Effect of Kaff Maryam aqueous extract in mice Kidneys

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Abstract:

Thirty mice were used in this study in the ages of (4-8 weeks) and body weight of (25±2 g) for the investigation of the histological effects of *Anastatica hierochuntica* aqueous extract in mice kidneys. Mice were divided into three groups where the first group was the control group that received daily orally administration of (1ml) of distilled water for two months. The second group were orally administered with a daily dose of (0.1g/ml) of the plant aqueous extract for one month. While, the third group had received the same dose of the second group but for two months. Animals were slayed at the end of the first and the second month and kidneys were autopsied and their histology section slides were examined by light microscope and photographed. Then, images were assessed histopathologically and comparisons were made between the treated groups of mice with the control group. Results showed that, the administration of daily oral doses of (0.1g/ml) of *Anastatica hierochuntica* aqueous extract for one week did not bring any change to the macroscopic appearance nor to the histology sections of the kidney tissues of the treated animal. whereas, the treatment with daily oral doses of(0.1g/ml) of the aqueous extract for one month had significant effects ($P \leq 0.05$) on the mice kidney tissues that included lymphocyte infiltration, necrosis, degeneration and swelling of the glomeruli where the kidneys appeared congested upon macroscopic examination. While, the continual treatment of the animals with the same doses of the plant extract for a second month resulted in an increased damage to the kidney tissues with a complete loss in kidney tissue architecture.

أثر المستخلص المائي (*لكف مريم*) في كلى الفئران

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قسم تقنيات التحليلات المرضية, الكلية التقنية, كركوك, العراق

الكلمات المفتاحية: التأثيرات النسجية , مستخلص مائي , انسجة كلى الفئران

الخلاصة:

استخدم في هذه الدراسةثلاثون فأرة تراوحت اعمارهم ما بين (٤-٨أسابيع) ووزن الجسم من حوالي (٢٥ ±٢ جم) للتحقق من التأثيرات النسجية للمستخلص المائي لنبات *Anastatica hierochuntica* في كلى الفئران. تم تقسيم الفئران إلى ثلاث مجموعات ، حيث كانت المجموعة الاولى هي مجموعة السيطرة حيث تم معاملة الفئران بجرعات يومية فموية من (١مل) من الماء المقطر. وعوملت المجموعة الثانية بجرعات يومية فموية من (٠,١غم / مل) من المستخلص المائي لنبات *Anastatica hierochuntica* لمدة شهر و احد في حين عوملت المجموعة الثالثة بذات الجرعات الفموية من المستخلص المائي للنبات ولكن لمدة شهرين. تم قتل الحيوانات في نهاية الشهر الاول والثاني وتم تشريح الكلى ومن ثم فحص الشرائح النسجية بواسطة المجهر الضوئي و تصويرها. ثم تم تقييم التغيرات المرضية النسجية في الكلى بالمقارنة بين المجموعات المعالجة من الفئران مع مجموعة السيطرة .وأظهرت النتائج أن إعطاء جرعات يومية فموية من (٠,١غم / مل) من المستخلص المائي لنبات *Anastatica hierochuntica* لمدة اسبوع

واحد لم يحدث أي تغييرات في مظهر الكلية الخارجي أو في نسيجها. في حين أظهرت معاملة الفئران بجرعات يومية فموية من (٠,١غم / مل) من المستخلص المائي للنبات لمدة شهر و احد الى ظهور تأثيرات معنوية ($P \leq 0.05$) في نسيج كلى الفئران حيث تضمنت انسلال الخلايا اللمفاوية، تنخر، تدهور وانتفاخ الكبيبات الكلوية حيث أظهرت الكلى احتقانا عند الفحص المجهري . في حين أظهر الاستمرار في معاملة الحيوانات بنفس الجرع من المستخلص النباتي لشهر ثاني الى زيادة الاضرار في نسيج الكلى مع فقدان كامل في بنية الكلية.

Introduction:

Herbal medicine was used by about 80% of the world´s population, primarily in developing countries for routine health care, and also enter the therapeutics in the developed countries[1]. These herbs escape toxicity testing before they are marketed as traditional medicine due to inadequate drug laws. Yet many reports reveal that drugs of plant origin are not free from toxic effects[2-4].*Anastatica hierochuntica*, belongs to the family Crucifera and the only member of the genus *Anastatica*[2] . It is a small, gray winter annual herb that grows to a maximum height of 15 cm, and produces small white flowers and it is found in the arid regions of Saudi Arabia, Egypt, Jordan, Iraq, the UAE, Iran, Kuwait, and North Africa, and can survive without water for long periods[3].

The whole plant contains flavonoids: luteolin-7- glucoside, isovitexin, kaempferol 7-glucoside, kaempferol 3-rhamnoglucoside, quercetin and lucitin. It also contains glucosinolates: glucoiberin and glucocheirolin. The fruits contain glucose, galactose, fructose, sucrose, raffinose and stachyose[1,2].

In Arab countries *Anastatica hierochuntica*, is known as Kaff Maryam , where it is widely consumed as a tea beverage. It is powdered, mixed with honey and taken for the treatment of many conditions, in particular as a remedy for difficult childbirth and uterine hemorrhage[4,5]. In addition, it is used to treat asthma, gastrointestinal disorders, depression, high blood pressure, indigestion, headache, cold, fever, malaria, epilepsy, fatigue, diabetes, heart disease, and infertility[4-6]. However, many these studies had identified and quantified a number of minerals and phenolic compounds from this plant used for treating health disorders. But fewer studies were available on the histological effect of the prolonged use of Kaff Maryam on different body organs[3].

Therefore, this study was aimed to investigate the histological effects of *Anastatica hierochuntica* plant in mice kidneys.

Materials and methods:

1. Animals management:

Healthy, adult mice animals of *Mus musculus* strain were used in this study, ranging in age between (4-8) weeks old and their weight were about (25± 2g).Animals were divided into three equal groups (each group consisted of 10 females) and were kept in an air condition room at a temperature of (22-24 ºC),with about (12-14) hours of day light exposure. Animals were housed in cages measuring (29*15*12 cm) and each ten animals were kept in one cage contained wooden shave. Water and Feed composed of (wheat, barely mixed with 250mg of milk powder) were freely accessible and animals were kept for at least two weeks for adaptation. Animal cages were cleaned and sterilized with 70% ethanol once a week regularly according to standard procedure[7].

2. Plant used in the study:

Samples of whole dried *Anastatica hierochuntica* plant were brought from Kingdom of Saudi Arabia. The aerial parts of the plant were isolated and kept in airtight glass containers till the time of the experiment.

3.Treatment:

The dried plant was ground to fine powder and about (1g) of the powder was mixed with (10ml)of distilled water and were incubated for 3hrs at (60° C). Suspension was then filtered and the water extracts were prepared daily just before administration orally to the experimental animals in a dose of (1ml/mouse of 25 g) according to standard procedure[8].

4.Experimental protocol:

Experimental animals were divided into three groups (10 mice each):

1.The control group mice were orally administered with a daily dose of (1ml) distilled water for 2 months.

2.Plant –treated group1 were orally administered with a daily dose of (0.1g/ml) of *Anastatica hierochuntica* plant water extract for one month.

3.Plant –treated group2 were orally administered with a daily dose of (0.1g/ml) of *Anastatica hierochuntica* plant water extract for two months.

Two animals from both the control and the plant - treated group 1 were slayed after one week of the treatment and their kidney tissues were assessed histological. then, the rest of the animals of the Plant –treated groups 1 and 2 were slayed after one and two months for kidney tissue histological assessment with regard to the control group.

5.Histological assessment:

Animals were autopsied and kidney tissues were excised, fixed in 10% formalin and were embedded in paraffin. Tissue sections were cut at 5µm, mounted on slides, stained with hematoxylin- eosin (H-E) for general kidney structure examination. The sections were examined by light microscope (Optika- Italy) and photographed by (Optica- Italy 4083-B5-camera) according to standard procedures[9,10].

6.Statistical analysis:

Data from the study were analyzed using T- test by using SPSS program Ver.10 for Windows. The P value of ≤ 0.05 was considered indicative of a statistically significant difference .

Results:

Results of table (1) showed no significant macroscopic changes noted in the control untreated mice and their kidney tissues that appeared normal in shape and size. Whereas, the continued treatment of animals with (120g/ Kg) of the aqueous extract of the plant *Anastatica hierochuntica* for one and two months respectively resulted in the congestion of the kidneys of the treated animals.

On the other hand, the histopathological findings of this study showed that, a daily oral dose of (0.1g/ml) for one week of the aqueous extract of the plant *Anastatica hierochuntica* produced no kidney tissue lesions and the kidney sections of the treated mice appeared as normal as the control group kidney sections (Figures 1&2),while, the treatment of mice with daily oral doses of (0.1g/ml) for two weeks of the aqueous extract of the plant resulted in the appearance of lymphocyte infiltration and necrosis of the kidney tissue glomeruli (Figure 3).

Whereas, (Figure 4) showed the histological changes in the kidney tissues after treatment with daily oral doses of (0.1g/ml) aqueous extract of *Anastatica hierochuntica* for one month where the a significant increase ($P \leq 0.05$) in the necrosis of kidney tissue glomeruli was recorded and necrosis was spotted in about (82.58%) in the kidney tissues of animals slayed from group2. The histological changes resulted from the continual administration of the plant extract for six and seven weeks were shown in (Figures 5&6) respectively, where a degeneration and swelling of the kidney tissue glomeruli was also detected after seven weeks of treatment.

In addition, (Figure 7) showed the result of mice treatment with daily oral doses of (0.1g/ml) aqueous extract of *Anastatica hierochuntica* for two months where a complete loss in kidney

tissue architecture was noted. In addition, results of table (2) revealed that, kidney tissue sections of the control group (Group1) showed no histopathological changes. While, kidney tissue sections biopsied from mice treated with a total concentration of (120g/Kg) for one month presented many histopathological changes ranged from mild to severe where (61.22%) of the autopsied animals showed inflammatory cellular infilteration mainly lymphocytes and neutrophils. While, (82.58%) of them showed necrosis of the glomeruli in their kidney tissue sections. Yet, about (23.30%& 36.44%) of Group2 animals showed degeneration and swelling of the glomeruli. Whereas, no loss in kidney architecture was detected in any kidney histology sections of group 2 being examined. Furthermore, results of table (2) also showed the significant increase ($P \leq 0.05$) in lymphocyte infiltration, necrosis, degeneration and swelling of the glomeruli in a percentage of (86.65%, 92.11%,89.14% and 57.75%) respectively. Besides, about (91.78%) of mice females from group 3 showed a complete loss of kidneys architecture.

Discussion:

Many studies had identified and quantified a number of minerals and phenolic compounds extracted from the plant *Anastatica hierochuntica* used for the treatment of many health disorders[1-3]. But, there are very limited studies available on the side effects and safety of the prolonged use of *Anastatica hierochuntica* on different body organs especially pregnant women and children[11&12].

Results of this study had shown that, there were no significant macroscopic or histological changes in the kidney tissues of mice treated with a single dose of (0.1g/ml) for one week of the aqueous extract of the plant *Anastatica hierochuntica* .This result however, came in agreement with the results obtained in[5&13] where they declared that, the administration of fewer , low doses of extracts from the plant Anastatica hierochuntica associated with no side effects. Yet, they affirmed on the hepatoprotective effect of the plant where, the (anastatins A and B flavonoids) isolated from the methanolic extract of the Anastatica hierochuntica plant were found to show hepatoprotective effects14&15.But, an elevation in the degenerative lesions where an increase in the lymphocyte infiltration, necrosis and swelling were all associated with the continual administration of the aqueous extract of the plant Anastatica hierochuntica for two months. Consistent with these findings were the observations of16-18 where they declared that, the plant Anastatica hierochuntica contain a combination of silica, Zinc, iron and aluminum minerals. Consequently, the sever hepatotoxicity noticed after the prolonged administration of the aqueous extract of the plant Anastatica hierochuntica might be probably due to the accumulation of high concentration of these minerals in the vital organs over long periods of time of plant extract administration19-22.Subsequently, adverse reactions were also been reported in some clinical trials where a review of 30 studies involving 506 subjects reported a total of 246 adverse events, thus representing an adverse reaction rate of approximately 2%. The major reactions reported included acne, changes to the menstrual cycle, dizziness, gastrointestinal distress, increased menstrual flow, nausea, skin reactions, urticaria and weight gain23&24.Minor adverse events include fatigue, hair loss, increased intraocular pressure, palpitations, polyurea, sweating and vaginitis24.

Conclusions:

1. In particular, an oral dose of (0.1g/ml) for one week of the aqueous extract of the plant *Anastatica hierochuntica* had no detectable effect on mice kidney histology (OSL- observed safe level).
2. Prolonged use of the aqueous extract of the plant *Anastatica hierochuntica* for more than one month is associated with significant side effects included a hallmark of cellular infiltration and lesions of necrosis.

3. Kidneys are vital organs in the body. Thus, the administration of any herb for the treatment of any medical conditions should be considered carefully to avoid kidney damage of the patient .
4. Treatment of women especially the pregnant with the aqueous extract of the plant *Anastatica hierochuntica* should be taken seriously due to the renal side effects of this extract to avoid complications.

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This study was implicated under the approval of the (Medical Laboratory Techniques Department board- College of Technology/ Kirkuk/ Iraq) and the maintenance and care of experimental animals complied with National Institutes of Health guidelines for the humane use of laboratory animals.

Table (1):Macroscopic changes observed in the kidneys of mice.

Animal Groups	*A. hierochuntica* Total Concentration/Kg	Macroscopic changes in mice females kidneys
Group 1	0 mg/kg	Normal
Group 2	120g/Kg	Congested
Group 3	240g/Kg	Congested

*** Group 1= Control group (1ml) distilled water, Group 2= Plant-treated mice for one month, Group 3 =Plant-treated mice for two months.**

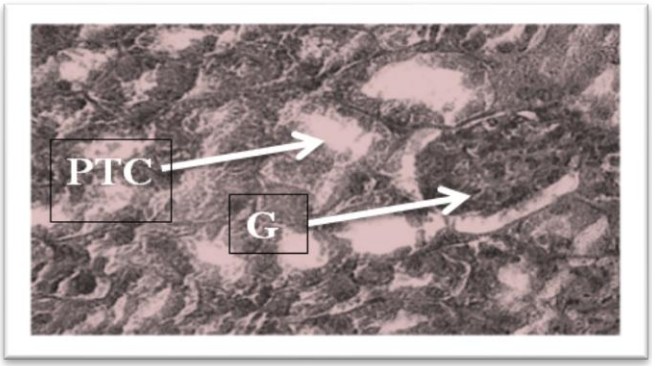

Figure (1): Histological kidney section from a control mouse showing normal morphology where; PTC= proximal tubule convulsion and G= Glomeruli , following (H-E Staining at 40X).

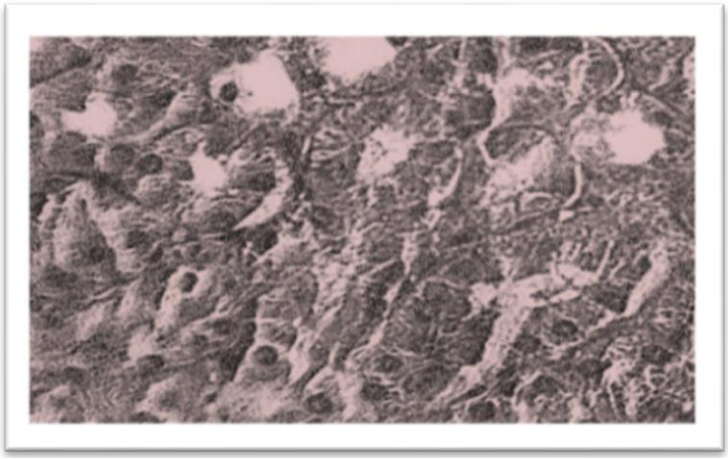

Figure (2): Histological kidney section of a mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* showing normal morphology when compared to the control following (H-E Staining at 40X).

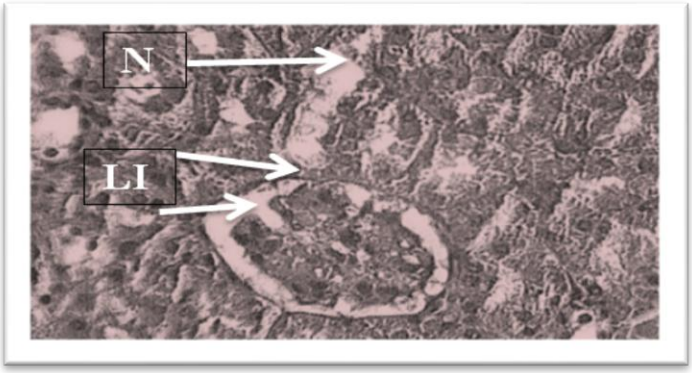

Figure (3): Histological kidney section from a mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* for two weeks showing Lymphocyte infiltration (LI) and Necrosis(N) following (H-E Staining at 40X).

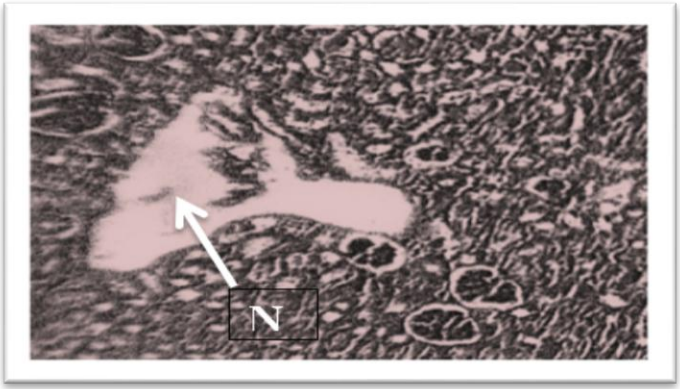

Figure (4): Histological kidney section from a mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* for one month showing the increase in the tissue Necrosis(N) following (H-E Staining at 40X).

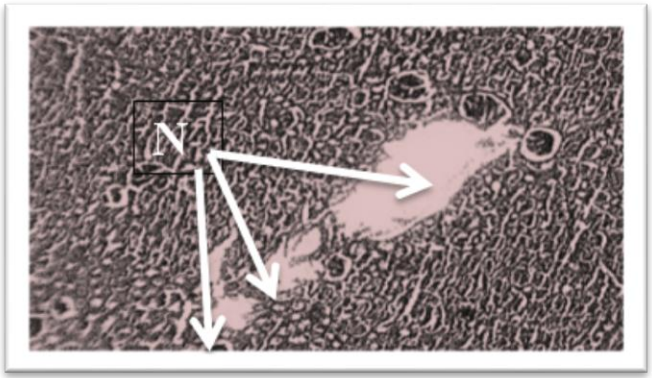

Figure (5): Histological kidney section from a mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* for six weeks showing a significant increase in the tissue Necrosis(N) following (H-E Staining at 10X).

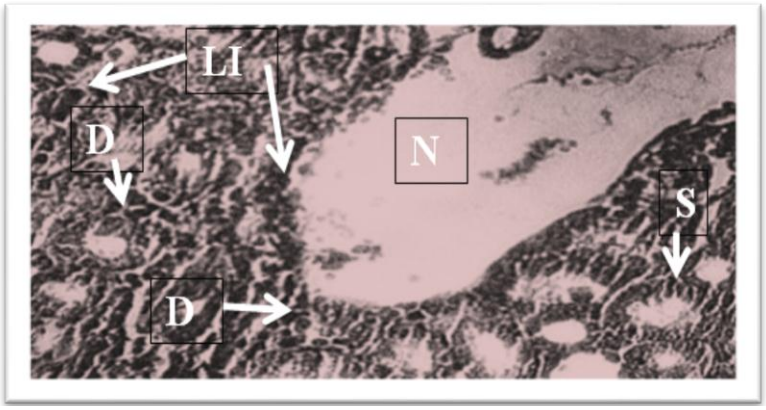

Figure (6): Histological kidney section from a mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* for seven weeks showing Lymphocyte infiltration (LI), Necrosis(N), Degeneration of the glomerulus (D) and Swelling (S) following (H-E Staining at 40X).

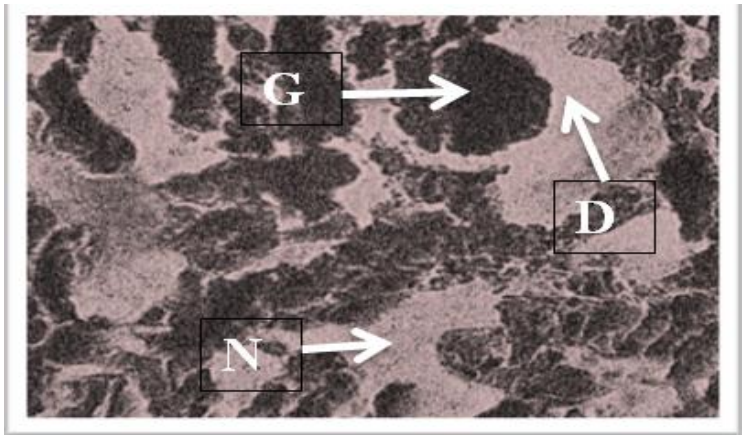

Figure (7): Histological kidney section from a female mouse treated with daily oral doses of (0.1g/ml) of the aqueous extract of the plant *Anastatica hierochuntica* after two months showing a severe Degeneration of the glomerulus (D) with severe Necrosis(N) in the kidney tissue and a complete loss of kidney architecture following (H-E Staining at 40X)

Table (2): A comparison in the histopathological changes scored in the kidneys autopsied from the three groups of the experimental animals.

Groups of Mice	*A. hierochuntica* Total Concentration/Kg	The percentage of histopathological changes in Mice Female Kidneys				
		LI%	N%	D%	S%	Complete Loss Of Architecture %
Group 1	0mg/Kg	0	0	0	0	0
Group 2	120g/kg	61.22	82.58	23.30	36.44	0
Group 3	240g/Kg	86.65	92.11	89.14	57.75	91.78

* **Group1= Control group. mice treated with (1ml) distilled water for 2 months.**
Group2= mice treated with (0.1g/ml) of the aqueous extract of the plant for one month
Group3 = mice treated with (0.1g/ml) of the aqueous extract of the plant for two months
LI= Lymphocyte Infiltration, N=Necrosis, FD= Fatty Degeneration, CDCV= Congestion and dalitation of the central hepatic vein and C&D= Congestion and Dalitation.

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