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Effect of hydroxychloroquine and *Artemisia herba-alba* administration on liver enzymes and kidney functions in laboratory male mice

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Abstract

Objective: study the effect of hydroxychloroquine (HCQ) and Artemisia extract on liver enzymes and kidney function tests.
Methods: seventy-two male mice are used in Lab as equal four groups:

- A control group diagnosed with distilled water,
- 1st group diagnosed with hydroxychloroquine (HCQ)
- 2nd group diagnosed with hydroxychloroquine and Artemisia extract
- 3rd group was diagnosed with Artemisia extract.

The time of investigation was around 6-18 days for each group of mice orally.
Results: AST, ALT, and ALP as liver enzymes increased in 1st and 2nd groups but these enzymes were low at 3rd group. Urea level increased in 1st group and decreased in 2nd group while no change was noticed in 3rd group. Also, Creatinine levels showed no significant change in all groups in comparison with the control group. After 6,12and 18 days of oral administration, the liver enzymes increased specially in 1st and 2nd groups. Also, urea concentration increased after 6, 12 and18days in 1st group. While, Creatinine levels showed no significant change in all periods.
Conclusion: Treatment of mice body at our lab with HCQ, Artemisia extract or both led to increment of liver enzymes, while urea level in mice body was high as we treated with HCQ and with low level in both HCQ and Artemisia extract.

Keywords*: hydroxychloroquine (HCQ), Artemisia extract, liver enzymes, kidney function tests*

1. Introduction

Hydroxychloroquine (HCQ) is an antimalarial drug, and it is one of the synthetic analogues of chloroquine and differs from it by a group of hydroxyl [1, 2]. HCQ was synthesized In 1946 by Alexander Surrey and Henry Hammer by adding a hydroxyl group to the main compound of chloroquine, [3]. It is metabolized in the liver by Cytochrome P 450 (CYP450) and its isoforms [4]. It also used in treatment of rheumatic and autoimmune diseases such as rheumatoid arthritis, lupus erythematosus and Sjögren's syndrome, also used in the skin diseases such as cutaneous porphyria, and it acts as an antitumor and treatment of cancerous diseases.[5-7]

Hydroxychloroquine is less toxic than its counterpart chloroquine, but there are some studies that have shown that hydroxychloroquine leads to elevate the liver enzymes and causes some cases of acute hepatotoxicity[8, 9]. Use of hydroxychloroquine does not affect the kidneys, as it is associated with a reduced risk of developing chronic kidney disease, [10] reduces proteinuria, and supports kidney function.[11-13]

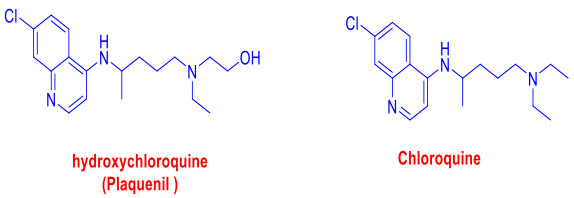

Figure 1: Hydroxychloroquine and Chloroquine [52]

Pons-Estel *et al* [14] demonstrated that hydroxychloroquine delays the onset of renal damage

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caused by lupus nephritis .Patients who received hydroxychloroquine showed less frequency of glomerulonephritis and had lower disease activity. After controlling for confounding factors, hydroxychloroquine protected against complete kidney damage.

On other hand, Artemisia is a herbaceous perennial, woody, plant it is one of the largest and most widespread genera of the family asteraceae it is a diverse genus consisting of more than 500 varied species and is exists in Europe, Asia and North America [15]. Artemisia used to treat inflammatory conditions, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and allergic disorders, furthermore, it has a malaria-killing effect. [16]. It is used to treat many diseases such as diabetes, liver disorders as antitoxin, some skin diseases and Cancer [17]. It contains many compounds, including glycosides such as santonin, Absinthin, and artemisinin. Also, it contains alkaloids, saponins, tannins, coumarins, and flavonoids. At study done by Irshaid *et al*. [18] showed that the oil extract of Artemisia has a significant role in protecting the heart, liver and kidneys in rats induced with diabetes, which is due to the strong antioxidants

Gilani and Janbaz, [19, 20] studied the effect of alcoholic and aqueous extract *A. absinthium* against hepatotoxicity caused by CCl4 and acetaminophen after the orally dose of 500 mg/kg body weight of the extract twice daily protects the liver from the effects of these compounds and reduces the level of AST and ALT .

Jayasimha *et al*.,[21] reported that *Artemisia absinthium* extract reduces high levels of urea and creatinine in diabetic rats, and this is due to its anti-diabetic effect.

According to above survey, the aim of our work is to study the effect of hydroxychloroquine (HCQ) and Artemisia extract on liver enzymes and kidney function tests.

2. Materials and Methods

2.1. Drug

Hydroxychloroquine sulfate (HCS) was used in this experiment, (HCS; quinoric tablets with an estimated dose of 200 mg/kg (manufactured by Bristol Laboratories, United Kingdom).

2.2. Plant collection and extract preparation

The aerial parts of the wormwood plant were obtained from a shop selling medicinal herbs in one of the local markets, the dry aerial parts were crushed in an electric grinder and 25g of dry plant powder was used with 250 ml of heated distilled water at a temperature of 40 °C and placed over the magnetic stirrer at a temperature of 40 °C for 24h. The mixture was filtered using several layers of medical gauze, and then the solution was separated using a centrifuge at 3000 rpm for 10 minutes. The filtrate was during using an incubator at a temperature of 40 °C. For 24-48 hours.

2.3. Animals

This study was done using 72 laboratory male mice of BALB/C dynasty weighing 20-25g, obtained from and raised in the animal house of the Department of Biology College of Sciences/ University of Misan, under controlled conditions in terms of temperature 20-25^{o}C and lighting cycle 12 hours light / 12 hours dark, for the duration of the study and the mice were placed in plastic cages.

2.4. The experimental design

The animal were divided into four equal groups 18 mice per each group and 6 mice for each sub group (after every 6 day a group of 6 mice were killed)

The drug and extract were determined according to previous researches and administered to mice (twice daily) for a maximum of 18days as below:

- The control group was given an oral dose of 0.2 ml of distilled water
- 1st group was oral dosed using HCQ, with a volume of 0.2 ml contains a concentration of 400 mg/kg for the first day, and a concentration of 200 mg/kg for the rest of the days.
- 2nd group was oral dosed 0.2 ml of HCQ with the same concentrations mentioned above and an evening dose of 0.2 ml (8000 mg/kg) of aqueous extract of *Artemisia herba alba*
- 3rd group was oral dosed with aqueous extract 0.2 ml (8000 mg/kg)

2.5. Blood samples collection

For assessment biochemical parameters, mice were euthanized by asphyxiation with chloroform, blood was collected from the heart of mice using a 5 ml syringe ,after collection, the blood was kept in a

gel tube and then centrifuged at 3000 rpm for 15 min ,serum was collected from the clear top layer after centrifugation and kept in Eppendorf tubes and stored in freezer until parameters were measured [22]. The samples were analysed by Cobas C111 device to obtain the data of liver enzymes AST, ALT and ALP in addition to urea and creatinine.

2.6. Data Analysis

Statistical analysis was performed by a one-way ANOVA (Analyses Variation) followed by LSD test. Data were expressed as Mean+SE. Statistical significance was set at P<0.05 (SPSS, 2001).

3. Results

The results of our study were depicted in Tables (1). These results showed a significant (P<0.05) increase in liver enzymes such as aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) serum concentration in groups under investigations (1^{st} -3^{rd}) in comparison with control group. Table 1 showed a significant increase(P<0.05) in AST level in the serum of first group, then in the second group then in the third group in comparison to control group (Table 1).

Table 1
The values of AST, ALT and ALP in serum of different groups (mean ± SE):

	AST (IU/L)	ALT(IU/L)	ALP (IU/L)
Control	153.26±4.20***	39.33±2.75**	51.14±2.11***
1^{st}	257.07±5.80*	72.96±10.23*	85.77±2.59*
2^{nd}	238.86±9.92*, **	50.46±3.18**	87.00±1.84*
3^{rd}	229.33±11.36**	47.16±2.12**	69.31±2.38**

A control group distilled water, 1^{st} group hydroxychloroquine (HCQ), 2^{nd} group hydroxychloroquine and Artemisia extract, 3^{rd} group Artemisia extract.
Different * refer to a significant difference among groups at level of (P<0.05).
Similar * refer to non-significant differences among groups.

The results in the Table (2) showed a significant (P<0.05) increased in urea concentration in 1^{st} group with elevation in its level in 2^{nd} group and increase in 3^{rd} group in comparison with control group. Also, the level of creatinine concentration in serum has no significant increment in first group but there was a significant increment(P<0.05) in the other two groups (Table 2).

According to period, the results showed a significant (P<0.05) increase in AST concentration in HCQ and HCQ +Artemisia extract groups compared to the control group after 6 days of treatment, while after 12 and 18 days of treatment, the results showed a significant(P<0.05) increase in AST enzyme level in all treatment groups compared to the control as shown in table (3).

Table 2
The values of urea, creatinine and C reactive protein in serum of different groups (mean ± SE):

	Urea(mg/dL)	Creatinine (mg/dL)
Control	38.02±1.40**	0.17±0.02*, **
1^{st}	52.16±4.33*	0.12±0.01**
2^{nd}	29.50±1.67***	0.27±0.06*
3^{rd}	44.83±2.708*, **	0.21±0.01*, **

A control group distilled water, 1^{st} group hydroxychloroquine (HCQ), 2^{nd} group hydroxychloroquine and Artemisia extract, 3^{rd} group Artemisia extract.
Different * refer to a significant difference among groups at level of (P<0.05).
Similar * refer to non-significant differences among groups.

The results in Table (3) showed significant (P<0.05) increased in ALT values in all treated groups after 18 days of dosing compared to the control group. While the results in the same Table showed that ALT concentration significantly increased (P<0.05) after 6 and 12 days of dosing group in group treated with HCQ only. Moreover, the results showed a significant (P<0.05) increase in ALP concentration in groups all treated groups after 6 and 18 days of dosing compared to the control group, the results also showed a significant(P<0.05) increase in ALP concentration in HCQ and HCQ +Artemisia extract

groups after 12 days of dosing compared to the control group as shown Table (3).

Table 3
level of liver enzymes in serum of different periods (M±SE)

	groups	After 6 days	After 12 days	After 18 days
AST (IU/L)	Control	145.48±8.90**	161.40±6.48**	152.90±5.82***
	1st	250.46±7.00*	249.53±12.97*	271.21±8.16*
	2nd	228.56±16.98*	262.00±12.47*	226.01±19.99**
	3rd	182.10±23.87**	250.08±4.28*	255.83±7.47*, **
ALT (IU/L)	Control	38.90±3.89**	44.60±3.95**	34.51±6.01**
	1st	102.06±26.79*	65.08±8.18*	51.73±3.29*
	2nd	45.21±5.02**	59.65±6.88*, **	46.53±2.32*
	3rd	38.60±3.17**	51.60±3.34*, **	51.30±1.71*
ALP (IU/L)	Control	50.05±3.62***	52.20±4.48**	51.18±3.4***
	1st	84.72±4.51*	83.90±6.35*	88.69±2.32*
	2nd	89.11±2.17*	86.06±4.55*	85.81±2.79*
	3rd	71.95±4.18**	63.28±3.74**	72.71±3.94**

A control group distilled water, 1st group hydroxychloroquine (HCQ), 2nd group hydroxychloroquine and Artemisia extract, 3rd group Artemisia extract.
Different * refer to a significant difference among groups at level of (P<0.05).
Similar * refer to non-significant differences among groups.

As for the urea and creatinine values Table (4) the results showed significant (P<0.05) decrease in the urea concentration in HCQ +Artemisia extract group after 6 days of dosing compared to the control and other groups, while after 12 days of dosing the results showed a significant (P<0.05) increase in the urea concentration in HCQ group compared to the control and other groups. While, the results showed a significant (P<0.05) increase in urea concentration in groups HCQ and Artemisia extract groups after 18 days compared to the control group.

The results showed that there was no significant (P>0.05) difference in creatinine concentration in all treated groups after 6, 12 and 18 days of dosing compared to the control group as shown in table (4).

Table 4
Urea, Creatinine level in serum of different periods (M±SE).

	groups	After 6 days	After 12 days	After 18 days
Urea (mg/dL)	Control	34.57±2.10*	43.15±1.34**	36.34±2.38**
	1st	34.90±3.66*	59.32±4.90*	62.25±7.99*
	2nd	22.02±1.18**	29.56±1.98***	36.91±1.10**
	3rd	34.44±1.60*	44.47±2.36**	55.58±4.68**
Creatinine (mg/dL)	Control	0.11±0.01*	0.25±0.07*	0.11±0.01*
	1st	0.18±0.04*	0.13±0.02*	0.11±0.01*
	2nd	0.16±0.02*	0.33±0.15*	0.30±0.12*
	3rd	34.57±2.10*	0.23±0.02*	0.23±0.04*

A control group distilled water, 1st group hydroxychloroquine (HCQ), 2nd group hydroxychloroquine and Artemisia extract, 3rd group Artemisia extract.
Different * refer to a significant difference among groups at level of (P<0.05).
Similar * refer to non-significant differences among groups.

4. Discussion

The results of the present study showed a significant increase in the level of liver enzymes ALT, AST, and ALP in mice that treated with hydroxychloroquine, it was similar to a study done by Elshishtawy *et al* [23]who showed that liver enzymes ALT, AST, and ALP were increased after daily administration of HCQ at a dose of 124 mg/kg to albino rats for 6 weeks. The results were also in agreement with another study by Abdel Galil [8], who observed an increase in liver enzymes AST and ALT in patient with systemic lupus erythematosus whom took 400 mg of HCQ per day for one year, after stopping the drug the enzymes returned to normal level.

Liver enzymes ALT, AST, and ALP are important, and the rise in these enzymes is an indication of liver damage, as these enzymes rise to weaken the normal level in the case of viral diseases and other liver diseases, in addition to taking some different medications. The main indicator for assessing liver function is the measurement of these enzymes. [24, 25]

Lin *et al* [26] indicated that the largest percentage of ALT enzyme is found in the liver, while AST is most found in the heart, the liver, and the kidneys.[27] Therefore, AST may increase when kidney and pancreatic tissue are damaged and liver cells necrosis, which supports an increase in AST, ALT, and ALP. The reason of rise in enzymes maybe due to the accumulation of drug doses in the liver, as the liver is primarily responsible for metabolizing these drugs, as the drug accumulates in Kupffer cells in the liver .Thus, overloading the liver lysosomes with indigestible substances and increasing their size and number this in turn leads to loss of function of plasma membrane. [28]

Also, the rise may be attributed to free radicals, as elevated liver enzymes are an indicator of cellular damage, loss of plasma membrane function, and liberation of enzymes into the interfluid and then into the blood [29]and oxidative stress causes an elevation in liver enzyme values. [30]

The results of the current study showed no significant change in the value of ALT and an increased in the values of AST and ALP compared to the control group in mice that were dosed with artemisia extract Table (1).

Our results agreed with Adam *et al* [31] they noted when rats fed a diet containing 10% leaf extract of *artemisia abyssinica* where showed a significant rising in levels of AST compared to control.

In another study, in agreement with ours there was no significant changes were observed in the levels of ALT enzyme and increased in value AST after dosing 1 g/kg of aqueous extract of Artemisia afra to rodents for three months.[32] The results contradicted with our results by Iriadam *et al*[22] who showed that giving the aerial parts extract of *Artemisia herba alba* at a dose of 85 mg/kg to diabetic rabbits reduced ALT and AST levels

Also, opposite to the results of the current study was reported by [33]that these results, as it indicated a significant decreased in the AST and ALT when Artemisia monosperma extract (5%) was given to rats that were suffering from high levels of AST, ALT enzymes due to being dosed with lead acetate,

In the groups that were dosed with HCQ + Artemisia extract, the results showed that the levels of AST and ALT decreased compared to the group of HCQ alone. In a study similar to ours by Sekiou *et al* [34]showed that the administration of *Artemisia herba alba* extract to alloxan -induced diabetic rats which received 400 mg/kg of the extract for 30 days led to reduction of liver enzymes concentration AST and ALT.

The decline in the values of AST and ALT is an indication of the restoration of hepatocytes, treatment of rats with plant extract enhanced the fight against free radicals which is widely used to inactivate reactive oxygen species (Ros) [35], where free radicals caused damage to cell membranes, including liver cells, which leads to the activity of liver enzymes inside the cytosol and leads to their entry into the blood circulation. Therefore, the increased in these enzymes indicates damage hepatocytes.[36, 37]

A study by Cordova *et al* [38] showed that polyphenols, especially flavonoids, have an inhibitory effect on the cytochrome p Cyp450 system, preventing the metabolism of drug compounds, thus reducing free radicals.[39]

As shown by Rezaei *et al* [53] that the injection of 100,200 and 300 mg/kg Artemisia extract +50 mg/kg thioamide to male rats led to a decrease in liver enzymes AST, ALT and ALP compared to the thioacetamide group, which increased liver enzymes significantly, and this study is identical to our study.

Artemisia plant contains many compound as (flavonoids, alkaloids, phenols, glycosides, terpenes) that have protective effects for the liver, as it led to a reduction in liver enzymes levels such as AST, ALT

that rise as due to diabetes [40] According to periods the results of the current study showed an increased in the values of AST, ALT and ALP in all treatment periods, after 6, 12 and 18 days Table (3), This agreement with study done by Galvan *et al* [41],which indicate increased in AST and ALT in woman with mixed connective tissue disease after treatment with 200 mg of HCQ with prednisone 40 mg led to increase in ALT and AST, normal liver function returned after discontinuation of HCQ.

The results of this study also agree with Chen *et al* [42] who noted that after conducting a marker on COVID-19 patients who received HCQ at 200 mg twice daily 10 days, a rise in ALP and AST enzymes occurred, and after 3 days of stopping taking HCQ there was decreased in these enzymes. The values of ALP and ALT in this study contradicted with Rajeshkumar *et al* [43], who did not observe a significant change in ALP and ALT values after rats were dosed with 60 mg HCQ once daily for 4 weeks.

These changes may be related to the dose, as the values of these enzymes were high when using higher doses, but they did not change when using a lower dose[44]. As for, the group treated with HCQ + Artemisia, ALP and AST values were significantly elevated in all treatment periods 6,12 and 18 days, while the ALT value was high in the last treatment period (18 days).

These results agree with Li *et al* [45] who observed that administration of a combination treatment of artemisinin-hydroxychloroquine sulfate to male Sprager Dawley rats at different doses 146, 219, 328 and 492 mg/kg resulted in an increased in AST and ALT at the highest dose of treatment (492 mg/kg).

The results of the current study showed an increased in the value of urea in mice treated with hydroxychloroquine compared to the control group table (2). The results of this study were similar to the results of ElShishtawy *et al* [23] which showed that hydroxychloroquine caused a relative increase in the urea value in experimental animals compared to the control group. Also, it was explained that the side effects of using HCQ were represented in the disturbance of liver and kidney functions, and one of the important indicators in evaluating kidney functions is the measurement of the concentration of urea and creatine in the blood serum, as the rise in urea is caused by a decrease in the glomerular filtration rate as a result of the disturbance of the renal tubules [46, 47]. Besides, current study showed that there is no difference in the level of creatinine, if creatinine is a useless product that is excreted from the blood into the urine by the kidneys. It is easily filtered through the glomeruli and is not recycled or metabolized, but it is only slightly excreted through the tubules. [48]

As for the group treated with Artemisia extract, the results showed that there was no significant difference in the values of urea and creatine in the mice treated with Artemisia extract compared to the control group Table (2). These results were similar to the results of the study done by Noori *et al* [49] which showed no significant differences in the levels of urea and creatine in rats treated intraperitoneally with Artemisia deserti extract 100 and 200 mg/kg respectively for 6 day. Also, the results of Mongi *et al* [50] which in agreement with our study, where the levels of urea and creatinine did not change in rats treated intraperitoneally with 200 mg/kg of Artemisia campestris essential oil (ACEO) by for two weeks, there were no significant differences between treated group and the control group.

According to periods the results of the current study, in the group treated with HCQ showed no change in urea concentration after 6 days of dosing, while the results showed a significant increase in urea values after 12 and 18 days compared to the control group (4). These results are conflicted with Singh *et al* [51] who explained that giving HCQ for a group of liver patients with a certain treatment for 6 months does not result in a significant change in its urea values. Also, Rajeshkumar *et al* [43]did not observe a change in urea after administration of 60 mg/kg HCQ to mice by intraperitoneal injection. While the results of the group treated with HCQ + Artemisia showed a significant decrease in the urea concentration after 6 and 12 days of dosing, and there was no significant change in the urea concentration after 18 days. This result agreed with Sekiou *et al* [34]who indicated that dosing 400 mg/kg of *Artemisia herba alba* extract for 30 days led to a decrease in the urea level that was raised by alloxan, while the urea level did not change after administration of the Artemisia extract to healthy mice in the same study. This result was also consistent with Li *et al* [45]. They indicated that administration male Sprager Dawley rats with a combination treatment of artemisinin-hydroxychloroquine for 14 days did not lead to a change in urea values. Creatinine did not show any significant change in all treated groups and this

agreed with the above studies [45] which indicated that the creatinine level does not change using both hydroxychloroquine or Artemisia extract.

5. Conclusion

Treatment of mice body at our lab with HCQ, Artemisia extract or both led to increment of liver enzymes, while urea level in mice body was high as we treated with HCQ and with low level in both HCQ and Artemisia extract.

6. Conflicts of interest

"There are no conflicts to declare".

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