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Effect of polyphenols which extracted from green tea in reduce toxic effects of cadmium sulfate in rat's livers

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Abstract: *This experimental was carried out in animal hosbital of veterinary medicine in Al-Qadisiyea university to investigate the positive role of polyphenols in liver protection from toxic effects of cadmium sulfate.twinty four of animals from femal rats were used .the animals were divided to four groups(six animal/group) which are control group (given water and food only) and three of treatment groups ,the first treatment group was given 50 mg/L from cadmium sulfate with drinking water,the second treatment group was given cadmium sulfate (50mg/L) and 400 mg/k.g of B.W from polyphenols ,and the third treatment group was given polyphenols only (400mg/kg of B.W) the drenching was lasting for thirty days for all groups ,all groups housed under same condition .The level of AST and ALT were measured in plasma.thehistlogical section of liver were examined The results of this study reveaeled to significant increase ($P\leq0.5$) level AST and ALT in first treatment group that was drenched cadmium sulfate compare with control group ,also the liver histological study was indicate damage in liver tissue resamble found sever congestion hemorrhage ,necrosis of hepatocytes ,thrombi in the central vein,and fatty degeneration in contrast with control group .while,in second treatment group that was given polyphenols and cadmium sulfate the result shown to improve in liver tissue and significant decrease ($P\leq0.05$) in level of AST and ALT in compare with first treatment group and the results of this study indicated a significant decrease($P\leq0.05$) in ASTand ALT in third treatment group that was given polyphenols only compare with control group and second treatment group*

I. Introduction

Herbal medicine has been commonly used over the years for treatment and prevention of diseases and health promotion as well as for enhancement of the span and quality of life (1.2). Many drugs used in conventional medicine were originally derived from plants (3). Green tea *(camellia sinensis*)is one of the most commonly consumed beverage worldwide. It's active components are reports to have several biological properties, including cancer chemoprevention, inti- inflammatory activities, antioxidant activity (4). The active phytochemicals in green tea are :-

1- pdyphenols (30-36%), principally flavanols, more commonly known as catechins the predominant catechins are epigallocatechin – 3- gallate (EG CG) epicatechin-3- gallate (ECG), epigallocatechin (EGC) and epicatechin (EC). (5).
2- Xanthic bases (affeine and theophylline).
3- Essential oils
4- Proteins (15 – 20 % dry weight).
5- Carbohydrates (5 – 7 % dry weight).
6- Vitamins.
7- Minerals and trace elements (5 % dry weight).
8- Lipids : As linoleic and linolenic acids, sterols as stigmasterol.
9-Volatile compounds: As aldehydes, alcohols, esters, lactones, hydrocarbons. (6).

Green tea is very rich in polyphenolic constituents which have high antiflammatory, antioxidant, and antimutagenic properties in various biological system (7).

Plolyphenols in green tea are potent antioxidants (8) and they have been linked with the hypothesis that their redox activities may confer specific health benefits(9). Polyphenols from green tea could be of special interest in the metabolic syndrome because epidemiologic observations and laboratory studies have shown that green tea has a variety effects including antioxidant and hypolipemic activities (10,11).

Green tea polyphenols can scavage active oxygen free radicals cells produced in many systems and protect cells from damage induced by free radicals (12).

The purpose of this study was to investigate the protective effect of polyphenol against liver damage induced by cadmium SulFate.

II. Material and Methods

Extraction of phenolic compound

The leaf of green tea was obtained from local market of Al-aadisyia city. Then dried and powdered, according to Gyon method(13)(200)gm of plant powder was weighted and added to (800)ml of 2% acetic acid and extracted, the mixture was left for 42 hours in an incubator at (50)c then filtered through filter paper to remove all the residual materials. The clear extracted solution treated with the same volumes of n.propand and then saturated with Nacl. The upper layer was separated by funnel, then dried at 45c using an incubator.

Experimental animals

Twenty-four femal Albino rats of wister strain of age(6-8 weeks) and weighting about (250 ± 13)gm were used for the experiment. These animals reared under controlled conditions. The period of this experimental was 30 days.

Experimental design

Animals were divided into four groups of six animals for each group 1- Control group : given distilled water for 30 days.

2- The first treatment group (T1) given (50 mg/L) of cadmium sulfatewith drinking water for 30 days

3- The second treatment group (T2) given (50 mg/L) of cadmium sulfate with drinking water, and orlly gavage (400 mg/kg of B.W) of polyphenol for 30 days.

4- The third treatment group (T3) orally gavage (400 mg/kg of B.W) of polyphenol for 30 day.

Blood and tissue collection

At the end of the experimental period (30 days), the animals were sacrified and blood samples were collected directly into tubes and centrifuged at 300 rpm for 20 minutes. The obtained serum was stored at 4c for estimation of AST and ALT. The liver was also quickly removed and washed with cold normal saline, cut and preserved in 10% neutral Formalin for the pathological studies for microscopy.

- **Estimation of AST and ALT**

AST and ALT were estimated according toAcolorimetric method by using kit (14).

- **Hitological examinations**

Liver tissues were cut in small pieces, placed in plastic cassettes, and immersed in neutral buffered Formalin for 24 hours. The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated using the standard techniques(15).

- **Statistical Analysis**

The one way analysis of variance(ANOVA) was used for analysis of study results, and then Duncan's test ($P \leq 0.05$) was detected to compare between groups (16).

III. Results and Discussion

The results obtained from this study showed a significant increase ($P \leq 0.05$) in AST (65±1.88) and ALT (205±1.46) enzymes (table1) in first treatment group (T1) that was administrated cadmium sulfate (50 mg/L) in contrast with control group, als this study revealed a significant decrease ($p \leq 0.05$) in AST (50 ± 2.25) and ALT (179 ± 4.29) in second treatment group (T2) that was administrated polyphenol (400 mg/kg of B.W) and cadmium sulfate (50 mg/L) comparing with first treatment group (T1). Also, this study seems to show a significant decreae ($p \leq 0.05$) in AST (39±2.54) and ALT (137.3±2.31) in third treatment group (T3) that was administrated polyphenols comparing with control group and second treatment group(T2) (table 1).The histological study for liver tissue of first treatment group showedsever hemorrhage ,congestion, Necrosis of hepatocytes. Also there is mild infiltration of inflammatory cells ofliver tissue(figure 3) andLarge thrombi in the central vein, fatty degeneration (steatosis) in the hepatocytes (hepatocytes appear as signet – like shape)(figure 4). compared with control group(figure 1,2). And there is improved in liver tissue of second treatment group that was administrated polyphenol and cadmium sulfate, it observed there is normal hepatic architecture (figure 6,7) Also, this study indicated normal hepatic architecture of liver tissue and normal hypatocytesin third treatment group that was drenched polyphenol.(figure 8)

The liver is the major organ responsible for metabolism of drugs and toxic chemicals, therefore it is the primary target organ for nearly all toxic chemicals toxins break down physicals barriers and cause tissues damage (17).

Nguyen etat., (18) found that is administration of cadmium caused cytotoxicity in hepatocytes and showed an increase in reactive oxygen species and reduce the activity of antioxidant enzymes such as superoxide dismutase and catalase (19).

Free radicals reduce goltathion in liver tissue, bind to the thiol group oxidation of cytochromes, reduce of calcium, break down DNA, and oxidation of faty acid\ +s(polyenic) that is found in plasma membrane of liver cell(20,21). This eventually leads to damage the liver tissue and this result agree with Markovic and james(22).

AST and ALT enzymes are located in various structure of the cells AST concentration mainly in liver, heart, skeletal, muscles, red blood cells, and kidney (23). ALT found with high concentration in liver and found with low concentration in pancrease and skeletal muscle(24) increase in activity of ALT and AST is sensitive sign of impaired organs membrane and well indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system(25,26). Therefore, the increase in AST and ALT in rats that drenched cadmium sulfate may be returned to increase lipid peroxidation and subsequent degradation of biomembranes, the permeability of the plasma membranes was severely affected and may lead to leakage of AST and ALT and increasing in their activities in the serum. Polyphenols are classified as antioxidants which mean they can protect cell and body chemicals against damage caused by free radicals(27,28) therefore administration of polyphenols lead to a significant decrease in AST and ALT and improved in liver tissue comparing with group that administered cadmium sulfate.

Several studies have indicated that polyphenol play important role in improved level of antioxidant enzymes such as Glutathione peroxides, catalase, Glutathione reductase, Glutathione transferase, superoxide, dismutase (29). These enzymes function to inhibit activity of free radicals.

Heshametal.(30) recorded that Gultathion peroxidase plays a central role in the defense against oxidative damage and toxins. Also, some studies indicated that increase activity of superoxide dismutase in serum are implicated in cellular protection against reactive oxygen species(29,31).

Also found that polyphenols inhibits lipid peroxide formation(32) , for this reason polyphenol may be improved liver tissue and decreased AST and ALT in plasma.

Table(1) effect of polyphenol on AST and ALT level of female wister rats treated with cadmium sulfate

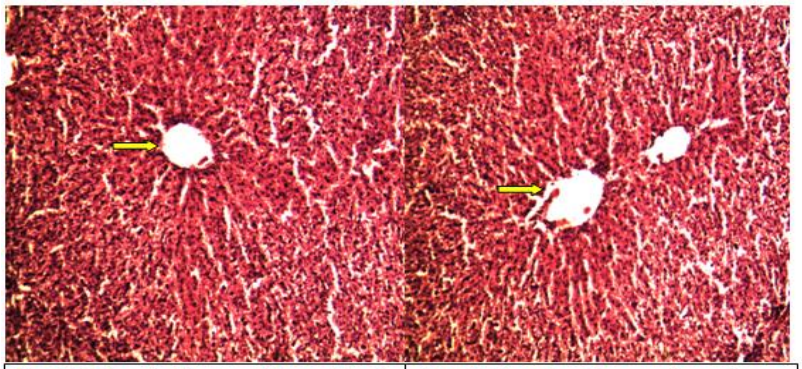
Test Group	AST	ALT
Control	**52.16 ± 1.52** ^{A}	**152.66 ± 3.10.** ^{A}
Treatment First (T1)	**65.0±1.88** ^{B}	**205.0 ± 1.466** ^{B}
Treatment Second (T2)	**50.0 ± 2.25** ^{A}	**179.0± 429** ^{C}
Third treatment (T3)	**39.0± 1.25** ^{c}	**137.33 ± 2.31** ^{D}

Number=average ± standard error (S.E)

Different litters = significant differences (P≤0.05)

T1=given cadmium sulfate (50 mg/L) with drinking water for 30 day

T2=given cadmium sulfate (50mg/L)with drinking water and orally gavage polyphenol (400mg/Kg of B.W)for



Figure(1):Liver of rat fromcontrol group :there is normal hepatic architecture ,radiating appearance of hepatocytes around the central veins(yellow arrow).10xH&E.

Figure(2):Liver of rat fromcontrol group:there is normal hepatic architecture ,radiating appearance of hepatocytes around the central veins(yellow arrow).10xH&E.

30 day T3= orally gavage polyphenol (400mg/Kg of B.W)for 30 day

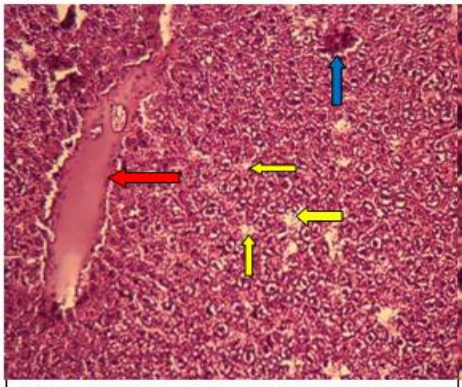


Figure (3):Liver of rat from T1 group: sever hemorrhage ,congestion and thrombus in the central vein(red arrow). Necrosis of hepatocytes(yellow arrows). Also there is mild infiltration of inflammatory cells(blue arrow). 20X H&E.

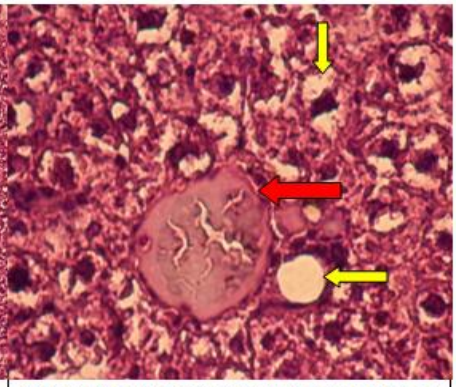


Figure (4): Liver of rat from T1 group: Large thrombi in the central vein(red arrow). There is fatty degeneration (steatosis) in the hepatocytes (hepatocytes appear as signet – like shape)(yellow arrow). 200X H&E.

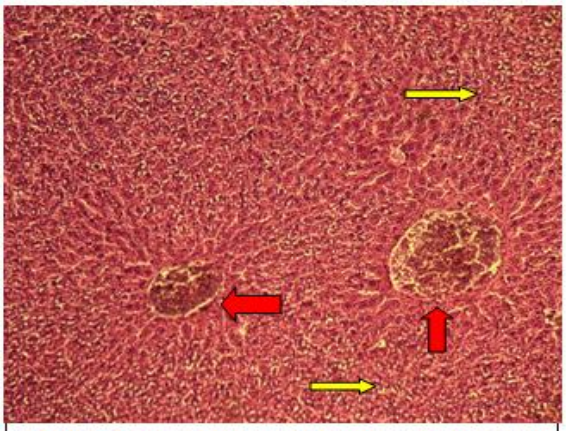


Figure (5):Liver of rat from T1 group: sever congestion in the central vein(red arrows). Necrosis and fatty degeneration of hepatocytes(yellow arrows) . 20X H&E.

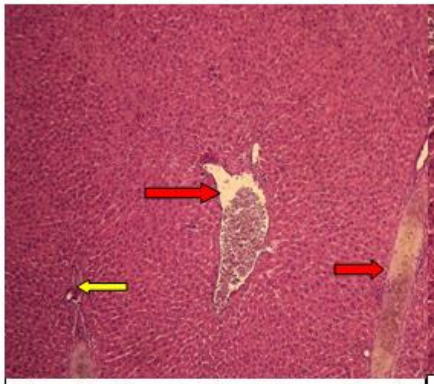


Figure (6): Liver of rat from T2group: There is congestion with thrombosis in the central vein (red arrows), infiltration of inflammatory cells (yellow arrow). Presence of normal hepatic architecture. 20X H&E.

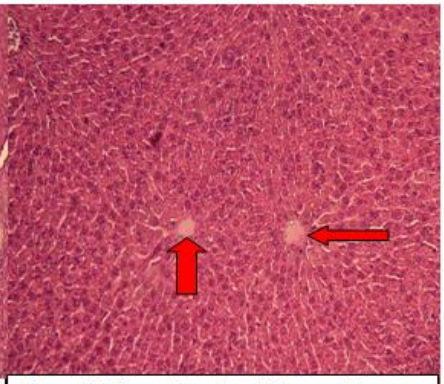
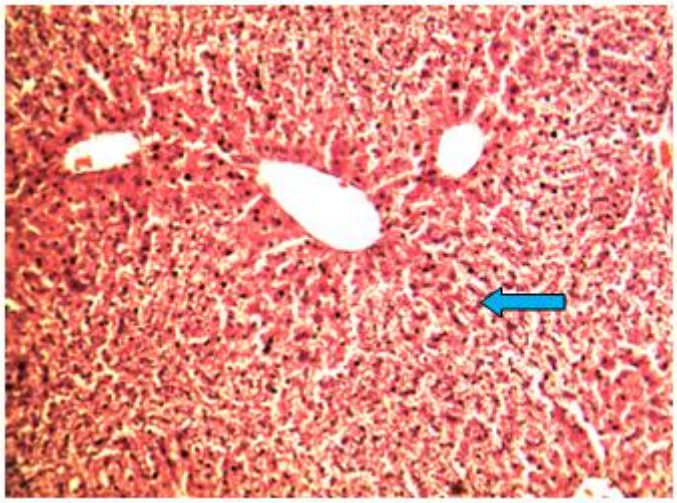


Figure (7): Liver of rat from T2 group: There are large thrombi in the central veins (red arrows). Presence of normal hepatic architecture. 20X H&E.



Figure(8):Liver of rat fromT3 group:there is normal hepatic architecture ,radiating appearance of hepatocytes around the central veins(blue arrow).10xH&E.

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