

## HEMATOLOGICAL PROFILE OF RATS TREATED WITH QUERCETIN DERIVATIVE AGAINST CARBON TETRACHLORIDE (CCL<sub>4</sub>) TOXICITY

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### ABSTRACT

The effect of quercetin derivative (1-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H –chromen-4-ylidene) thiourea coded (QTU) on the hematological profile of Wistar rats induced toxicity with carbon tetrachloride (CCl<sub>4</sub>) was investigated.

Administration intra peritoneal injection of 12.5 mg /kg and 25mg/kg of quercetin derivative (QTU) ,after four weeks of being exposure to CCl<sub>4</sub>, led to a significant decrease (p<0.05) in WBC count, with a corresponding increase on PCV and Hb. It was also observed that the functional recovery of these blood indices is concentration dependent.

Results of this study revealed a depletion of RBC and a decrease in Packed Cell Volume (PCV) and Hemoglobin (Hb),In addition, an elevation in the levels of White Blood Cell (WBC) caused by CCl<sub>4</sub> compared to control samples.

### INTRODUCTION

Quercetin (3, 5, 7, 3, 4-pentahydroxy flavon), is a flavonoid consider as a one of the most prominent dietary antioxidants (1). Quercetin occurs in glycosylated form in French beans, broccoli, apples and especially in onions (2). Quercetin is the most abundant antioxidant in the nature and has an antioxidant potential four times that of vitamin E (3).

Quercetin has been reported to increase antioxidative defense system by up regulating antioxidant enzymes (4). It has many beneficial effects in human health,

including cardiovascular protection, anticancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity and anti-inflammatory effects (5). Quercetin prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions (6).

## **MATERIALS AND METHODS**

### **1- Chemical Materials:-**

Quercetin derivative (1-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H –chromen-4-ylidene) thiourea coded (QTU) was prepared in the department of pharmaceutical Chemistry College of Pharmacy university of Basra as a new antioxidant compound

### **2-Experimental Animals**

Forty adults male rats of about 5 months age, weighting about  $225 \pm 25$ g were used in this experiment they were obtained from the animal house unit of the Department of physiology, Pharmacology and Biochemistry, University of Basra. The animals were maintained in cages (at room temperature) fed with standard laboratory chow (pellets) and water given ad libitum

### **Experimental design**

The animals were divided randomly into 5 equal groups (6 rats in each) and treated for 4 weeks as following:

1. Control group: In which rats were injected intraperitoneal with olive oil (0.5 ml/day).
2. QTU group: In which rats were injected intraperitoneal QTU only (25mg/kg of QTU (dose corresponding to 1/10 of QTU LD<sub>50</sub>) dissolved in DMSO (0.5ml /day)
3. CCl<sub>4</sub> group: In which rats were injected intraperitoneal with CCl<sub>4</sub> 1ml/kg dissolved in olive oil (0.5 ml/day).
4. QTU (12.5mg/kg): In which rats were injected intraperitoneal with 12.5mg/kg of QTU (dose corresponding to 1/20 of QTU LD<sub>50</sub>, dissolved in DMSO(0.5 ml/day)) and CCl<sub>4</sub> 1ml/kg dissolved in olive oil (0.5 ml/day).

5. QTU (25 mg/kg) : In which rats were injected intraperitoneal with 25mg/kg of QTU(dose corresponding to 1/10 of QTU LD<sub>50</sub> , dissolved in DMSO (0.5 ml/day)and CCl<sub>4</sub> 1ml/kg dissolved in olive oil (0.5 ml/day).

### **Blood sample collection:-**

At the end of experimental period (28 days) Rats were sacrificed by light chloroform anesthesia, a 'Y' shaped cut in the rat abdomen was done 'The samples of blood were collected from the heart by heart puncture by the use of the disposable syringes of 5-10 cc capacity, blood was collected and analyzed according to ) 7 '(ml of blood transferred into EDTA as an anticoagulant tube for hematological investigations :RBCs 'Hb 'PCV, WBCs and differential WBCs analysis.Hematological parameters

Red blood corpuscles count (RBC), white blood cell (WBC), differential leukocyte count and hemoglobin (Hb), Packed cell volume (PCV) or hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) . All this parameters were measured by the methods below:

#### 1.Red Blood Corpuscles Count (RBC) (corpuscle /mm<sup>3</sup>)

The RBC count was obtained according to (8).

#### 2.Estimation of Hemoglobin Concentration (Hb) (g/dl)

The Hb concentration was measured manually (Sahli method), and read the result in gm/dl. (8).

#### 3.Packed Cell Volume (PCV) (%)

The procdure was done manually by using Microhaematocrit centrifuge (8).

#### 4.The absolute values

These were calculated by the following equations:

Mean Corpuscular Volume (MCV)

MCV indicates the average volume of a single RBC in femtoliter (ft)

$$\text{MCV} = \frac{\text{PCV (in L/L)}}{\text{RBC count / L}}$$

i. Mean Corpuscular Hemoglobin (MCH)

MCH indicates the average weight of Hb in a single RBC in picogram (pg)

$$\text{MCH} = \frac{\text{Hb (in gm/L)}}{\text{RBC count / L}}$$

ii. Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC indicates the average concentration of Hb in the RBCs and it is expressed as %.

$$\text{MCHC} = \frac{\text{Hb (gm/dl)}}{\text{PCV \%}} \times 100$$

5. Total White Blood Cells Count (WBC) (Cell/mm<sup>3</sup>) and Differential WBC Count were done according (8).

## RESULTS

The current study revealed that QTU has no any sort of any effectiveness on RBCs count, hemoglobin concentration and hematocrit ratio, compare with control group (1).

Treated rats with CCl<sub>4</sub> led to significantly decreased (p<0.05) in RBC compared with the control group

The administration of (12.5mg/kg) of QTU led to a significant increase (p<0.05) in RBCs count compare with CCl<sub>4</sub> treated rats which reach to its almost normal value compared with the control value.

Hemoglobin concentration (Hb) and hematocrit ratio (Hct) were also increased significantly ( $p < 0.05$ ) compared with  $\text{CCl}_4$  treated rats but they were still less significantly ( $p < 0.05$ ) compared to control group .

Administration of (25mg/kg) of QTU also led to significantly increased ( $p < 0.05$ ) in blood picture parameters compared to the  $\text{CCl}_4$  treated rats .It seems from the results that the RBCs and hemoglobin return to its almost normal values compare to control value ,whereas Hct value still less significantly ( $p < 0.05$ ) compare with control group .

The QTU dosages results showed that dose (25mg/kg) acted better significantly ( $p < 0.05$ ) than the (12.5mg/kg) on the  $\text{CCl}_4$  treated group.

**Table (1): Effects of QTU on blood parameters ( RBCs count , haemoglobin concentration (Hb), haematocrit ratio (Hct) on  $\text{CCl}_4$  treated male rats .**

Group	RBCs $\times 10^6/\text{mL}$	Hb (g/dL)	Hct (%)
Control	9.07 a 0.71	15.60 a $\pm 0.77$	60.57 a $\pm 1.63$
QTU (25mg/kg)	8.87 a 0,32 $\pm$	15.88a $\pm 0.31$	61.36 a 0,83 $\pm$
$\text{CCl}_4$ (1ml/kg)	7.39 b $\pm 0.39$	12.13c $\pm 1.13$	42.17d $\pm 1.06$
(12.5mg/kg) QTU & $\text{CCl}_4$	8.50a $\pm 0,46$	14,36b $\pm 0.62$	53,75c $\pm 1.10$
(25mg/kg) QTU & $\text{CCl}_4$	8.87a $\pm 0.96$	15.29a $\pm 0.54$	58.52b $\pm 2.89$
LSD	0.727	0.864	1.99

Results are expressed as mean  $\pm$  standard deviation , Different letters indicate significant differences among groups at ( $P \leq 0.05$ ).

It seems that MCHC, MCH and MCV were not affected by QTU injection compared with the control group .

Administration of CCl<sub>4</sub> led to a significant increase in MCHC compare to control, whereas no effect was recording on MCH compered to control group . The MCV value significantly decreased( $P \leq 0.05$ ) due to CCl<sub>4</sub> compere with the control group

Treated rats with a dose of QTU 25mg/kg led to a significant decrease in MCHC compared to CCl<sub>4</sub> treated rats , whereasMCHC value was not affected significantly by dose12.5mg/kg,comperd to the CCl<sub>4</sub> treated rats. MCH value was not affected significantly by dose 12.5mg/kg of QTU, but the MCH was affected significantly by dose 25mg/kg QTU compered to CCl<sub>4</sub> treated rats.

Injection of dose 12.5mg/kg of QTU led to significantly increased in MCV compere to CCl<sub>4</sub>treated rats .

Injection of dose 25mg/kg QTU led significantly decreased in MCHC compere to CCl<sub>4</sub> treated rats ,but both MCH and MCV were not affected by dose 25 mg/kg QTU .

Table (2) Effects of QTU on blood picture parameters ( mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) on CCl<sub>4</sub> treated male rats .

Group	MCHC	MCH	MCV
Control	25.76 b 0.73±	17.29 ab 0.83±	68.78 a 3.43±
QTU (25mg/kg)	25.88 b 0.67±	17.91 a 0.69±	69.22 a 1.18±
CCl <sub>4</sub> (1ml/kg)	28.69 a 0.96±	16.23 b 0.60±	57.29 c 1.87±
(12.5mg/kg) QTU & CCl <sub>4</sub>	26.97 ab 0.60±	16.88 b 0.23±	63.24 b 1.71±
(25mg/kg) QTU & CCl <sub>4</sub>	26.05 b 0.53±	17.24 ab 0.57±	66.15 a 0.87±
LSD	2.399	0.737	0.849

Results are expressed as mean  $\pm$  stranded deviation , Different letters indicate significant differences among groups at ( $P \leq 0.05$ ).

QTU has not significant different ( $P < 0.05$ ) on total leukocytes count, lymphocytes and neutrophil percentage compared to control group. Treated rats with CCl<sub>4</sub> led to a significant increase in total leukocyte ,neutrophils and eosinophils, whereas lymphocyte percentage significantly decreased ( $P < 0.05$ ) in CCl<sub>4</sub> group compared with control and QTU alone group. On the other hand, monocyte and basophil percentages were not affected.

The effects induced by CCl<sub>4</sub> on total and differential leukocytes count are reversed to almost its normal values when the rats treated by QTU (12.5mg/kg) and 25mg/kg .It seems that higher dose of QTU (25mg/kg) was acted better on CCl<sub>4</sub> toxic of total leukocytes count, neutrophil , and lymphocyte percentages.

**Table (3) Effects QTU on total and differential leukocyte count and precentage.**

Group	leuko	lympho	mono	Nut	Espino	Baso
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Control	6.21b 0.63±	69.05 a 3.59±	8.90 0.69±	20.78 c 2.01±	1.42 b 0.37±	0.67 0.26±
QTU (25mg/kg)	6.41b 0.59±	68.71a 2.53±	9.22 0.68±	21.82 c 1.97±	1.35 b 0.36±	0.42 0.34±
CCl <sub>4</sub> (1ml/kg)	8.63 a 0.61±	56.40 d 2.38±	8.65 0.65±	29.82 a 2.10±	1.66 a 0.42±	0.62 0.26±
(12.5mg/kg) QTU & CCl <sub>4</sub>	7.45 b 0.79±	61.08 c 4.05±	9.03 0.55±	26.13 b 1.29±	1.60 a 0.24±	0.62 0.26±
(25mg/kg) QTU & CCl <sub>4</sub>	6.87 b 0.65±	64.98 b 3.49±	8.52 0.94±	24.35 b 1.02±	1.50 a 0.36±	0.50 0.33±
LSD	0.783	3.89	NS	2.06	0.2493	NS

Results are expressed as mean ± stranded deviation , Different letters indicate significant differences among groups at ( $P \leq 0.05$ ).

## DISCUSSION

The results clearly demonstrated that CCl<sub>4</sub> administration produced pancytopenia (a general reduction in the blood cellular elements) as shown by microcytic hypochromic anemia, thrombocytopenia and lymphopenia in the blood as evidenced by a significant decrease ( $P < 0.05$ ) in RBC count, haematocrit ratio PCV, haemoglobin concentration (Hb) values and platelets count. There was a significant decrease ( $P < 0.05$ ) in mean cell volume (MCV), mean cell hemoglobin (MCH), However a significant increase ( $P < 0.05$ ) in mean cell hemoglobin concentration (MCHC) with the exception of total WBC counts, although, there was lymphopenia.

These findings are in agreement with the previous studies (9-13). The source of ROS in erythrocytes is the oxygen carrier protein hemoglobin, oxyhemoglobin (Oxy-Hb) that undergoes autoxidation to produce  $O_2^{\bullet-}$  (14). Oxy-Hb undergoes a slow autoxidation, producing  $O_2^{\bullet-}$ , which yields hydrogen peroxide ( $H_2O_2$ ). Therefore, Hb is constantly exposed to an intracellular flux of  $H_2O_2$  as well as to an extracellular flux, due to the high permeability of this metabolite. Exposure of Oxy-Hb to  $H_2O_2$  leads to oxidative modifications that have been proposed as selective signals for proteolysis in erythrocytes (15). Occasional reduction of  $O_2$  to  $O_2^{\bullet-}$  is accompanied by oxidation of Oxy-Hb to methemoglobin (Met-Hb), a rust brown-colored protein that does not bind or transport  $O_2$  (14). CCl<sub>4</sub> intoxication caused a significant increase the autoxidation of oxyhemoglobin to methemoglobin, which indicated that CCl<sub>4</sub> induced oxidative stress on erythrocytes (16). Increased methaemoglobin concentration in the



blood predisposes to hypoxia consequent to inactivation of a fraction of hemoglobin as well as to the increased hemoglobin oxygen affinity and possible changes in the oxygen permeability of erythrocyte membranes during acute methemoglobinemia (17). Alternatively, (18) have been found that the erythrocytes membrane alterations and the loss of functional integrity precede the onset of CCl<sub>4</sub> -induced liver cirrhosis.

The exposure of erythrocyte to chemical and some drugs has been associated with erythrocyte distraction and hemolytic anemia (19). The depletion in erythrocytes count and Hb level leads to iron deficiency anemia which is characterized by a microcytic-hypochromic anemia, also hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation this could be another reason for decreasing hematological values (20).

The depression in erythrocytes count, haematocrit ratio, hemoglobin level and microcytic-hypochromic recorded in this study could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the erythropoiesis rate and their enhanced removal from circulation as a consequence of the toxic effect of CCl<sub>4</sub> toxicity on bone marrow, spleen and liver. Counteracting the effects of CCl<sub>4</sub> toxicity on the above parameters by (1-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H – chromen-4ylidene) thiourea (QTU) could be attributed to inhibition of hemolysis of RBCs and/or toxic effects of Carbon tetrachloride on the hemopoietic organs

This action may be due to bioactivity of this compound according to the specific molecular structure, in which five chemically active group, phenolic hydroxyl substituent (which donates a hydrogen atom to radicals, stabilizes them and produce relatively flavonoid radical). Unsaturated double bond give it a strong antioxidant ability from accepting oxygen and to inhibition of ROS action on RBC membrane by inhibition of lipid peroxidation in the erythrocytes membranes and increasing its resistance to spontaneous haemolysis or to the amelioration of the toxic effect of CCl<sub>4</sub> on the bone marrow.

Protective action of QTU on the erythrocytes agree with previous study (21) who synthesized four C-8-aminomethyl derivatives of quercetin and study the proactive action of this new derivatives of quercetin on the oxidative hemolysis on mice

erythrocytes and showed that the derivatives C-8position have the ability to protect cells from acute oxidative stress.

Also the present study results agree with ( 22) who investigated that the protective effect of new quercetin derivatives named 2-Chloro-1,4- naphthoquinone and its potential metabolite 2-chloro -3 hydroxy-[1,4]-naphthoquinone on osmotic fragility of erythrocytes, and resulted this derivative have the ability to protection effect on osmotic hemolysis

Data in the present study showed a significant increased ( $P<0.05$ ) in leukocytes count and neutrophils percentage and a significant decrease ( $P<0.05$ ) in lymphocytes percentage in rats administered  $CCl_4$  compared with control. these findings are agrees with previous studies, (10,23,24). Intoxication with  $CCl_4$  induced a highly significant increase of WBC<sub>s</sub> count ,This increase may be attributed to the defensive mechanism of immune system (25 ), It has been found that leukocytosis ( increase of white blood cell counts ) which might not have been a result of significant increase of WBC production but the release of marginated neutrophils and other neutrophil pool into the circulation which produced the neutrophilia in rats treated with  $CCl_4$  (11) . Neutrophils are the most abundant circulating granulocytes and their granules contain numerous microbicide molecules chemotactic factor is produced as a result of infection or injury in an extracellular site, these cells enter the tissues (26) .

The abnormal hematologic parameter changes in total and differential leukocytes count caused by  $CCl_4$  may be attributed to the inflammatory response . This induce release of a large number of cells from bone marrow, including neutrophils, which subsequently release  $H_2O_2$  that might induce damage to surrounding tissues and cells (27). Improvement of these changes by concomitant administration of (1-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H –chromen-4-ylidene) thiourea (QTU) with  $CCl_4$  may be related to its anti-inflammatory effect resulted from free radical scavenging activity according to its properties as a flavonoid compound , a protective agent form  $CCl_4$  intoxicated group may be due to the role of one of its active components, are known to be vasculo-protector and powerful antioxidant (28) ,as well as the flavonoids probability did so by reducing the accumulation of toxic  $CCl_4$  derived metabolites( 29). Blood parameters were found to be positively affected by using as (1-(2-(3,4-

dihydroxyphenyl)-3,5,7-trihydroxy-4 H –chromen-4-ylidene) thiourea (QTU) as therapeutic agent.

This finding is in agree with previous study (30) by using quercetin tetraacetylderivative and concussed that the beneficial treatment of quercetin derivatives in inflammatory disease

## CONCLUSION

Hematological parameters ( RBC, PCV, Hb, WBC and diferential WBC) are used to provide useful information for diagnosis in routine clinical evaluation of the state of health. The current study revealed the reversal effect of (1-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4 H –chromen-4-ylidene) thiourea (QTU) on some hematological parameters of rats had changed by carbon tetrachloride, which was observed to have decreased the levels of PCV and Hemoglobin and raised of WBC count , Taken together the QTU is an effective compound against the toxic effects of CCl<sub>4</sub> on hemopoeticsystem and also demonstrated that the higher dose of the QTU significantly lowered the elevated parameters and increased the reduced indices back to levels close to their control level.

الصورة الدموية للجرذان المعاملة بمشتق الكورستين ضد التسمم برابع كلوريد الكربون

### الخلاصة

تأثير مشتق كورستين (١ - (٢ - (٣،٤ - ديهيدروكسيفينيل) -٣،٥،٧- تريهيدروكسي-٤ -H-كرومين-٤-يليدين) ثيوريا ويرمز له (QUA) على الصورة الدموية للجرذان المختبرية المعاملة برابع كلوريد الكربون (CCl<sub>4</sub>).

أظهرت نتائج هذه الدراسة انخفاض في عدد خلايا الدم الحمراء وانخفاض في حجم الدم المضغوط (pcv) وتركيز الهيموجلوبين (Hb)، بالإضافة إلى ارتفاع في مستويات خلايا الدم البيضاء (WBC) الناتجة عن CCl<sub>4</sub> مقارنة مع بيانات مجموعة السيطرة.

حقن البريتوني داخل ١٢.٥ ملغم / كغم و ٢٥ ملغم / كغم من مشتقات الكورستين (QTU)، بعد أربعة أسابيع من التعرض ل CCl<sub>4</sub>، أدى إلى انخفاض معنوي في عدد خلايا الدم البيض ، مع زيادة مقابلة على حجم الدم المضغوط و الهيموغلوبين. ولوحظ أيضا أن الانتعاش الوظيفي لمؤشرات الدم هذه يعتمد على تركيز المشتق.

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