

Histological Study of the Effects of the Watery Chili Pepper Extract on Kidney in White Mice

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Abstract

The current study designed to investigate the main role of hot chili pepper extract on the weight loss and other effects on some biological parameters on white mice. The present study carried out sixty white mice which divided into three treated groups and one group as control. Each group composed of fifteen mice. The treated groups received three doses that differed in concentration from watery hot chili pepper extract for four weeks as treatment period. The high dose noted have prominent effect on the weight loss compared with other treated groups and control. The kidney tissue so have significant differences in the biometric dimensions of renal corpuscles exactly after treated with moderate and high doses of water hot chili pepper extract. So the high concentration of watery chili pepper extract displayed tissue architectures with very many histological alterations, in contrast to the prior group that received a low dose of chili pepper treatment.

INTRODUCTION

Since ancient times, medicinal herbs have been identified and used in traditional medical procedures. The Sumerian culture left behind the earliest written records of herbs, which are found on clay tablets that list hundreds of medicinal plants, including opium. Medicinal herbs are often used in non-industrialized cultures, mostly because they are more readily available and less expensive than modern drugs [1].

As a result of their distinctive flavor and sporadic antioxidant and antimicrobial activity, plant extracts of herbs and spices are becoming increasingly popular as natural antibacterial food preservation agents. [2].

For millennia, people have employed herbs and spices for both culinary and therapeutic

purposes. Besides enhancing the flavor, aroma, and color of food and beverages, spices can prevent both acute and chronic ailments. There is now plenty of evidence that, in addition to their antioxidant, anti-inflammatory, antitumorigenic, glucose- and cholesterol-lowering properties, spices and herbs also have cognitive and mood-affecting properties. [3].

Research over the past ten years has revealed a wide range of health benefits due to its bioactive components, including sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins, particularly flavonoids and polyphenols. Herbs and spices high in polyphenols and clove. Spices and herbs including clove, rosemary, chili pepper, sage, oregano, and cinnamon are excellent

sources of antioxidants due to their high phenolic component content [4].

any of the numerous chili pepper species and cultivars, which are very hot and sour peppers from the nightshade family (Solanaceae). Although they are grown in warm climates all over the world, chili peppers are indigenous to the Americas. A few of the *Capsicum annum* cultivars that are among the most popular chili peppers include the cayenne, jalapeño, serrano, and Thai chili peppers. While some of the hottest chili peppers, including the habanero, Carolina reaper, and ghost chili pepper, or bhut jolokia, are cultivars of *C. chinense*, tabasco is a variety of *C. frutescens*. Chili peppers can be eaten fresh or dried and are used to flavor foods like barbecue, spicy curries, and other savory sauces [5].

Methods and Materials

Experimental animal:

Sixty adult white mice, average weighing was 30-32gm, animals housed at a temperature of 25–28°C and a relative humidity of 40–45% while being fed regular pellets and water. Before beginning the trial, mice spent two weeks getting used to the lab environment in an animal house of science college in Al-Muthanna university.

Experimental design: Each of the four groups, which each had fifteen mice, was made up of the animals. Fifteen animals were used in Group A as the control group. Group B: 15 animals who received a low dose (concentration 0.4%) of chili pepper (0.5 ml) daily for 30 days. 15 mice in Group C received 0.5 ml (concentration 0.8 %) of a medium dose of chili peppers orally, and 15 mice in Group D received 0.5 ml of (concentration 1.6 %) high

dose of a watery chili pepper extract daily for 30 days.

Chili pepper collection: On the same day that the extract was created, the chili pepper fruits were washed, allowed to dry at room temperature, and then blended. The chili peppers were bought from a neighborhood market in Al-Samawah city.

Preparation of watery extract of chili pepper : 10 grams of plant powder were added to 200 milliliters of distilled water in a sterile glass beaker, shaken for 24 hours, and then used, according to Arabi et al,(2020). To create the extract, the material was dried in an electric oven at 40 °C after being run through layers of sterile soft cotton to determine its candidacy. The filtrate was then separated using a centrifuge (3000 rpm). After that, the extract was gathered, placed in a clean bottle, and stored in the freezer until required.

Preparation of histological slide: Formalin was used to preserve the tissue samples for 48 hours. the samples were dehydrated in graded ethanol concentrations. After cleaning with xylene then imbedding them in paraffin wax for cutting. Then, tissue sections with a 5 µm thickness were mounted on glass slides and stained for light microscopic examination with hematoxylin and eosin Fereidouni et al,(2017) .

Results and Discussion:

The physiological results of weight loss rate

The hot peppers extract have many effects on experimental mice weight, where the rate of weight loss was 1-3 gm per month.

The table (4.1) showed the mice weight after first week of the experimental time, the weight average of control group (A group) was (30.44± 0.70 gm), while the group that had only received a low dose (B group) was (30.32±

0.47gm), whereas the mice weight of the group after treated with the moderate dose (Cgroup) was (30.33 ± 0.62 gm), the average weight of the group which treated with high dose (D group) was (30.15 ± 0.67 gm). The statistical analysis revealed that mice treated with deferent doses of extract noted no significant differences in the average of weight less in all treated groups compared with control group.

Table (4.1) showed the average of mice weight after second week, the control group was normal in the second week (30.44 ± 0.71 gm), while was (30.34 ± 0.47 gm) in the group that received a low dose of watery chili pepper extract, the mice weight after treated with moderate dose was (29.5 ± 0.61 gm), whereas the weight of the mice that treated with high dose of watery chili pepper extract was (28.74 ± 0.66 gm). The statistical analysis showed there are no significant differences in weight less in the B group compared with control, the C group have significant decreased in weight less compared with (B group) and control, the D group have significant decrease compared with all previous groups.

The weight of mice in control group was (30.44 ± 0.71 gm), while the weight of mice treated with a low dose of watery chili pepper extract after third week was (30.36 ± 0.55 gm), a middle dose was (28.02 ± 0.63 gm), and a high dose was (27.76 ± 0.64 gm), according to Table (4.1). The statistical analysis revealed that the weight less didn't have significant differences between B group and control while have significant decrease compared C and D groups respectively with control.

Table (4.1) showed the weight less in mice after forth week, the average weight of control group was (30.45 ± 0.77), whereas after treated with a low dose was (29.38 ± 0.46 gm), while the average weight of the mice after treated with a middle dose was (27.54 ± 0.59 gm), the average weight after treated with high dose was (26.78 ± 0.63 gm). The statistical analysis revealed that the B group havea significant decrease compareda with control groupa, the C group have significant decreased in weight less compared with B and control groups, the D group have significant decreased in weight less compared with previous treated and control groups.

Table 4.1: weight loss rate (g), SE: standard error

	Low dose weekly				<i>P value</i>
	First week	Second week	Third week	Fourth week	
Mean $\pm$ SE Weight measurement	30.32 ± 0.47^A	30.34 ± 0.47^A	30.36 ± 0.55^B	29.38 ± 0.46^B	p = 0.001 NS
Middle dose weekly					
Mean $\pm$ SE Weight measurement	30.33 ± 0.62^A	29.5 ± 0.61^B	28.02 ± 0.63^C	27.54 ± 0.59^D	p = 0.001 S N
High dose weekly					
Mean $\pm$ SE Weight measurement	30.15 ± 0.67^A	28.74 ± 0.66^B	27.76 ± 0.64^C	26.78 ± 0.63^D	p = 0.001 S N
Control group weekly					

Mean ± SE Weight measurement	30.44± 0.70 ^A	30.44± 0.71 ^A	30.44± 0.71 ^A	30.45 ±0.77 ^A	p = 0.994 NS
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SE: standard error; P: Anova; Different latters denote to the significant differences at $p < 0.05$

The histological results of a kidney

Some anatomical description of kidney:

The mouse kidney has two sides, a bean-like form, and a reddish brown tint. The hilum is situated at the convexa surface, where the renal artery enters and exits the renal vein and uretera. The kidney was discovered to be surrounded by a thin capsule of connective tissue which have two layers: an outer layer mostly composed of collagenous fibers with a small amount of elastic fibers and an interior layer primarily composed of smooth muscle cells (Fig.4.1).

Histological results in control group:

Each kidney have many nephron which consider the functional and structural unit of kidney, the nephron have many parts which included (renal corpuscle, proximal convoluted tubule, henle loop, and distal convoluted tubule), the main site of nephrons in the cortical region of kidney.

The renal corpuscle have spherical structures, composed of glomerular capillary surrounded by connective tissue capsule called Bowman's capsule, which have a significant diameter ($77.38 \pm 0.03 \mu\text{m}$) in (Table.4.1). The Bowman's capsule is made up of two layers (parietal and visceral layers), the visceral layer of which protects the glomerular capillaries in the renal corpuscle and the parietal layer of which is held up by a large foundation membrane. Podocytes and mesangial cells may be seen between the glomerular capillaries (Fig.4.2).

The histological results of a kidney after treated with a low concentration of chili pepper extract:

The kidney was shown to be surrounded by connective tissue capsule in the tissue section after being treated with low doses of chili pepper extract after 30 days. The connective tissue capsule was divided into two layers, each of which had prominent spaces that were home to connective tissue fibers and small cells (Fig.4.4).

The composition of the cortical region of the kidney following treatment with a low concentration of hot pepper extract was reported to be normal without any pathological conditions; all other findings were comparable to those of the kidney in the control group.

The Bowman's capsule has prominent visceral and parietal layer with prominent space (Bowman's space) between two layers, the glomerular capillaries have normal in shape without blood congestion, and the mesangial cells between glomerular capillaries have dark irregular nuclei. The diameter of the renal corpuscles ($63.29 \pm 0.05 \mu\text{m}$) is significantly smaller than that of the control group (Fig.4.3). There were no any significant differences in the tissue structures of the renal corpuscles through all the period time of administration, this is due to the active compounds in hot peppers, most notably carotene and capsaicin, which have been shown to have antioxidant, anti-inflammatory, anti-necrosis and anti-cancer effects when consumed in the appropriate quantity and manner, and this is in agreement with (Villa-Rivera et al., 2020).

The histological results of a kidney after treated with a moderate concentration of chili pepper extract:

The histological result of renal corpuscle after one month from treated with hot chili pepper extract noted the partial layer of Bowman's capsule had a noticeable deficiency in the tissue section of the kidney, and the renal corpuscle of the treated group's kidney had a noticeable diameter ($45.40 \pm 0.08 \mu\text{m}$) (Table.4.1). Compared to the control group, the renal corpuscle's diameter had dramatically shrunk (Fig.4.5). These findings supported the hypothesis put forth by (Rios-Silva et al.,2018) that chili peppers can cause acute renal injury and hemorrhage in the kidney. The mesangial cells in these studies had irregular, black nuclei and were situated between glomerulus capillaries.

The tissue section in (Fig. 4.6) revealed severe damage to the renal corpuscle, prominent Bowman space degeneration, aberrant mesangial cell growth, and patches of bleeding in the kidney parenchyma, that due to capsaicin in chili which responsible for risk effects on blood pressure and body weight when its concentration is increased, it leads to tissue diseases and damage to vital parts, and this is in agreement with (Segawa et al.,2020).

The histological results of a kidney after treated with a high concentration of chili pepper extract:

The kidney's cortical region in the mice getting a high concentration of watery chili pepper extract for 30 days showed tissue structures with very many histological alterations (Fig 4.8), in contrast to the prior group that received a low dose of chili pepper treatment. The diameter of the renal corpuscle was ($22.76 \pm 0.15 \mu\text{m}$) (Table.4.2), and it significantly decreased when compared to both

the control group and the group receiving low and middle doses of chili pepper extract.

The Bowman's capsule is not clearly visible, and the renal corpuscle is abnormally shaped. The kidney's histology revealed a partial layer of Bowman's capsule that was opaque with inconspicuous smooth cells, had elongated black cells, and was filled with thick secretion. The layer of Bowman's capsule that encircled the glomerular capillaries was completely degenerated (Fig.4.7), the capsaicin compound is associated with pain receptors, and if you overeat hot peppers and allow large amounts of capsaicin to enter the body, capsaicin can increase the level of salts inside the body. This process prevents the kidneys from performing their functions properly and naturally because of the infections that they may suffer from. In fact, it is also possible for a layer of pus or pus to form on them that affects them negatively, this is in agreement with (Shi et al.,2018).

Table.4.2: Diameter of renal corpuscles in mice. μm

Treatment \ diameter	Renal corpuscle mean $\pm$ S.E
Control group	77.38 ± 0.03^A
Low dose of chili pepper	63.29 ± 0.05^A
Middle dose of chili pepper	45.40 ± 0.08^B
High dose of chili pepper	22.76 ± 0.15^C
Total p value	0.001

Different letters denote to the significant differences at $p < 0.05$

Fig.4.1: Transverse section of kidney in control group which showed A-kidney capsule, B-renal tubule, C-renal corpuscle. H&E stain 40x.

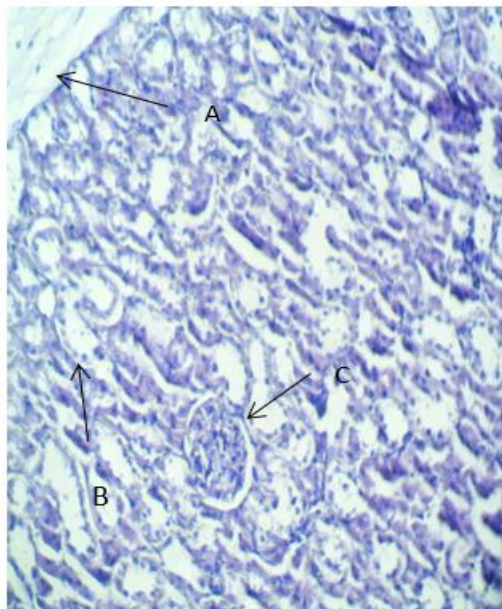


Fig.4.2: Transverse section of kidney in control group which showed A-Renal corpuscle, B-glomerular capillaries, C-bowman's space, D-mesangial cells, E-Proximal convoluted tubule, F-Distal convoluted tubule. H&E stain 20X

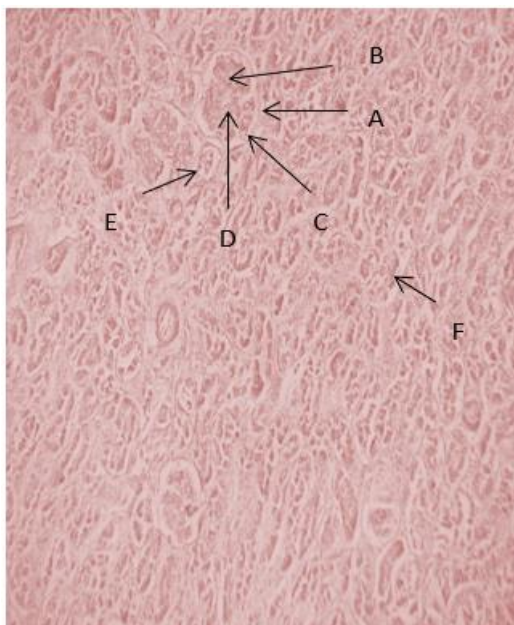


Fig.4.3: Transverse section of kidney after treated with low concentration of chili pepper which showed composition of cortical region normally without any pathological condition. H&E stain 4x.

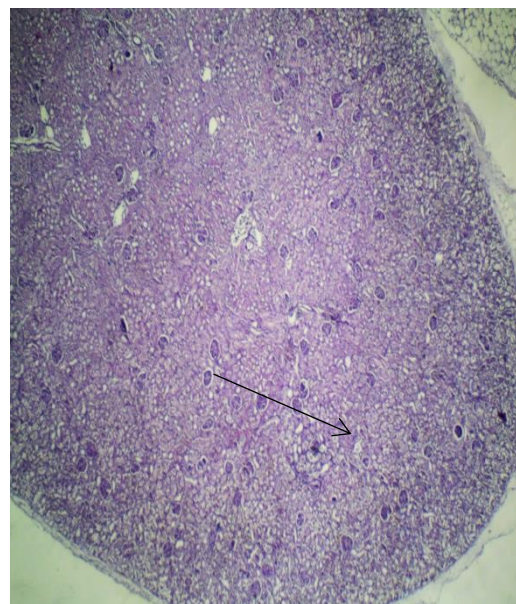


Fig.4.4: Transverse section of kidney after treated with low concentration of chili pepper which showed must A-(P.C.T) were empty and other fill with secretion , ascend branch of (H.L) have lumen wider from the descend branch and B- (D.C.T) have prominent space without secretion.H&Estain20x.

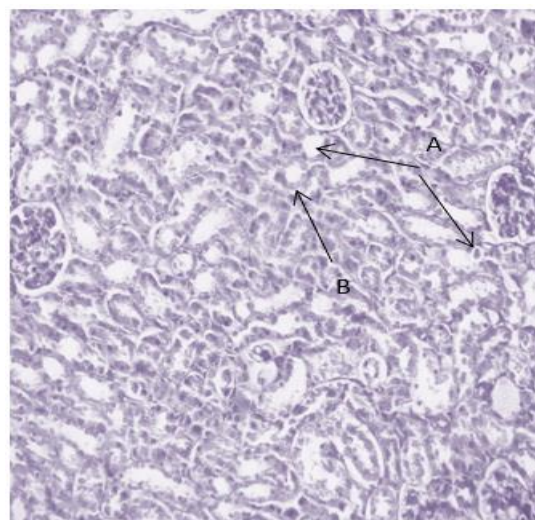


Fig.4.5: Transverse section of kidney after treated with middle concentration of chili pepper which showed structurala changing marked by the thickening of the interstitium with compresses of the flocculus capillary and disappearance of gloerular room (FC).H&E stain 20x.

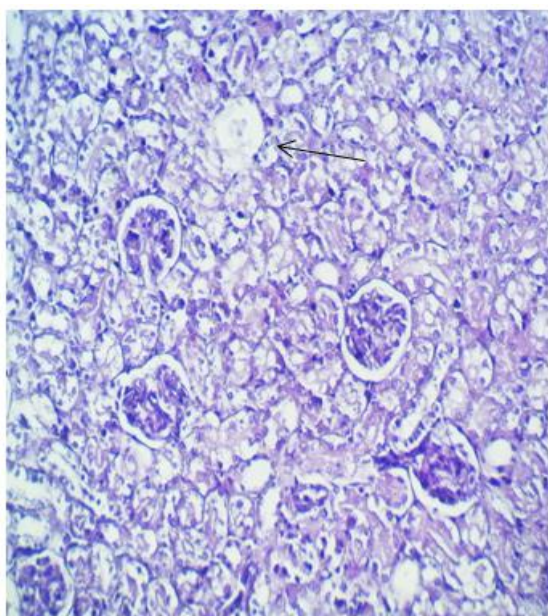


Fig.4.7: Transverse section of kidney after treated with high concentration of chili pepper which showed showing glomerular vacuolation, obstruction of the glomerular renal space, vascular congestion (arrowhead) and cellular infiltration .H&E stain 20x.

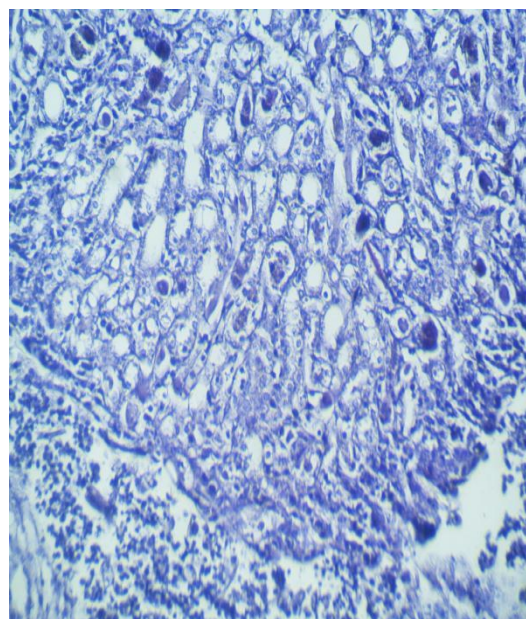


Fig.4.6: Transverse sectiona of kidney in middle dose group which showed A-cystic dilation filled with blood, B- inflammatory cell aggregation and some necrosis lesions H&Estain20x.

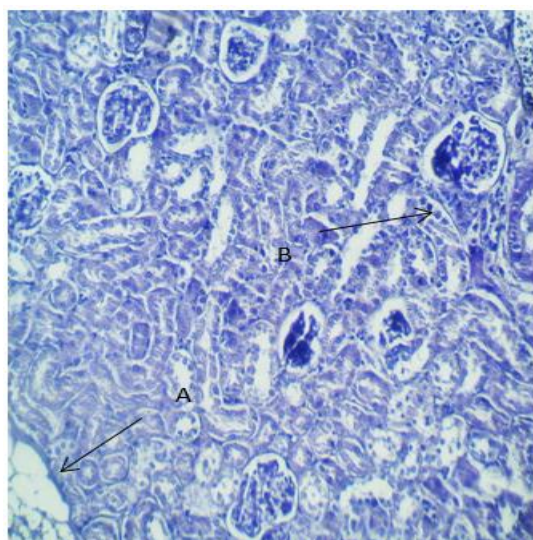
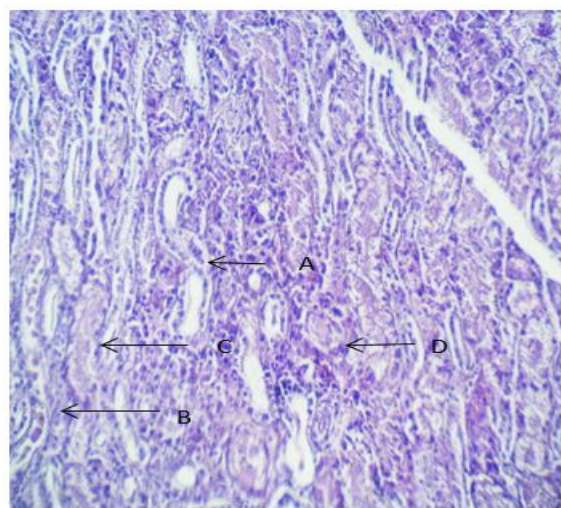


Fig.4.8: Transverse section of kidney after treated with high dose of chili pepper which showed A-destruction of epithelial layer of Henley loop, B-exfoliated epithelial layer, C-hemorrhage, D-blood congestion. H&E stain 20x.



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