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Effects of Aqueous Extract of Cumin Seed (*Cuminum cyminum*) on the Structure and Function of Albino Rat Kidneys Treated with Dibutyl Phthalate

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Abstract

This study was aimed to investigate the effects of *Cuminum cyminum* seed aqueous extract on the function and histological structure of rat kidney treated with dibutyl phthalate (DBP). Eighteen albino rats weighing 190-240g and aged 10-12 weeks were used for this study which went on for 28 days. The rats were divided into three groups (each with six animals): 1st group: control rats were given food and tap water, 2nd group was given DBP (420mg/Kg. B.W.) with tap water and diet, 3rd group were fed cumin plant orally at a daily dose of (200 mg/kg B.W.) and DBP (420mg/kg. B.W.) was given with drinking water and fed with diet daily. Kidney and liver function parameters were measured and histological structure of kidney was examined. Results showed that DBP significantly increased creatinine, urea as well as liver parameters AST and ALT, while decreased ALP level, destruction, hemorrhage and inflammation in kidney. Cumin decreased AST and ALT level but increased ALP level and elevated kidney hemorrhage which concluded that DBP may cause oxidative stress for rat liver and kidney. Despite hepatoprotective role of cumin, it interacts with DPB and increases bleeding in kidney.

Keywords: *Cuminum cyminum*; Dibutyl phthalate (DBP); Histological structure, Kidney, Rat.

تأثيرات المستخلص المائي لبذور الكمون *Cuminum cyminum* على تركيب ووظائف الكلى في الجرذان البيض المعاملة بثنائي بيوتيل الفثاليت

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الخلاصة:

هدفت الدراسة الى التحري عن تاثير للمستخلص المائي لبذور الكمون *Cuminum cyminum* على تركيب ووظائف الكلى في الجرذان المعاملة ب dibutylphthalate. تم استخدام ثمانية عشر من الجرذان البيض وزنها تراوح بين 190–240 غم وعمرها 10–12 اسبوعاً . استمرت هذه التجربة لمدة 28 يوماً. تم

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تقسيم الجرذان الى ثلاث مجاميع (كل مجموعة تضمنت ستة حيوانات): المجموعة الأولى: مجموعة السيطرة: تم تزويدها بالغذاء مع ماء الحنفية، المجموعة الثانية: اعطيت DBP (420 مغم / كغم وزن الجسم) مع الغذاء و ماء الحنفية. المجموعة الثالثة: زودت بنبات الكمون فموياً بجرعة يومية (200مغم/كغم وزن الجسم) مع DBP (420 مغم / كغم وزن الجسم) مع الغذاء و ماء الشرب يومياً. تم قياس معايير وظائف الكلى والكبد فضلا عن الفحص النسجي للكلى. أظهرت نتائج المصل الكيموحيوية ان مادة الDBP سببت ارتفاعا معنوياً في مستويات كل من الكرياتنين و اليوريا وكذلك معايير وظائف الكبد AST و ALT ،لكن سببت انخفاضاً في مستوى ALP و كذلك أحدثت التهابات و تكسر ونزف في الكلى. سبب الكمون انخفاضاً معنويا في مستويات AST و ALT لكنها رفعت مستوى ALP وسببت ازدياد النزف الكلوي. يمكن الاستنتاج بأن DBP قد يسبب أجهاد تأكسدي لكل من الكلية والكبد .بالرغم من الدور الوقائي الذي أظهره الكمون للكبد، الا انه سبب ازدياد في النزف الكلوي بسبب تداخله مع DBP.

1-**Introduction:**

Plant kingdom is a real source of various medicines and other pharmacologically effective compounds, as well as one of the best sources of chemical variation to find new drugs [1].
An imbalance between free radicals and antioxidants leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates. 1, 2
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An imbalance between **free** radicals and antioxidants leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates.1,2 Antioxidants have protected the body from the harmful effect of the free radicals.2,3An imbalance between free radicals and antioxidants leads to oxidative damage of proteins, fat, nucleic acids, and carbohydrates.

Oxidative stress can be defined as an inequality of free radicals and antioxidants causing oxidative destruction for various structures like carbohydrates, oils, fats, nucleic acids and proteins. [2].

Dibutyl phthalate (DBP), one of the phthalates, has gained a lot of attention from the scientific community and general public because of its large annual production volume in millions of tons [3]. DBP is a phthalate diester that is primarily used as a lubricant, antifoaming agent and skin emollient for nitrocellulose, polyvinyl acetate and polyvinyl chloride [4]. Phthalates such as DBP, di(2-ethylhexyl) phthalate (DEHP) and di-isononyl phthalate (DINP) cause a number of kidney problems in animals, including renal cysts, decreased creatinine clearance and transitional cell carcinoma [5]. In rodents, phthalates increase liver weight, affect liver function and change liver enzymes. After being exposed to these compounds, these effects have been observed [6]. In rats DBP induced impairment of spermatogenesis, lower serum FSH and testosterone levels and lower levels of enzymatic antioxidants which were associated with histopathological abnormalities [7].

The cumin plant, which is one of the aromatic herbs for which this study is devoted to, is one of the plants that is used in many fields and in traditional medicine, especially in the veterinary medicine practice, as a stimulant. It is a carminative, and cumin seeds are anti-oxidants and have the ability to suppress free radicals and fat peroxides [8, 9]. They have the ability to reduce plasma cholesterol level in diabetic rats. A decrease in cholesterol and triglyceride levels has also been noticed when treated with this plant [10]. It has been detected

that when the kidney of female albino mice was exposed to profenofos, cumin was effective in lowering uric acid and creatinine levels [11].

Due to the great role of cumin plant as free radical scavenger, this study was aimed to investigate the protective effects of *C. cyminum* seed aqueous extract against the damage induced by DBP in albino rats.

2 - Materials and Methods:

Plant Collection and Aqueous Extract Preparation:

After obtaining the seeds of the cumin plant from the local city market, they were exposed to fresh air away from the sun rays, as they had already been dehydrated and ground in a dry electric mixer [12]. The powder was then homogenized with distilled water (1g /5 ml) for 15 accurate minutes using an electric vibrator before being left for 24 hours. The resulting mixture was filtered through gauze, after which the filtrate was placed in special 100 ml bottles and centrifuged at a speed of (3000) round / minute (for 15 minutes). The filtrate was taken and put in glass bottles and the bottles were placed in an oven at 40 ^{0}C to dry the extract. Later on, the solid material represented by the dry raw material of the extract was taken and weighed to prepare the required concentrations [13].

Experimental Design

Eighteen adult female albino rats (*Rattus norvegicus*) with a body weight of 190-240g and age ranging between 10-12 weeks, were used in this study. The experiment was conducted in the animal house at Biology Department, Education College, Salahaddin University, Erbil and it lasted for 28 days. The experimental rats were randomly divided into three groups (each with six animals) as follow:

G1: control rats were given diet and tap water.
G2: This group was given DBP (420mg/kg. B.W.) with drinking water and diet.
G3: This group was given cumin aqueous extract orally in regular doses of (200 mg/kg B.W) and DBP (420mg/kg. B.W.) was given with drinking water and fed with diet daily.

Anesthesia, Dissection, Blood Sample Collection and Removal of Kidney

After 28 days of the experiment, rats were fasted for 24hours. The animals were then given ketamine (35 mg/kg B.W.) and xylazine (5 mg/kg B.W.) to make them unconscious [14]. Blood samples were collected from the heart before removing each kidney from the body which were then divided into two equal parts. The pieces were washed with normal saline and finally kept in neutral buffered formalin for further processing.

Serum Preparation

For biochemical analysis, blood samples were taken by cardiac puncture into chilled tubes without ethylene diamine trichloro acetic acid (EDTA) and centrifuged at 3000 rpm for 15 minutes [15]. Then the serums were taken for biochemical determination of kidney function parameters (creatinine and urea), as well as liver function parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

Histological Preparation

Ten percent formaldehyde was used for fixation of kidney specimens for 24 hours and then paraffin method was used by dehydrating through serial dilutions of alcohol (50%, 70%, 95%, 100%), xylol was used for clearing. The organs had already been dried and later on infiltrated as well as embedded in paraffin wax. Paraffin blocks were prepared for sectioning at 5 µm

thickness per section by using microtome (Hunting Don, Bright. UK.). The obtained tissue slices were collected on glass slides, deparaffinized by xylol and rehydrated by descending serial of ethanol, and then stained by hematoxylin and eosin [16].

Statistical Analysis

Data was expressed as mean ± standard error (mean ± S.E.) and statistical analysis was performed using the statistical package for the social sciences (SPSS version 20) software. Duncan test for multiple comparisons was used to determine statistical differences following analysis of variance (ANOVA). Statistical significance was determined at $P<0.05$ [17].

3 - Results:

Effect of Cumin and DBP on Renal and Liver Function Parameters:

As shown in Table 3.1, the level of serum urea **(38.25 ± 6.70** mg/dl) significantly increased at ($P \leq 0.05$) in group 2 rats when they received DBP in a dose of (420mg/kg. B.W.) in comparison with the control group rats. While rats treated with cumin aqueous extract (200 mg /kg B.W) failed in recovering urea level to normal status which significantly increased **(36.75±3.20** mg/dl**)** as compared to the control group. Serum creatinine level significantly increased ($p\leq 0.05$) both in the second and third group of experiment in comparison to control and were (**0.625 ± 0.095, 0.875 ±0.050** mg/dl**)** respectively.

On the other hand, serum ALT and AST levels significantly increased **(20.50 ±5.69** IU/L**)** and **were(26.50 ± 5.57** IU/L**)** respectively at ($p\leq 0.05$) in DBP group as compared to control group whereas the levels decreased significantly **(9.25 ± 3.40** IU/L**)** and **(21.50 ± 2.08** IU/L**)** respectively at ($p\leq 0.05$) in rats treated with cumin as compared to DBP. Finally, serum ALP level was measured which had significantly decreased **(61.75 ± 8.4** IU/L**)** at ($p\leq 0.05$) in DBP group as compared to the control group. However, treating rats with cumin did not show significant changes in comparison to the control group but significantly increased serum ALP levels **(87.25± 9.29** IU/L**)** as compared with DBP group.

Table 3.1: Effects of cumin and DBP on serum renal and liver function parameter in albino rats

Parameters/Groups	Blood urea concentration mg/dl	Serum creatinine concentration mg/dl	Serum ALT level IU/L	Serum AST level IU/L	Serum ALP level IU/L
G1: Control group	29.50 ± 1.29 b	0.575 ± 0.050 c	8.75 ± 0.95 b	19.0 ± 2.94 b	90.25 ± 8.4 A
G2: DBP (420mg/Kg. B.W.)	38.25 ± 6.70 a	0.625 ± 0.095 b	20.50 ± 5.69 a	26.50 ± 5.57 a	61.75 ± 9.22 B
G3: Cumin aqueous extract (200mg/Kg B.W.) + DBP (420mg/Kg. B.W.)	36.75± 3.20 a	0.875 ± 0.050 a	9.25 ± 3.40 b	21.50 ± 2.08 b	87.25 ± 9.29 A

- **Data is viewed as mean ± S.E.**
- **The same letters indicate that there are no statistical variations in the data.**
- **The various letters denote statistical differences.**
- *** = P<0.05**
- **n = 6 animals in each group.**

Histological Study

Sections through kidney prepared and examined under light microscope showed normal structure of Bowman's capsule with proximal and distal convoluted tubule (Figure 4.1 A and B). While kidney of rats infected with DBP without treatment caused dilatation of proximal and distal convoluted tubules, inflammatory cell infiltration, destruction of endothelial cells of Bowman's capsule and hemorrhage was detected both in glomeruli and convoluted tubules (Figure 4.2 A and B). When treated with DBP and cumin aqueous extract, hemorrhages were also detected in the kidney in a high amount with inflammatory cell infiltration, glomerular atrophy as well as tubular dilatation (Figure 4.3 A and B) in the rats. All these histopathological changes were due to reactive oxygen species produced by DPB.

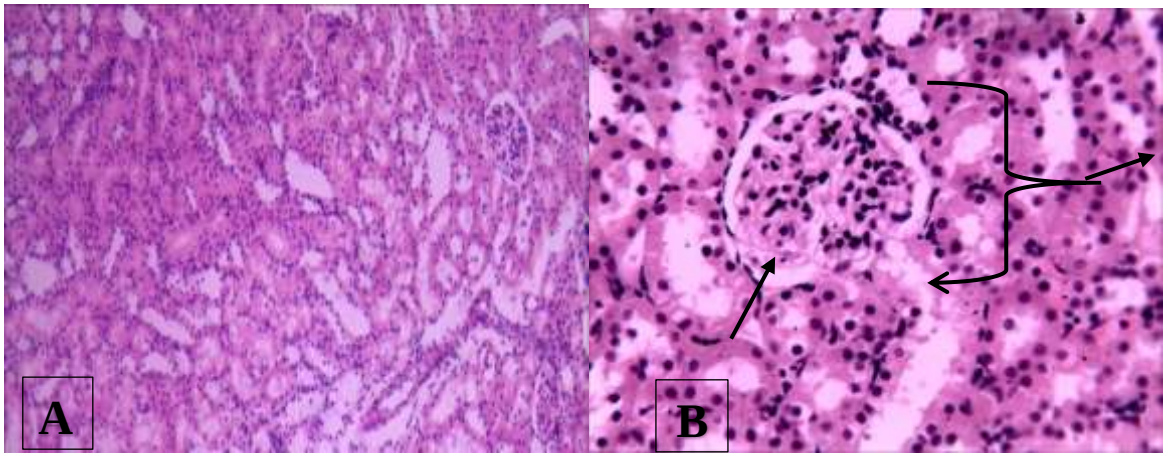

Figure 4-1: A and B: Section through kidney of control group rat shows normal histological structure of glomerulus } and convoluted tubules ↗. 10 x and 40 x respectively, H&E.

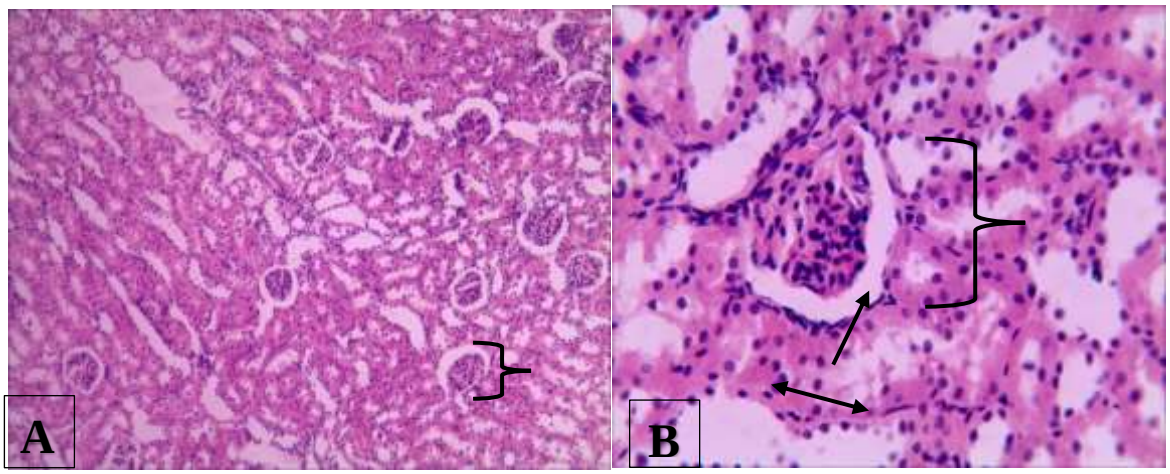

Figure 4-2: A and B: Section through kidney of rats treated with DBP shows glomerular atrophy and endothelial cells destruction of Bowman's capsule } ↑, bleeding ↕ with inflammatory cell infiltration inside it and dilated tubules . 10x and 40x respectively, H&E.

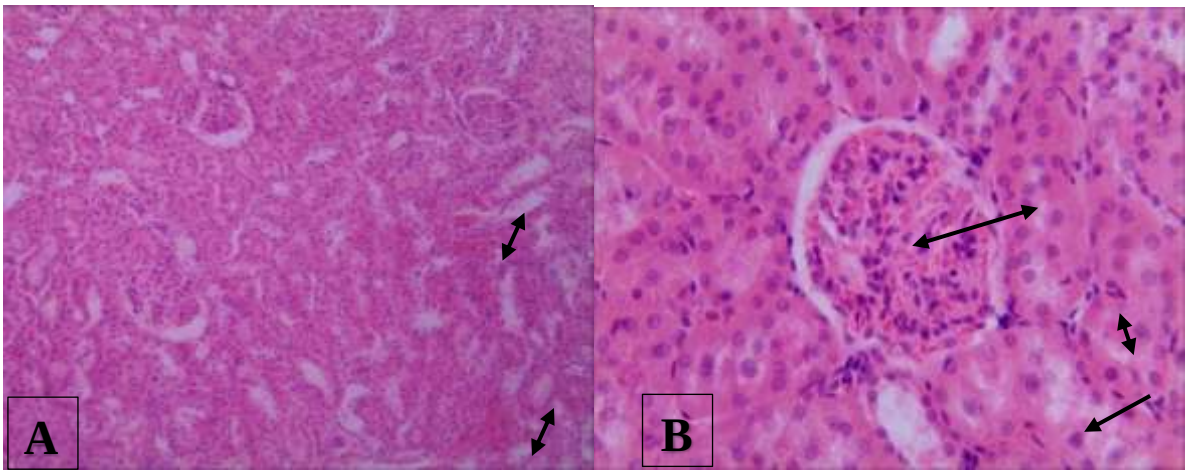

Figure (4-3) A and B: Section through kidney of rats treated with cumin a long with DBP, shows hemorrhage ↑ in glomeruli and convoluted tubules with inflammatory cell infiltration due to DBP intake, severe bleeding observed when DBP interact with cumin. 10x and 40x respectively, H&E.

4 – Discussion:

Effect of Cumin and DBP on Renal and Liver Function Parameters: Baralic *et al*. [18] performed a study on DBP effects alone and when mixed with other chemicals like DEHP and bisphenol A (BPA),and found that the serum urea level significantly increased in mixture group, while creatinine level showed non-significant changes. They concluded that the effect of mixture is higher than the effects of the substances like DBP alone in low dose. On the other hand, serum ALP, AST and ALT increased non significantly in DBP as compared with the control group. However, mixing it with other compounds significantly increased serum ALT and AST levels.This somewhat agrees with our study since creatinine and urea level significantly increased in combination group as compared to control rats. But infecting rats with DBP also had a significant increase in serum creatinine and urea level due to high dose of DBP in our experiment. This made it clears that cumin interacts with DBP and has adverse effects on kidney. So, it is better to use it in lower doses to prevent these interactions. DBP increased ASTand ALT levels but the elevation was significant due to different concentration used in our experiment, while significantly decreased ALP level. As well as treating rats with cumin significantly decreased hepatic enzyme levels AST and ALT as compared to DBP group, but a significant increase was detected in serum ALP level of the combination group as compared to DBP rats. This indicates that interaction of cumin with DBP also has adverse effect on some liver parameters. Hormonal fluctuations in female rats may play a great role in variation of the results.

Additionally, Kumar, *et al*. [19] investigated the effects of *C. cyminum* on kidneys exposed to profenofos in female Swiss albino mice. Cumin was found to be effective in lowering uric acid and creatinine levels.

On the contrary, this result is somewhat similar to the study of Kubo and Kinst [20]. Their experimental study extended for 6 weeks where male Wistar rats were fed *C. cyminum* fruits at 2% or 10% of their normal diet. Rats were also fed a mixture (5 percent *C. cyminum* fruits + 5 percent *T. vulgaris* leaves) for the same amount of time. Rats were not harmed by diet containing 2% *C. cyminum* fruits. In rats that were fed a diet containing 10% *C. cyminum* fruits, growth impairment and enterohepatonephropathy were detected. These changes were also observed in rats fed a mixture of the two plants, and they were followed by leukopenia,

anemia, increased serum AST activity and urea, as well as lower total protein and albumin levels.

DBP is a commonly used plasticizer in flexible polyvinyl chloride (PVC) materials manufacture. Phthalates leach out of PVC because they are noncovalently bound, making them usable for biological exposure [21, 22].
Exposure of humans to DBP principally happens through contaminated food and water, especially high-fat foods that may come into contact with DBP-containing plastics, adhesives, or other packaging materials. Since multiple plasticizers are used to cover medicines such as antibiotics, antihistamines, and laxatives, pharmaceutical formulations often result in substantial human exposure [23].
Exposure to chemical mixtures has been linked to a variety of negative health effects as a result of multiple forms of toxicity, including neurotoxicity, cardiotoxicity, hematotoxicity, nephrotoxicity and hepatotoxicity, according to epidemiological and biomonitoring reports [24, 25, 26, 27, 28, 29, 30].

Histological Study:

In several studies DEHP and DBP for example, have been shown to inhibit hepatic apoptosis at low doses while increasing the frequency of apoptotic hepatocytes at higher doses [31]. Oral animal investigations have reported minimal effects on the liver and a slight decrease in kidney weight [32]. This study supports the statement since glomerular atrophy was detected which leads to renal atrophy. Zhu *et al*. [33] reported that oxidative stress caused renal fibrosis and dysplasia in adult rat offspring treated with DBP.

Cumin seeds contain a variety of phytochemicals with antioxidant, carminative and antiflatulent properties. The seeds are a good source of fiber in the diet. Cuminaldehyde (4-isopropylbenzaldehyde), pyrazines, 2-methoxy-3-secbutylpyrazine, 2-ethoxy-3–isopropylpyrazine, and 2-methoxy-3–methylpyrazine are among the essential oils found in its seeds. Cumin active ingredients can boost gut motility and aid digestion. Although cumin is a common and medicinal plant with a variety of beneficial and healing properties, it also has some negative side effects. Contact dermatitis (skin rash caused by contact with an allergen or irritant), respiratory reactions, liver cancer (above dietary levels) and lower blood sugar levels are all side effects of cumin often raising the chances of bleeding [34, 35]. This is supported by our study since the level of hemorrhage increased when cumin was used with DBP. Arachidonate-induced platelet aggregation was inhibited by cumin extract. It also reduced the production of thromboxane B2 from exogenous ($14C^0$) arachidonic acid (AA) in washed platelets, while simultaneously increasing the formation of lipoxygenase-derived products [36].

On the other hand, hepatotoxicity and nephrotoxicity were observed in the rats given 500 mg/kg paracetamol orally for four weeks. However, when the animals were given a mixture of paracetamol 500 mg/kg and 6% *C. cyminum* fruit, the recovery of paracetamol hepatotoxicity was demonstrated by a rise in body weight, the absence of hepatocellular fatty vacuolation, and a substantial improvement in serochemical and hematological parameters [19].

Dose and duration of using herbs is very important as well as their interaction with other substances. So overdoses and duration must be prevented to avoid side effects of interactions among various substances.

5 - Conclusion:

Dibutyl phthalate may cause oxidative stress for rat liver and kidney. In spite of hepatoprotective role of *Cuminum cyminum*, it may interact with DPB and has side effects on kidney, like increasing the risk of bleeding due to high anticoagulant property. Hence, overdose of seed must be avoided at all cost.

Ethics Approval

Institutional guidelines for the care and use of animals were followed. All procedures performed in the study and involved animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

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Conflict of interest

There was no conflict of interest among the authors in presenting this article for publication.

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