained by other authors (Novy *et al.*, 2013). These outcomes proposed that herbal teas for bacterial infections could be a prophylactic or first base therapy agent.

The antibacterial behavior of alkaloid extracts was tested against *K. Pneumonia* from the plant extract found in Iraq (Jasim *et al.*, 2015).

Herbal teas are the most common natural alternatives for the treatment of infectious diseases due to growing antibiotic resistance, and are gaining more popularity at the moment. The findings of the current study showed that *Camellia sinensis* ' hot and cold green and black teas extract indicates the existence of strong antibacterial activity, confirming its use against infection. Antimicrobial activity analysis was based on bacterial growth inhibition.

#### **The minimum inhibition concentration of the green and black tea extracts (cold and hot)**

Although Minimum inhibitory concentration (MIC) is still the gold standard for determining the antimicrobial activities of agents (Hacioglu *et al.*, 2017).

Black, and green teas (hot and cold extracts) are effective against bacterial growth especially (*K. pneumoniae*). We demonstrated the best MIC for hot green tea extract (800 µg/ml) followed by cold green tea extract and hot black tea extract (1000 µg/ml) and the less activity is cold black tea extract (2000 µg/ml) are effective against *K. pneumoniae* (Table 4), 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 aqueous 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
<i>K. pneumoniae</i>	2000	1000	1000	800

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). *K. pneumoniae* was from organisms which found to be sensitive to green and black tea extracts (hot and cold) (table 4).

It is understood from the results obtained that cold black tea extract undergoes the process of oxidation and loses the compounds of polyphenols (catechin). This can be attributed to the lower concentration

of cold black tea antimicrobial activity (Saikia *et al.*, 2006). Black tea and green tea could be used effectively and safely in antibacterial treatment according to these results (Hacioglu *et al.*, 2017).

These our results were found to be similar with the results of earlier researches, Archana and Abraham, (2011), who exhibited the green tea extract have greater antimicrobial activity than black ones. And Othman *et al.*, (2019), found that green tea extract have high antimicrobial activity and the least activity was found in black tea extract. Polyphenols and catechin compounds contains in GTE more that found in BTE could, have been found to possess antibacterial properties, which are highly sensitive to the oxidation process. Thus, polyphenols have a huge impact on the inhibition bacterial growth (Abbas *et al.*, 2017; Othman *et al.*, 2019).

Extracts of the native Syrian plants included (black and green teas) demonstrated antimicrobial activity against *K. pneumoniae* strains. When evaluating the synergistic effects of antibiotics, interactions between plant extracts were more effective against resistant strains, the strongest effect of teas against *K. pneumoniae* (Haroun and Al-Kayali, 2016).

Some polyphenol extracts from different types of teas were found to inactivate heat-labile enterotoxin-induced diarrhea *K. pneumoniae* (Verhelst *et al.*, 2013). Similarly, Darabpour *et al.*, (2011), exhibited effect of extracted teas against *K. pneumoniae*.

These our results were found to be contravene with earlier results, where *K. pneumoniae* were to be resistant to aqueous extracts of green tea and black tea (Kaur *et al.*, 2015). Also, in previous study done by Kumar *et al.*, (2012), where aqueous extracts of teas have shown little antibacterial activity against gram negative bacteria isolated. Subhashini *et al.*, (2010), detected aqueous extracts of tea samples were not effective to kill the bacteria.

In addition, earlier study by Hacioglu *et al.* (2017) reported the black and green teas were not effective against most of the microorganisms studied. The findings of the teas were bactericidal in their concentrations of use; nevertheless, they showed some bactericidal effect and that tea catechins had an antibacterial effect on bacteria and polyphenol concentration did not show much difference between different extractions of green tea.

### **Effect of green and black tea extracts on rat liver and kidney tissue infected with pathogenic bacteria:**

Green and black tea extracts 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 (Chan *et al.* 2010). In our existing research, rats were more hardly affected after infected with *K. pneumoniae* bacteria, but when treated with green and black tea extracts lead to improve histopathological lesions in the liver and kidney. *Klebsiella pneumoniae* found in gastrointestinal tract of humans and animals and associated with urinary tract infection, wound infection, bacteremia and nosocomial infections. Acute inflammation was indicated by the inflammatory reactions, which consisted mainly of mononuclear cells (Ki and Rotstein, 2008).

Pores may be found in the epidermis, contributing to folliculitis, furuncles, or carbuncles. Infection of the skin's surface layers is called erysipelas, while the deeper involvement of the dermis and/or subcutaneous tissues is labeled cellulite. Eventually, involving even deeper structures of the skin can lead to fasciitis and even myositis. Involvement of fat tissue causes panniculitis for people with thick adipose tissues (e.g., overweight or obese individuals). The development of an STI depends on three steps: bacterial adherence to host cells, tissue invasion with host defense evasion and toxin development (Kumar *et al.*, 2014). Virulence genes encode special proteins that confer these properties in most pathogenic bacteria (Ki and Rotstein, 2008).

Hepatic tissue of rats when infected by *K. pneumoniae* lead to drainage of the liver is severely affected. Cell membrane breakage occurs. The sinusoids have been largely separated and some lymphoid tissue aggregations have also been formed on the cell surface. Additionally, this bacterium caused mild cellular hemorrhage, and there is vascular congestion, caused by inflammation of the vascular tissue. The mild hemorrhage may because of destruction of cortical radial vein. These are as

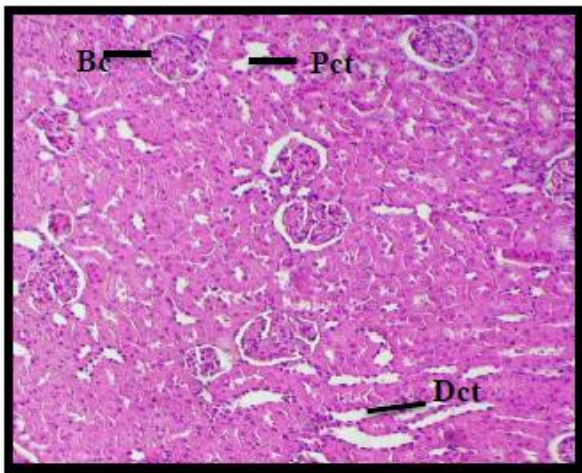


a result of effect of pathogenesis of *K. pneumoniae* (Anibijuwon *et al.*, 2017), same result obtained by our study.

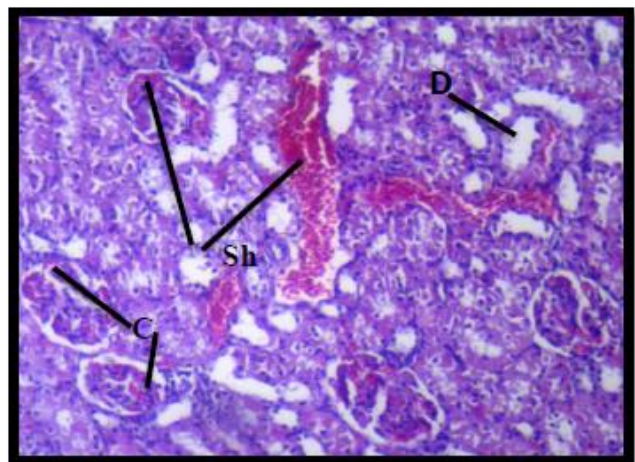
**Effect of green and black tea extracts on rat liver and kidney tissue:**

Histologically, the 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). But kidney of the second cohort of rats which infected with *K. pneumoniae* showed pathological changes consisted of sever hemorrhage, dilatation, congestion in tubules and glomerulus with reduced cellularity (figure 2). Also show in tubules and glomerulus with increased inflammatory cell infiltration and neutrophil, and destruction of endothelial cells of Bowman's capsule (figure 3). And shows sever hemorrhage, dilatation, in tubules with inflammatory cell infiltration and pyknotic cells of tubules, cellular vacuolation (figure 4).

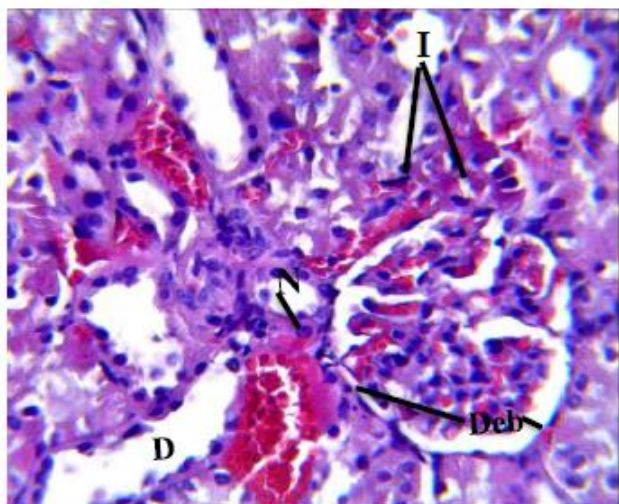
While the third cohort of rats that infected with *K. pneumoniae* bacteria are treated by green tea extract, the kidney showed some hemorrhage, tubular dilatation and some congested Bowman's capsule and some restored normal histological architecture (figure 5). The fourth cohort of rats which infected with *K. pneumoniae* bacteria, when treated with black tea extract, the kidney shows nearly normal histological architecture of glomeruli and tubules, mild hemorrhage and mild dilatation of convoluted tubules as compared to control rat kidney infected with *K. pneumoniae* (figure 6).



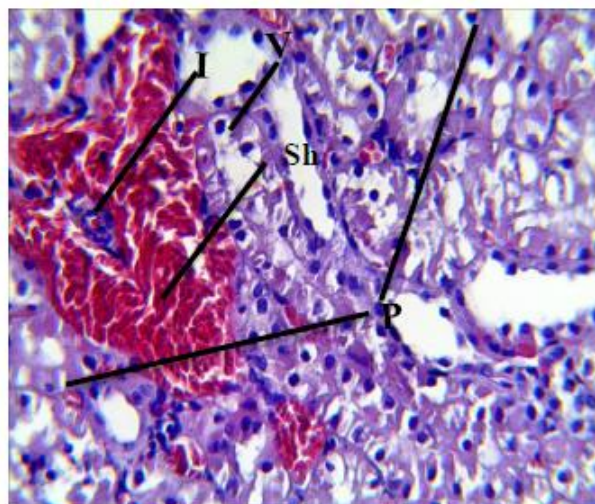
**Fig.1.** Photomicrograph from control rat shows the normal histological structure of kidney include: Bowman's capsule (Bc), proximal (P ct), distal convoluted tubules (Dct), (100x. H and E).



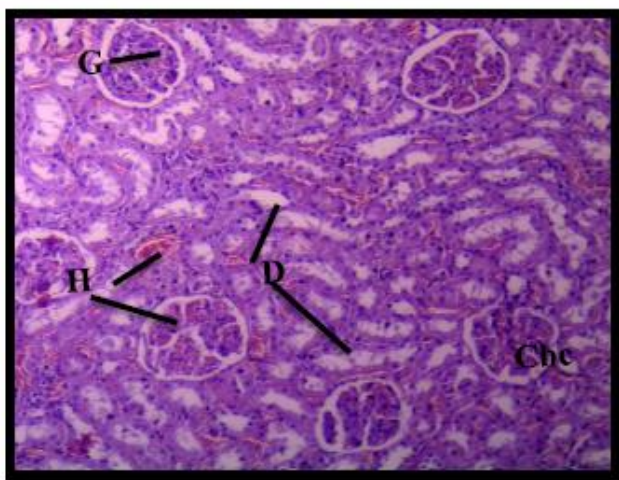
**Fig.2.** Photomicrograph from control rat kidney infected with *K. pneumoniae* bacteria shows sever hemorrhage (Sh), dilatation (D), (C) congestion in tubules and glomerulus with reduced cellularity (100x. H and E).



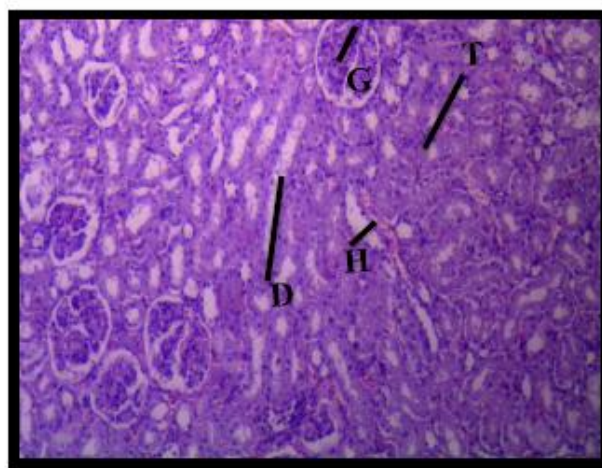
**Fig.3.** Photomicrograph from control rat kidney infected with *K. pneumoniae* bacteria shows sever hemorrhage (Sh), dilatation (D), in tubules, and glomerulus with inflammatory cell infiltration (I) and neutrophil N, destruction of endothelial cells (Deb). (400x. H and E).



**Fig.4.** Photomicrograph from control rat kidney infected with *K. pneumoniae* bacteria shows sever hemorrhage (Sh), dilatation (D), in tubules with inflammatory cell infiltration (I) and pyknotic cells of tubules (P), cellular vacuolation (V). (400x. H and E).



**Fig.5.** Photomicrograph from control rat kidney infected with *K. pneumoniae* bacteria and treated green tea extract shows some hemorrhage (H), tubular dilatation (D) and some congested Bowman's (Cbc) capsule and some restored normal histological architecture of it (G), (100x. H and E).



**Fig.6.** Photomicrograph from control rat kidney infected with *K. pneumoniae* bacteria and treated with black tea extract shows nearly normal histological structure of glomeruli (G) and tubules (T), mild hemorrhage (H) and mild dilatation (D) of convoluted tubules as compared to control rat kidney infected with *K. pneumoniae* (100x. H and E).

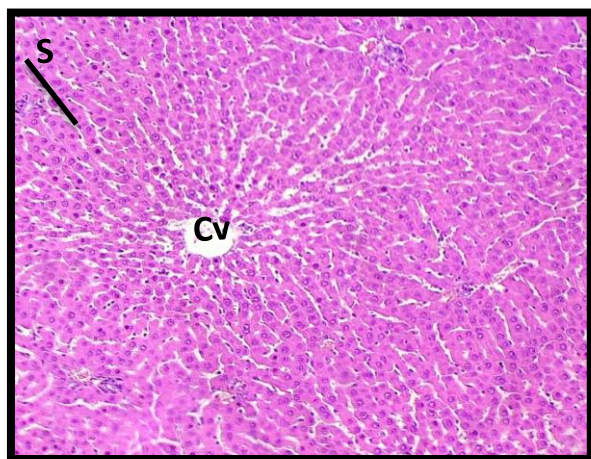
The cohorts of rats that infected with *K. pneumoniae* bacteria, when treated with green tea extract and black tea extract, the kidney shows restoration of approximately normal features of glomeruli proximal and distal convoluted tubules, and decreased hemorrhage level. In line with this our findings, previous study done by Hasanein *et al.*, (2012) that concluded, aqueous extract prepared from green tea, black tea with improved kidney functions. Bakr and Header, (2014), who found in

them study that administration of green tea extract to rats recorded in an improvement of renal function. Also, agreed with Dufresne and Farnworth (2001); and Rhee *et al.*, (2002) who found that rats receiving green tea upgrading renal functions. In this respect, Choi *et al.* (2004); and Khan and Mukhtar, (2007), documented that green tea catechins are obviously effective in mitigating oxidative stress and inflammatory reactions in the kidney tissue, catechins and their derivatives have also been identified as antioxidants that scavenge free radicals to protect cells in normal and pathological conditions. And Yokozawa *et al.*, (2003), found the black tea polyphenol is effective against renal failure in rats. Also, Sara *et al.*, (2009), elicited the nephrotoxicity and oxidative damage when treated with green tea, and black tea were improving antioxidant defense, tissue integrity and energy metabolism.

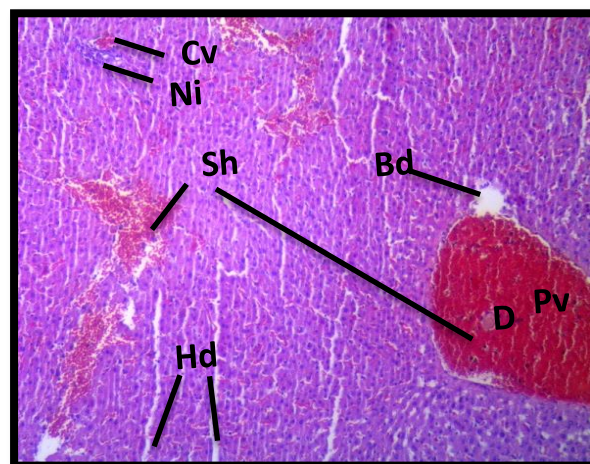
### Effect of green tea extract and black tea extract on rat liver tissue

When made photomicrograph to control cohort of rats (first cohort) the liver appearance the normal histological structure of hepatic lobule, normal central vein sinusoids (figure 7). While the pathological changes of the rat's liver infected with *K. pneumoniae* bacteria (second cohort), observed by photomicrograph, consisted of sever hemorrhage in central vein, with necrotic inflammation, sever hemorrhage and dilatation in (portal vein), bile duct and hepatic drainage (figure 8). Also appears hemorrhage in central vein with hemorrhage and dilatation in sinusoids, abnormal enlarged hepatocytes, pyknosis, karyorrhexes and karyolysis, cellular vacuolation (figure 9).

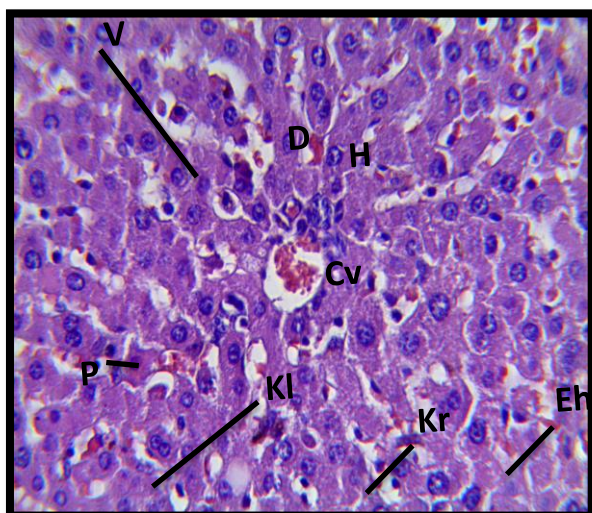
The liver of infected rat with *K. pneumoniae* that treated with green tea extract (third cohort) appears restoring of the nearly normal histological structure of hepatic lobules and central vein (figure 10). While the liver of infected rat with *K. pneumoniae* bacteria when treated with black tea extract (fourth cohort), shows mild sinusoidal dilatation, somewhat restored central vein structure (figure 11)



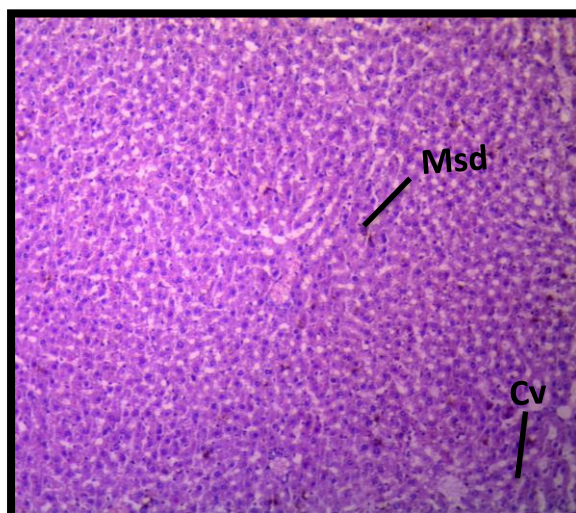
**Fig.7.** Photomicrograph from control rat liver shows the normal histological structure of hepatic lobule, normal central vein (Cv) sinusoids (S) (100x. H and E).



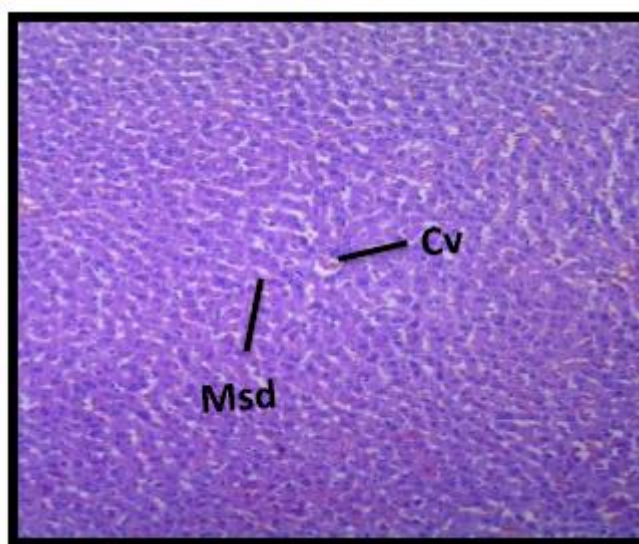
**Fig.8.** Photomicrograph from control rat liver infected with *K. pneumoniae* bacteria shows hemorrhage in central vein with necrotic inflammation (Ni), sever hemorrhage (Sh) and dilatation (D) in (portal vein Pv), bile duct (Bd) and hepatic drainage (Hd) (100x. H and E).



**Fig.9.** Photomicrograph from control rat liver infected with *K. pneumoniae* bacteria shows hemorrhage in central vein (Cv) with hemorrhage (H) and dilatation (D) in sinusoids (D), abnormal enlarged hepatocytes (Eh), pyknosis (P), karyorrhexes (kr) and karyolysis (Kl), cellular vacuolation (V)(400x. H and E).



**Fig.10.** Photomicrograph from control rat liver infected with *K. pneumoniae* and treated with green tea extract shows restoring of nearly normal histological structure of hepatic lobules and central vein (Cv). Mild sinusoidal dilatation (Msd) (100x. H and E).



**Fig.11.** Photomicrograph from rat liver infected with *K. pneumoniae* bacteria and treated with black tea extract shows mild sinusoidal dilatation (Msd), somewhat restored central vein structure (Cv) (100x. H and E).

The effect of different doses from aqueous extract prepared from green and black tea for treatment the rats infected with *K. pneumoniae* 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), finding is congruent with our findings, and his study data demonstrated that the therapy of green tea extract significantly improves the histopathological status of liver function rats. Also, these our result is consistent with several previous studies have reported a protective effect of green tea on rats 'liver, mainly due to its antioxidant effects (Chen *et al.* 2004; Dobrzynska *et al.*, 2004; Fiorini *et al.*, 2005;

Zhang *et al.*, 2006). Certain report documented an antineoplastic effect in the hepatocellular carcinomas by triggering apoptosis of neoplastic cells (Nishikawa *et al.* 2006). Although some experiments did not show any adverse hepatic effects after rats were given green tea (Bun *et al.*, 2006; Isbrucker *et al.*, 2006). Also, our result agrees with Abolfathi *et al.*, (2012), how found treatment with green tea in rats no considerable change was observed indicating the protective effect of green tea against hepatic complications and antioxidant enzymes. And hepatic toxicity, observed in rats, was one of the most significant effects. The effects of green tea or its components on the liver *in vivo* and *in vitro* are contradictory (Takami *et al.*, 2008; Chan *et al.*, 2010). Green tea polyphenols caused hepatic necrosis in rats (Chan *et al.*, 2010). It was proposed, however, that the hepatotoxicity components are the catechins and their esters of gallic acid (Mazzanti *et al.*, 2009). Hepatotoxicity was suggested because of oxidative stress induced in the liver by polyphenon E including 400 mg/kg EGCG because it increases the level of liver transaminase that leads to liver damage (Shanaflet, 2013).

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### داخل الجسم الحي، التأثير المضاد البكتيري لمستخلصات الشاي الأخضر والأسود لكبد وكلى الجرذان المصابة

إسراء محمود محمد<sup>1</sup> وسوسن محمد عبد الله سورجي<sup>1</sup> و ولاء طه يوسف<sup>2</sup>

1 قسم علوم الحياة، كلية التربية، جامعة صلاح الدين

2 كلية التقنيات الصحية والطبية/ بغداد، الجامعة التقنية الوسطى

#### المستخلص

الهدف من الدراسة هو معرفة التأثير المضاد البكتيري لمستخلصي المائي الساخن والبارد للشاي الأخضر والأسود وتقييم النشاط المضاد لبكتيريا *Klebsiella pneumoniae* اضافة الى ذلك دراسة تأثير هذه المستخلصات على كبد وكلى الجرذان بعد اصابتهم بهذا النوع من البكتيريا جدياً. تم تقسيم 16 من ذكور الجرذ إلى أربعة مجاميع (4 لكل منهما)، المجموعة الاولى: بدون معاملة (السيطرة)، المجموعة الثانية: تمثل الجرذان المحروقة جدياً والمصابة ببكتيريا *K. pneumoniae*  $1.5 \times 10^8$  /مل، المجموعة الثالثة: تمثل الجرذان المصابة التي عولجت بالشاي الاخضر (1000 مايكروغم/مل)، المجموعة الرابعة: تمثل الجرذان المصابة التي تمت معالجتها بالشاي الاسود (2000 مايكروغم/مل). تم تحليل النشاط المضاد للميكروبات من مستخلصات الشاي بطريقة الانتشار أجار والحد الأدنى للتراكيز المثبطة. أظهرت النتائج إن بكتيريا *K. pneoumoniae* بشكل عام لديها معدلات عالية من المقاومة للعوامل المضادة للميكروبات المستخدمة بشكل شائع. تم استنتاج إن المستخلص الساخن بنوعيه أفضل تأثيراً كمضاد بكتيري من البارد. أظهرت الجرذان المصابة نزف حاد وإرتشاح الخلايا الالتهابية في كبد وكلى الجرذان ولكن عند استخدام مستخلصات الشاي الأخضر والأسود كعلاج عن طريق الجلد، وجدنا الكلى استعادت التراكيب النسيجية الطبيعية للكبيبات والأنابيب تقريباً. والكبد في الجرذان المصابة التي عولجت بمستخلصات الشاي الأخضر والأسود استعاد البنية النسيجية الطبيعية للفصيصة الكبدية والوريد المركزي تقريباً. كما أظهرت الدراسة ان الشاي الاسود كان له تأثير أفضل من الشاي الاخضر في استعادة البنية النسيجية للأعضاء المذكورة.

الكلمات المفتاحية: مضادات البكتيريا، المضادات الحيوية، *K. Pneoumoniae*، الشاي الأخضر، الشاي الأسود، كبد، كلى، جرذ.