SEBHA UNIVERSITY JOURNAL OF PURE & APPLIED SCIENCES VOL.21 NO. 4 2022
DOI: 10.51984/JOPAS.V21I4.2116

مجلة جامعة سبها للعلوم البحتة والتطبيقية
Sebha University Journal of Pure & Applied Sciences

Journal homepage: www.sebhau.edu.ly/journal/index.php/jopas

The effect of Moringa oleifera leaf powder on hydroxychloroquine -induced kidney tissues injury

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Keywords:
Creatinine
Hydroxychloroquine
Moringa oleifera
Rats
Urea

ABSTRACT

The present study was conducted to investigate the possible protective effect of Moringa oleifera leaves powder on Hydroxychloroquine induced kidney tissue injury. White male rats were used, and dried Moringa leaf powder was given to them with lunch for 3 weeks to play a protective role in the body, they were dosed orally by tube prepared daily for 5 days with hydroxychloroquine in different doses (1000, 500,250 mg /kg b.wt). Urea and creatinine levels were determined in the serum in addition to the histopathology of the kidney, and the hematological effects were determined for this medicine in the rats. The results showed a significant increase in the urea level and the concentration of creatinine in rats that had been given hydroxychloroquine compared to normal and treated groups with M. oleifera. Also, treatment with M. oleifera showed a very significant drop in urea level. There was an apparent change in the urea level with experience. A significant increase in the count of RBCs was recorded for the hydroxychloroquine group concentration of 1000 mg/kg, compared to the control group and the other groups. And that recorded significant increase in the content of Hb and count of Platelet was recorded for the hydroxychloroquine group concentration 1000 mg/kg, compared to the control group and the other group's .at P value < 0.05. The effects of tissue degradation by hydroxychloroquine were improved when treated with Moringa oleifera leaves powder.

تأثير مسحوق أوراق المورينجا أوليفيرا على إصابة أنسجة الكلى التي يسببها هيدروكسي كلوروكين.

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الكلمات المفتاحية:
جرذان
مورينجا
أوليفيرا
كرياتينين
هيدروكسي كلوروكين
يوريا.

الملخص

أجريت هذه الدراسة للتحقيق في التأثير الوقائي المحتمل لمسحوق أوراق المورينجا أوليفيرا على إصابة أنسجة الكلى التي يسببها عقار هيدروكسي كلوروكين، واستخدمت ذكور الجرذان البيضاء ، وأعطيت مسحوق أوراق المورينجا مع الغداء لمدة 3 أسابيع لتلعب دورًا وقائيًا في إصابة الجسم ، تم تناولت عن طريق الفم بواسطة انبوب معدي يوميًا لمدة 5 أيام عقار هيدروكسي كلوروكين بجرعات مختلفة (1000 ، 500 ، 250 مجم / كجم من وزن الجسم). تم تحديد مستوى اليوريا في المصل. علاوة على ذلك ، تم فحص مستويات الكرياتينين في الدم، بالإضافة إلى التشريح المرضي للكلى، وتم تحديد التأثيرات الدموية لهذا الدواء في الجرذان. أظهرت النتائج زيادة معنوية في مستوى اليوريا وتركيز الكرياتينين في الجرذان التي تم إعطاؤها هيدروكسي كلوروكين مقارنة بالمجموعات الطبيعية والمعالجة بـ المورينجا أوليفيرا. كما أظهر العلاج بـ المورينجا أوليفيرا انخفاضًا كبيرًا جدًا في مستوى اليوريا، وكان هناك تغيير واضح في مستوى اليوريا ضمن التجربة. تم تسجيل زيادة معنوية في عدد كرات الدم الحمراء في مصل الدم لتركيز مجموعة هيدروكسي كلوروكين 1000 مجم/كجم، مقارنة بمجموعة التحكم والمجموعات الأخرى. وسجلت زيادة معنوية في محتوى الهيموغلوبين وعدد الصفائح الدموية لمجموعة هيدروكسي كلوروكين بتركيز 1000 مجم / كجم مقارنة بمجموعة السيطرة والمجموعة الأخرى بقيمة.P <0.05

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Article History : Received 13 May 2022 - Received in revised form 25 July 2022 - Accepted 03 October 2022

Introduction

Hydroxychloroquine is an antimalarial medication that has been used as a first-line treatment for rheumatic diseases for decades [1] Hydroxychloroquine (HCQ)can have a variety of effects on a variety of animal organs, including the liver, kidney, and intestinal tissues, as well as the immune system and the brain [2]. And, according to [3], HCQ use has become negatively associated with the likelihood of developing chronic kidney disease CKD sooner. Perhaps the flares of lupus nephritis were restricted.

And the impact of HCQ on chronic renal disease has been the subject of previous studies (CKD) [3]. Studied 256 lupus patients for up to twenty-five years and discovered that HCQ use was negatively associated with the likelihood of developing advanced CKD. We assumed that evidence of HCQ's Effectiveness in preventing end - stage renal disease ESKD and death, or possibly lupus nephritis flares, was limited. As a result, we created a retrospective cohort to observe from a population-based statistics set to see if there's a link between HCQ use and the risk of developing CKD in SLE patients [3].

Moringa oleifera is a member of the Moringaceae family of plants. Its leaves are high in macronutrients, micronutrients, polyphenols, phenolic acids, vitamins, carotenoids, flavonoids, and alkaloids, as well as polyphenols, phenolic acids, vitamins, carotenoids, flavonoids, and alkaloids [4]. As a result, the *Moringa oleifera* plant is used to nourish both animals and humans as a superior nutritional supplement. It has long been used as a traditional medicinal resource and is employed to treat a variety of ailments. [5] The purpose of this study was to determine the efficacy and protective benefits of *Moringa oleifera* leaves against hydroxychloroquine-induced kidney tissue damage in rats.

Materials and Methods:

Plant material: Fresh *Moringa oleifera* leaves were collected during the month of January 2021 from the home garden, Libya and was identified and confirmed by Leaves were dried in the shade,,and crushed and kept in a tray until use.

Chemicals: Hydroxychloroquine sulphate ($C_{18}H_{26}ClN_3O$) used in this work was obtained from EVA Group Limited, Egypt.

Experimental animals:

Adult male rats weighing 180 g were used in the experiments, which were purchased from Helwan Animal Station in Egypt. 5 rats per cage were housed at 24°C and were subjected to a 12 hour light/12 hour dark cycle. They were provided a regular meal and have unrestricted access to water. For acclimatisation, the animals were kept in these usual circumstances for two weeks prior to the experiment.

Experimental design:

Animals were randomly separated into 8 groups of 5 rats at the end of the acclimation phase. **Group I** rats served as the control group and were fed a normal diet, while **Group II** rats were given *Moringa oleifera* leaves powder (2.7g/kg b.wt) mixed with meal, **Group III** rats were given orally HCQ (1000 mg/kg b.wt) daily for 5 days, and **Group IV** rats were given *Moringa oleifera* powder (2.7g/kg b.wt) daily for 5 days. After 3 weeks of mixing in the diet, HCQ (1000 mg/kg b.wt) was given orally for 5 days.

For 5 days, **Group V** rats were administered HCQ (500 mg/kg b.wt) orally, while **Group VI** rats were given *Moringa oleifera* powder (2.7g/kg b.wt). After 3 weeks in the diet, **Group VII** rats were given HCQ (500 mg / kg b.wt) orally for 5 days, **Group VII** rats were given HCQ (250 mg / kg b.wt) orally for 5 days, and **Group VIII** rats were given *Moringa oleifera* powder (2.7g/kg b.wt) 3 weeks of mixing in the diet, HCQ (250 mg/kg b.wt) was given orally for 5 days.

Collection of blood and tissues specimens:

Rats were fasted for 12 hours before being slaughtered under chloroform anesthesia at the end of the experiment. Using sterile syringes, blood samples were taken directly from the heart. Anticoagulants were added to whole blood collected in clean, dry centrifuge tubes (EDTA). For biochemical studies, the remaining blood was collected in clean, dry centrifuge tubes. Serum samples were then produced by centrifugation for 15 minutes at 4000 rpm. These samples were maintained at -20 °C in clean Stoppard plastic vials until they were analysed. The kidney was excised and rinsed in normal saline solution to remove the excess blood, then weighed and fixed in 10% formalin for anatomical study.

Physiological study: This study includes determination of serum urea and creatinine levels, using commercial diagnostic kits supplied by diamond Diagnostics, Cairo, Egypt.

Principle of action urea:

The method is based on the following reaction:

$Urea + H2O \rightarrow 2NH3 + CO2$

The ammonium ions formed are measured by the bertha lot reaction absorbs light between 530nm and 560nm proportional to initial urea concentration[6].

Calculation:

Urea concentration = A Sample / A Standard × Standard conc.

$$\text{Urea in urine (g/dl)} = \frac{\text{A sample}}{\text{A Standar}} \times 5$$

Principle of action creatinine:

Creatinine in alkaline solution reacts with picric acid to form colored complex. The amount of the complex formed is directly proportional to the creatinine concentration[7].

Calculation:

$$A_2–A_1 = \Delta A_{Sample} \text{ or } \Delta A_{Standard}$$

$$\text{Creatinine in serum. (mg/dl)} = \Delta A_{Sample} / A_{Standard} \times 2$$

$$\text{Creatinine in urine (mg/dl)} = \Delta A_{Sample} / A_{Standard} \times 100$$

Creatinine clearance (ml/min)

$$= \frac{(\text{mg ceratinine / dl urine} \times \text{ml urine / 24 hrs})}{(\text{mg creatintine /dl serum} \times 1440)}$$

Haematological study: In rats,[8]approach was used to determine the characteristics of the blood picture: red blood cell count(RBCs), white blood cell count(WBCs), content haemoglobin (HB), and mean platelet count(PLT)[8]

Histopathological examination:

The kidney tissues were collected from different groups and fixed in 10% neutral buffered formalin then tissues were dehydrated in ascending grades of ethyl alcohols, 70%, 90% and 100% (to prevent shrinkage of tissues and to remove water gradually from the fixed tissues), cleared in xylol (to remove alcohol and to allow the fixed tissues to be miscible with paraffin wax in the following step) and embedded 60°C in paraffin wax The Cambridge Rocking Microtome was then used to cut serial transverse sections at a thickness of 5-6 microns and affix them to slides. Sections were stained in Haematoxyline and Eosin (H&E) for the general histological research, then washed, dehydrated, clarified, and mounted with Canada balsam for permanent preparations [9]. Light microscopes were used to inspect and photograph the sections.

Statistical analysis:

The results were represented as mean standard deviation, and statistical analysis was carried out using ANOVA and a post hoc test (Duncan). It's a type of parametric statistical analysis that evaluates between- and within-group variance in order to determine differences between two or more groups. Statistical significance was defined as a P value of less than 0.05. SPSS version 20 was used to analysis the data.

Results:

Kidney enzymes

1- Serum urea level:

The hydroxychloroquine group, which received 500 mg/kg and was processed by moringa, had a substantial increase in serum urea levels as compared to the control group and the other groups, with a P value of 0.01. Rats given HCQ with *Moringa oleifera*, on the other hand, exhibited significant improvements in kidney function tests when compared to rats given HCQ alone. **Table 1 and Figure 1** show the results.

2- Serum creatinine level:

When comparing the rats with different doses of hydroxychloroquine to the control group, there was a substantial drop in creatinine concentration. In contrast, hydroxychloroquine caused a substantial rise in creatinine concentration in moringa processing groups as compared to the control group, with a P value of 0.01. **Table 1 and Figure 2** show the results.

Table (1): Mean values of serum urea (g/dl), and creatinine (mg/dl), in the serum of different experimental groups.

Groups Parameters	Urea (g/dl) mean ± S.D	Creatinine (mg/dl) mean ± S.D
Control	64.60 ± 7.70^{b}	0.46 ± 0.13^{d}
Moringa	53.80 ± 4.65^{a}	0.50 ± 0.12^{d}
HCQ(1000mg/kg)	53.00 ± 6.27^{a}	0.41 ± 0.09^{b}
MO+HCQ(1000mg/kg)	50.00 ± 6.00^{a}	0.26 ± 0.04^{a}
HCQ (500mg/kg)	54.40 ± 2.60^{a}	0.28 ± 0.11^{a}
MO+ HCQ(500mg/kg)	63.75 ± 2.21^{b}	0.30 ± 0.10^{a}
HCQ(250mg/kg)	53.50 ± 3.00^{a}	0.24 ± 0.06^{a}
MO+HCQ(250mg/kg)	51.60 ± 8.32^{a}	0.43 ± 0.11^{c}
P-value(P)	0.002**	0.002**
Decision	**=Very-Sig (P<0.01)	**=Very-Sig (P<0.01)

The data are presented as mean standard deviation (N=5 in each group). (**) Very significant, with a p value of 0.01 when compared to the control group. HCQ = hydroxychloroquine, *MO* = *Moringa oleifera*, a, b, c, d, superscript letters in the same column means having different significantly (P<0.05).

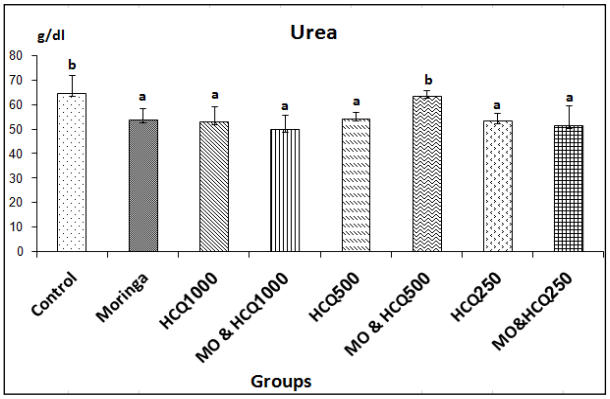

Figure (1): The serum urea levels of different treated groups, a,b, means having different superscript letters in the same row differ significantly (P<0.05).

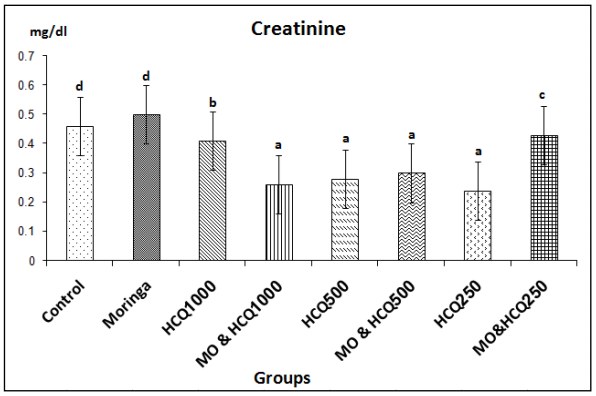

Figure (2): The serum creatinine concentrations of different treated groups,a,b, means having different superscript letters in the same row differ significantly (P<0.05).

Haematological studies

1. Red blood cells (RBCs):

Table 2 shows that there was no significant difference in the number of RBCs in the blood of the experimental and control groups as compared to the control group. When compared to the control group and the other groups, the hydroxychloroquine group concentration of 1000 mg/kg showed a significant increase in the count of RBCs in serum, with a P value of 0.05. **Graph 3**.

2. White blood cells (WBCs):

When comparing the counts of WBCs in the blood of the different doses of HCQ to the control group, no significant differences were found. When the P value is more than 0.05, it is considered significant. **Table 2** and **Figure 4** show the results.

3. Haemoglobin concentration (Hb):

Table 2 and **Figure 5** show the results. The content of Hb in the blood of the experimental groups was not significantly different from the control group. When compared to the control group and the other groups, the hydroxychloroquine group concentration of 1000 mg/kg showed a significant increase in Hb content, with a P value of 0.05.

4. Platelet:

Table 2 shows that there was no significant difference in platelet count in the blood of the experimental groups compared to the control group. When compared to the control group and the other groups, the hydroxychloroquine group concentration of 1000 mg/kg showed a significant increase in platelet count with a P value of 0.05. **Graph 6.**

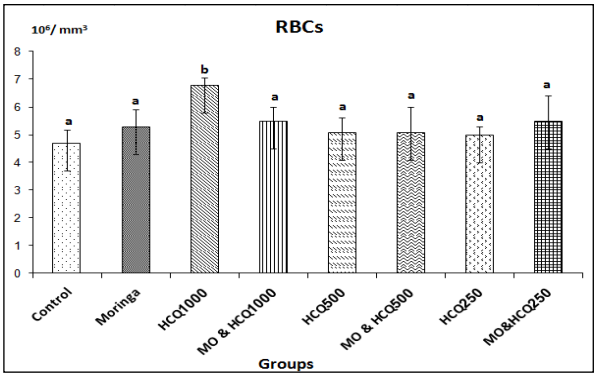

Figure (3): Showing count of RBCs of different treated groups, a, b, means having different superscript letters in the same row differ significantly (P<0.05).

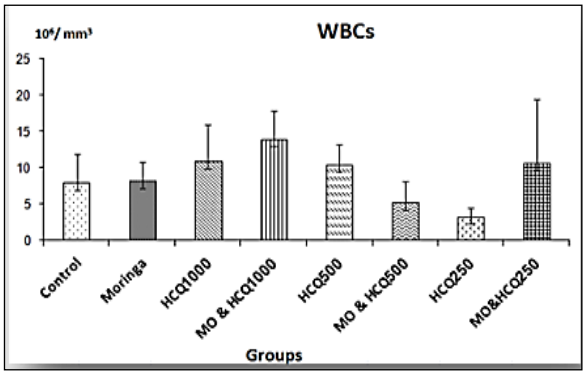

Figure (4): Showing count of WBCs of different treated groups.

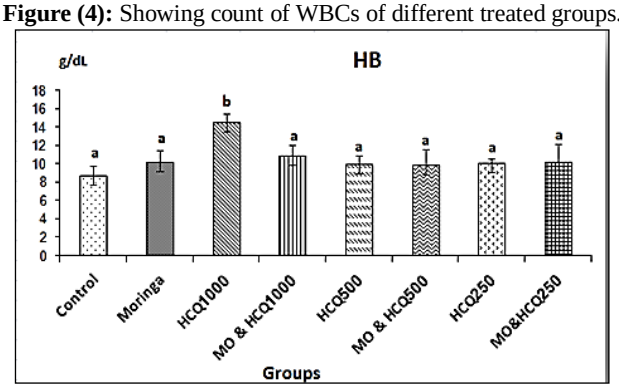

Figure (5): Showing count of Hb of different treated groups,a,b, means having different superscript letters in the same row differ significantly (P<0.05).

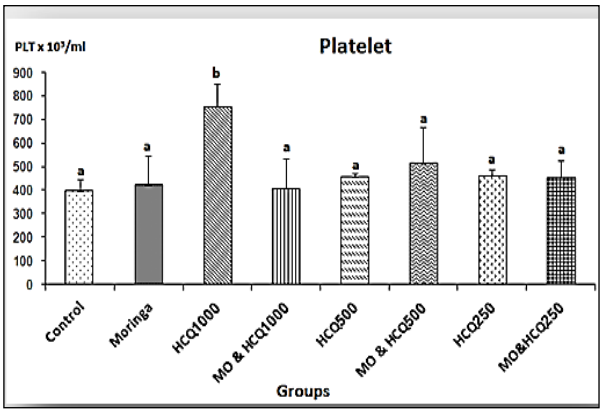

Figure (6): Showing count of platelet of different treated groups, a, b, means having different superscript letters in the same row differ significantly (P<0.05).

Table (2): In the sera of separate experimental groups, the mean count of (RBCs) (10^6 mm3), (WBCs) (10^6 mm^3), (HB) level (g/dl), and (PLT) 10^3/ml).

Groups Parameters	RBC 10^6/ mm^3) mean ± S.D	WBC 10^6/ mm^3) mean ± S.D	HB (g/dL) mean ± S.D	PLATL PLT x 10^3/ml) mean ± S.D
Control	4.7 ±0.47[a]	7.9 ± 4.0	8.7 ± 1.12[a]	398.0 ± 50.2[a]
Moringa	5.3 ± 0.62[a]	8.2 ± 2.6	10.2 ± 1.29[a]	425.4 ± 124.3[a]
HCQ(1000mg/kg)	6.8 ± 0.27[b]	10.9 ± 5.0	14.6 ± 0.88[b]	758.2 ± 94.9[b]
MO+HCQ (1000mg/kg)	5.5 ± 0.51[a]	13.9 ± 3.9	10.9 ± 1.13[a]	411.0 ± 124.6[a]
HCQ(500mg)	5.1 ± 0.51[a]	10.4 ± 2.8	10.0 ± 0.92[a]	459.0 ± 14.7[a]
MO+ HCQ(500mg/kg)	5.1 ± 0.89[a]	5.2 ± 2.9	9.9 ± 1.68[a]	517.7 ± 151.8[a]
HCQ(250mg/kg)	5.0 ± 0.28[a]	3.3 ± 1.2	10.1 ± 0.46[a]	464.6 ± 25.6[a]
MO+HCQ(250mg/kg)	5.5 ± 0.92[a]	10.7 ± 8.7	10.3 ± 1.89[a]	458.0 ± 71.1[a]
P-value(P)	0.01**	0.1^{ns}	0.000***	0.000***
Decision	**=Ver.Sig (P≤ 0.01)	Not. Sig (P>0.05)	***=Ex.Sig (P<0.001)	***=Ex.Sig (P<0.001)

The data are presented as mean standard deviation (N=5 in each group). (**) Compared to the control group, very significant, p<0.01 and (***) extremely significant, p≤ 0.001 and (ns) not significant. HCQ = hydroxychloroquine, MO = Moringa; a, b, indicate that distinct superscript letters in the same column differ significantly (P<0.05).

Histopathological studies: -

1-Histopathological of the kidney in the control group:
Histopathological examination of control renal tissue **(Figure. 7, A&B)** revealed an intact normal renal architecture and well components of the glomeruli (renal corpuscles), renal convoluted tubules and interstitial tissues of both the cortex.

2- Histopathology of the kidney in rats that were given *Moringa oleifera* for 3 weeks:
Sections of the kidney from rats and treated with Moringa showed normal histology with no evidence of pathological damage, normal renal glomeruli and tubules, **(Figure.8 , C&D).**

3- Histopathological of the kidney in the rats given HCQ doses of 1000 mg/kg:

The kidney of rats treated with HCQ revealed histological alterations including the glomeruli, renal tubules and interstitial tissues. Many of the renal corpuscles had disrupted histological structure in the form of shrunken and lobulated capillary tuft renal corpuscles, dilated Bowman`s space **(Figure.9, E&F).**

4- Histopathological of the kidney in the rats given HCQ doses of 1000 mg/kg and treated with *Moringa oleifera* for 3 weeks:

Histopathological examination of the kidney of rats given Moringa for 3 **weeks** and then HCQ showed an almost normal kidney structure. However, few dilated renal tubules and a dilated Bowman's spaces were detected. (**Figure. 10, G&H).**

5- Histopathological of the kidney in the rats given HCQ doses 500 mg/kg:

Histopathological examination of kidney sections revealed glomerular tuft congestion, oedema of the Bowman's space, and degenerative changes within the renal tubular epithelium, as well as lumenal dilation in a few proximal and distal convoluted tubules **(I&J in Figure 11).**

6- Histopathological of the kidney in the rats given HCQ doses 500 mg/kg and treated with *Moringa oleifera* for 3 weeks:

Moderate improvement of histopathological changes was observed in treated group with Moringa. These was confirmed by the detection of some shrunken, lobulation, congestion and hyalinization of glomerular capillary tuft, degenerated podocytes of visceral layer of Bowman`s capsule **(Figure. 12, K&L).**

7- Histopathological of the kidney in the rats given HCQ doses 250 mg/kg:
Most of the renal pellet structure appeared to have regained its normal organization and the proximal and distal convoluted tubules showed a normal appearance of renal endothelial

Epithelium in hydroxychloroquine rat's doses 250 mg/kg. However,

a few proximal and distal convoluted tubules showed a small dilation of the lumenal**. (Figure. 13, M&N).**

8- Histopathological of the kidney in the rats given HCQ doses 250 mg/kg and treated with *Moringa oleifera* for 3 weeks:
Histopathological examination of renal tissue **(Figure. 14, O&P)** revealed a intact normal renal architecture and well components of the glomeruli (renal corpuscles), renal convoluted tubules and interstitial tissues of both the cortex.

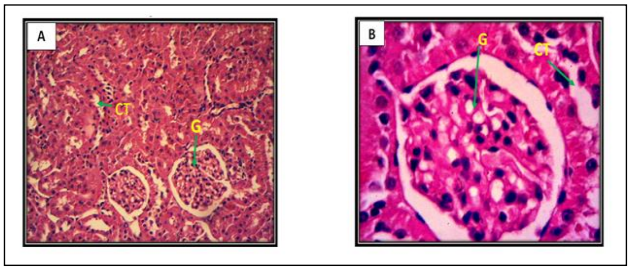

Figure. (7): Photomicrograph of rat kidney tissue. Control group I (A&B) showing glomeruli (G),renal convoluted tubules (CT), (H&E, A; 100X). (H&E, B; 400X).

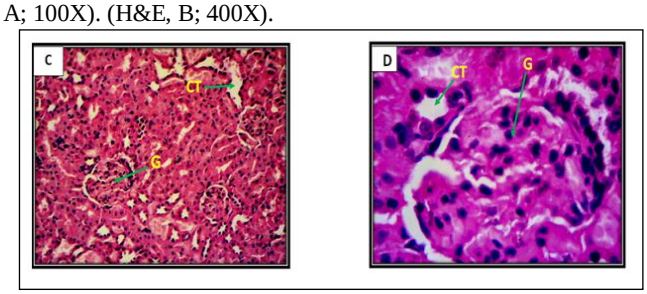

Figure (8): Photomicrograph of rat kidney tissue. Group II (Control Moringa)(C&D).showing glomeruli(G),renal convoluted tubules (CT), (H&E, A; 100X). (H&E, B; 400X).

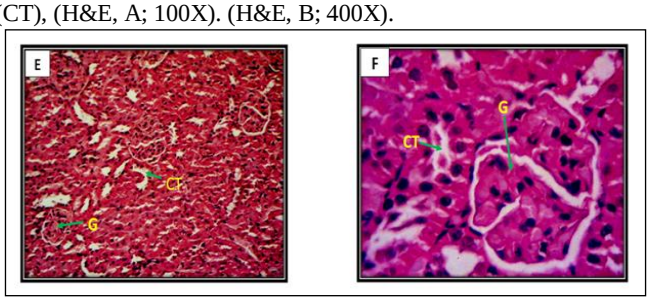

Figure. (9): Photomicrograph of rat kidney tissue. group III (E&F) rats with dose of hydroxychloroquine 1000 mg /kg) showing glomeruli (G), renal convoluted tubules (CT), (H&E, E ; 100X). (H& E, F; 400X).

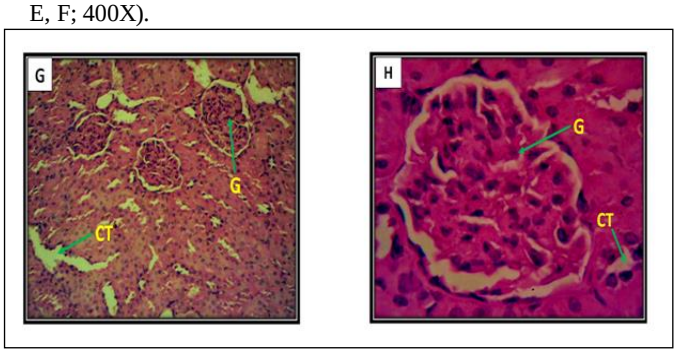

Figure (10): Photomicrograph of rat kidney tissue. Group IV (G&H) rats with dose of hydroxychloroquine 1000 mg /kg and treated with Moringa. Showing glomeruli (G), renal convoluted tubules (CT), (H&E, G; 100X). (H& E, H; 400X).

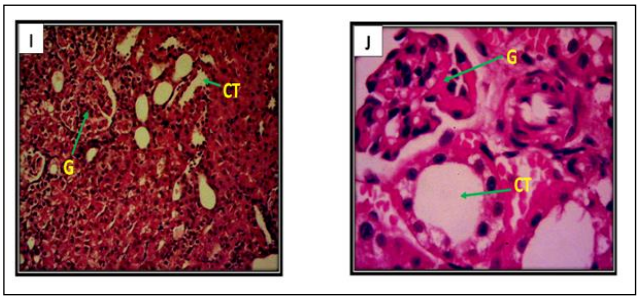

Figure (11): Photomicrograph of rat kidney tissue. Group V (I&J) rats with a dose of hydroxychloroquine of 500 mg /kg). Showing glomeruli (G), renal convoluted tubules (CT), (H&E, I;100X).(H&E,J;400X).

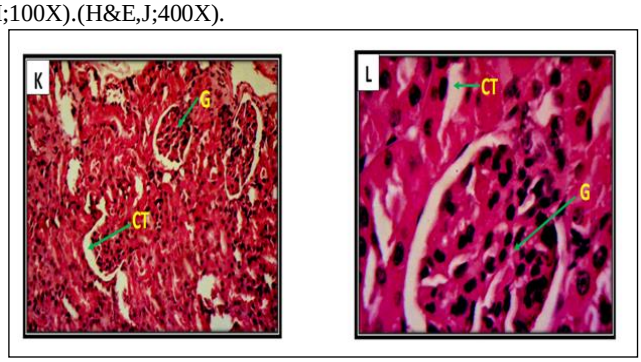

Figure (12): Photomicrograph of rat kidney tissue. Group VI (K&L) rats were given a dose of hydroxychloroquine 500 mg /kg and treated with Moringa. Showing glomeruli (G), renal convoluted tubules (CT), (H&E, K ; 100X). (H& E, L; 400X).

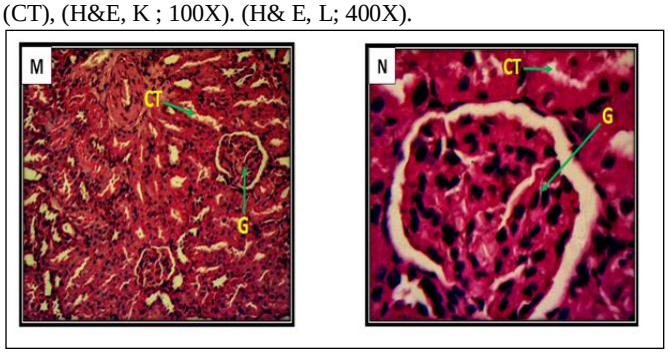

Figure (13): Photomicrograph of rat kidney tissue. Group VII (M&N) rats with dose of hydroxychloroquine 250 mg/kg). Showing glomeruli (G), renal convoluted tubules (CT), (H&E, M; 100X). (H& E, N; 400X).

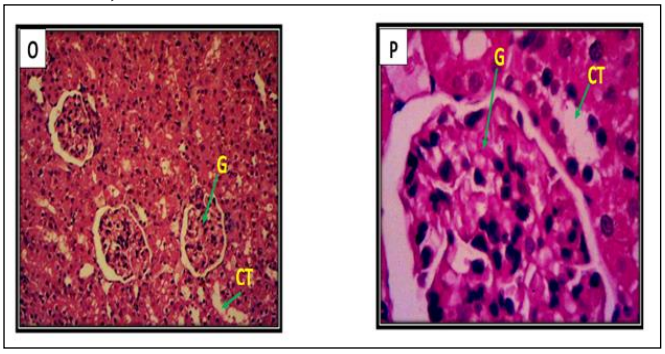

Figure (14): Photomicrograph of rat kidney tissue. Group VIII (O&P) rats with dose of hydroxychloroquine 250 mg/kg & treatment with Moringa. Showing glomeruli (G),renal convoluted tubules (CT), (H&E, O; 100X). (H& E, P; 400X).

Discussion:
A abrupt decrease in glomerular filtration rate, expressed by an increase in serum creatinine concentration or oliguria, is defined by kidney injury **[10].** Creatinine is more specific to the kidney and a better measure for identifying Kidney impairment **[11]**. In the current investigation, the hydroxychloroquine group received 500 mg/kg of hydroxychloroquine and was processed by Moringa, which resulted in a substantial increase in the level of urea in serum as compared to the control group and the other groups. When compared to animals

given HCQ alone, rats given HCQ with *Moringa oleifera* exhibited significant improvements in several Kidney function tests.

When comparing the rats with different doses of hydroxychloroquine to the control group, there was a substantial drop in creatinine concentration. In contrast, when the hydroxychloroquine group was compared to the control group, there was a substantial rise in creatinine concentration in the Moringa-processed groups.

The study's findings revealed that HCQ caused a substantial rise ($p<0.05$) in the number of RBCs, which was likewise linked to an increase in total haemoglobin. Unlike [12], who found a significant decrease ($P< 0.05$) in the haematological indices after exposure to HCQ in comparison to the control group, there was no significant increase in the number of WBCs in the blood of rats treated with HCQ.

Rats was treated HCQ With moringa for 3weeks, the RBC count and haemoglobin level were significantly increased ($P < 0.05$) with increase count platelet. It was compared to HCQ groups, while WBC amounts were non-significantly increased when compared to HCQ groups.

as well as glomeruli decrease, Bowman's space dilation, and renal tubule degeneration in the HCQ-treated group's kidney[8].When compared to the kidney tissue of the control group, *M.oleifera* treatment resulted in normal renal glomeruli, normal renal tubules, and a thin perivascular layer of fibrous tissue. The present findings demonstrated that moringa treatment reduced HCQ-induced inflammation in the kidneys, as evidenced by normal liver and kidney tissues and a thin coating of collagen fibres after treatment with *M. oleifera*.

The histopathological results of this study showed that rats given HCQ in different doses, developed severe kidney injury and fibrosis, as evidence of the marked inflammatory changes, and deformation in the structure of the liver and kidneys. However, pre-emptively treating rats with *M. oleifera* for (**3 weeks**), showed moderate improvement in inflammatory changes and fibrosis. These results indicated that treatment with *M. oleifera* attenuated the inflammation caused by treatment with HCQ, which may be due to its antioxidant effects. rats treated with *M. oleifera* for (**3 weeks**), showed normal histological changes, similar to those of normal rats, according to [13].

Conclusion:

Based on these results, one can conclude that this plant can prevent the haematological toxic effects of HCQ.

In HCQ-treated rats, histopathological results revealed congestion of the glomerular tufts with oedema of the Bowman's space and degenerative alterations within the renal tubular epithelium, as well as increased the thickness of the periglomerular and peritubular fibrous layer.

HCQ causes several histopathological changes in the kidney tissue of male albino rats, including congestion of the glomerular tufts with oedema of the Bowman's space and degenerative changes within the renal tubular epithelium, as well as increases the thickness of the periglomerular and peritubular fibrous layer.

Abbreviations and Acronyms:

EDTA = Ethylene Diamino Tetra Acetic.
HB = Haemoglobin**.**
HCQ = Hydroxychloroquine.
MO = *Moringa oleifera.*
RBC = Red Blood Cell.
WBC = White blood cells.

Acknowledgment

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