HEMATOLOGICAL PROFILE OF RATS TREATED WITH QUERCETIN DERIVATIVE AGAINST CARBON TETRACHLORIDE (CCL 4) TOXICITY

Preprint · January 2018

DOI: 10.13140/RG.2.2.25397.32483

CITATIONS
0

READS
39

4 authors, including:

Mohammed Al-Diwan
University of Basrah
30 PUBLICATIONS **9** CITATIONS

SEE PROFILE

Shaker A.N. Al-Jadaan
University of Basrah
64 PUBLICATIONS **64** CITATIONS

SEE PROFILE

HEMATOLOGICAL PROFILE OF RATS TREATED WITH QUERCETIN DERIVATIVE AGAINST CARBON TETRACHLORIDE (CCL_4) TOXICITY

Asmaa, S. Madthi[*] , Mohammed A. Al-Diwan[*] ,Shaker A. N. AL-Jadaan[**]

[*] College of Veterinary Medicine, University of Basrah , Basrah , Iraq
[**]College of Pharmacy , University of Basrah, Basrah , Iraq
(Received ,Accepted)

Key words: Hematology, quercetin derivative, Carbon tetrachloride toxicity

Corresponding Author:shakeraljadaan@yahoo.com

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 Wistarrats induced toxicity with carbon tetrachloride (CCl4) 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 CCl4, 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 CCl4 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 ± 25g 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_{50}) dissolved in DMSO (0.5ml /day)
3. CCl_4 group: In which rats were injected intraperitoneal with CCl_4 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_{50} , dissolved in DMSO(0.5 ml/day)) and CCl_4 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_{50} , dissolved in DMSO (0.5 ml/day)and CCl_4 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^3)

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)

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

i.Mean Corpuscular Hemoglobin (MCH)

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

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

ii.Mean Corpuscular Hemoglobin Concentration (MCHC)

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

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

5.Total White Blood Cells Count (WBC) (Cell/mm3) 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, compere with control grouptable (1).

Treated rats with CCl_4 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_4 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 CCl4 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 CCl_4 treated rats .It seemsfrom the results that the RBCs and hemoglobin return to its almost normal values compere to control value ,whereas Hctvalue still less significantly ($p<0.05$) compere 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 CCl_4 treated group.

Table (1): Effects of QTU on blood parameters (RBCs count , haemoglobin concentration (Hb), haematocrit ratio (Hct) on CCl_4 treated male rats .

Group	RBCs ´10^6/mL	Hb (g/dL)	Hct (%)
Control	9.07 a 0.71	15.60 a ±0.77	60.57 a ±1.63
QTU (25mg/kg)	8.87 a 0,32±	15.88a ±0.31	61.36 a 0,83±
CCl_4 (1ml/kg)	7.39 b ±0.39	`12.13c ±1.13	42.17d ±1.06
(12.5mg/kg) QTU & CCl_4	8.50a ±0,46	14,36b ±0.62	53,75c ±1.10
(25mg/kg) QTU & CCl_4	8.87a ±0.96	15.29a ±0.54	58.52b ±2.89
LSD	0.727	0.864	1.99

Results are expressed as mean ± stranded deviation , Different letters indicate significant differences among groups at ($P\leq0.05$).

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

Administration of CCl_4 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_4 compere with the control group

Treated rats with a dose of QTU 25mg/kg led to a significant decrease in MCHC compared to CCl_4 treated rats , whereasMCHC value was not affected significantly by dose12.5mg/kg,comperd to the CCl_4 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_4 treated rats.

Injection of dose 12.5mg/kg of QTU led to significantly increased in MCV compere to CCl_4treated rats .

Injection of dose 25mg/kg QTU led significantly decreased in MCHC compere to CCl_4 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_4 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_4 (1ml/kg)	28.69 a 0.96±	16.23 b 0.60±	57.29 c 1.87±
(12.5mg/kg) QTU & CCl_4	26.97 ab 0.60±	16.88 b 0.23±	63.24 b 1.71±
(25mg/kg) QTU & CCl_4	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 ± stranded deviation , Different letters indicate significant differences among groups at ($P \leq 0.05$).

QTU has not significantdiferent($P<0.05$) on total leukocytes count, lymphocytes and neutrophil percentage compared to control group. Treated rats with CCl_4 led to a significant increase in total leukocyte ,neutrophils and eosinophils, whereas lymphocyte percentage significantly decreased ($P<0.05$) in CCl_4 group compared with control and QTU alone group. On the other hand, monocyte and basophil percentages were not affected.

The effects induced by CCl_4 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_4 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

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_4 (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_4	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_4	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 clearlydemonstrated that CCl4 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 autooxidation, 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). CCl4 intoxication caused a significant increase the autoxidation of oxyhemoglobin to methemoglobin, which indicated that CCl4 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 CCl4 -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_4toxicity on bone marrow, spleen and liver. Counteracting the effects of CCl_4 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 tetrachloriedon the hemopoeitic 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 bound 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_4 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-8positionhave 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_s count ,This increase may be attributed to the defensive mechanism of immune system (25), It has been founed 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 marginatedneutrophils 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 CCl4 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 CCl4 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 tetraacetylederivative 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_4 on hemopoeiticsystem 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 (CCl4).

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 CCl4 مقارنة مع بيانات مجموعة السيطرة.

حقن البريتوني داخل ١٢.٥ ملغم / كغم و ٢٥ ملغم / كغم من مشتقات الكورسيتين (QTU)، بعد أربعة أسابيع من التعرض ل CCL4، 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.

REFERENCES

1. **Paolillo, R.; Romano, Carratelli, C.; and Rizzo, A. (2011).** Effect of resveratrol and quercetin in experimental infection by Salmonella enteric serovar Typhimurium. Int Immunopharmacol; 11: 149-156

2. **Pandey, K.B.; and Rizvi, S. Ibrahim. (2009).** Current understanding of dietary polyphenols and their role in health and disease. Curr Nutr Food Sci; 5: 249-263.
3. **Sangai, Neha P.; and Verma, Ramtej J. (2012).** Quercetin ameliorates bisphenol a-induced toxicity in mice. Acta Poloniae Pharmaceutica - Drug Research, Vol. 69 No. 3 pp. 557-563.
4. **Tieppo, J.; Vercelino, R.; Dias, A.S.; Silva, Vaz M.F.; Silveira, T.R.; and Marroni, C.A. (2007).** Evaluation of the protective effects of quercetin in the hepatopulmonary syndrome. Food Chem Toxicol; 45: 1140-1146.
5. **Reutrakul, C.; Ningnuek, N.; and Pohmakotr, M. (2007).** Planta Med. 73, 683.
6. **Inal, M.E.; Akgun, A.; and Kahraman, A. (2002).** Radioprotectiveeffects of exogenous glutathione against whole-body gamma-ray irradiation: age- and gender-related changes in malondialdehydelevels, superoxide dismutase and catalase activities in rat liver. Methods Find Exp Clin Pharmacol; 24:209-12.
7. **Sood, R. (1996).** Hematology for students and practitions. 4th, ed., Jaypee brothers Medicial Publishers, (p) LTD., India.: 318-325.
8. **Schalm, O. W.; Jain, N. C. and Carrol, E. J. (1975).** Veterinary hematology. Leo and Febiger, ed. Philadelphia.: 140-152.
9. **Sohair R. Fahmy and Salwa A. H. Hamdi . (2011)** Antioxidant effect of the Egyptian freshwater Procambarus clarkii extract in rat liver and erythrocytes . African Journal of Pharmacy and Pharmacology, Vol. 5(6), pp. 776-785.
10. **O.J.Sule,.R.Abdu and K.Kiridi(2016):**Effect of Carica papaya (L) Leaves on Haematological Parameters in Ccl4-induced Wistar Albino Rats, British Journal of Medicine & Medical Research ,16(3): 1-6, 2016, Article no.BJMMR.13686 ,ISSN: 2231-0614, NLM ID: 101570965.
11. **Saba AB, Oyagbemi AA and Azeez OI. (2010).** Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of Cnidoscolus aconitifolius in rats. Nig. J. Physiol. Sci. 25: 139 – 147 .

12. **Salman MMA, Randa, and Abdel-Rahman A (2016)** Patho-physiological studies on the Reverse Effect of Curcumin (curcuma longa, Zingiberaceae) and Ursofalk(Ursodeoxycholic acid) against the Toxicity of Carbon Tetrachloride on Albino Rats. J Liver 5:200 doi:10.4172/2167-0889.1000200.
13. **Firdaws A. AL-Mashhadani.(2017)** . Effect of Fenugreek Seed and Leaves on Some Hematological and Biochemical Parameters in CCl4-induced Liver Injury. Int.J.Curr.Microbiol.App.Sci. 6(4): 2328-2337.
14. **Johnson, R.M., Jr.G. Goyette, Y. Ravindranath, Y.S. Ho, 2005.** Hemoglobin autoxidation and regulation of endogenous H2O2 levels in erythrocytes. Free Radi. Biol. Med., 39: 1407-1417.
15. **Giulivi, C., K.J. Davies, 2001.** Mechanism of the formation and proteolytic release of H2O2-induced dityrosine and tyrosine oxidation products in haemoglobin and red blood cells. J. Biol. Chem., 276: 24129-24136.
16. **Kumaravelu, P., S. Suhramaniyam, D.P. Dakshinamoorthy, N.S. Devaraj, 1996**. The antioxidant effect of eugenol on CCl4,-induced erythrocyte damage in rats. Nutr. Biochem., 7: 23-28.
17. .**Shumilova, T. E.; Yanvareva, N. A.; Shereshkov, V. I. and Nozdrachev, A. D. (2006).** Effect of nitrite-induced methemoglobinemia on the kinetics of blood deoxygenation. . *Biology Bulletin*. 33 (2):167-71.
18. **Mourella,M and Teresa,M.F.(1991);**Erythrocyte defects precede the onest of CCl4-induced liver cirrhosis .Life .Sci.48:1083-1090 .
19. **Beutler E(2001);** Hemolytic anemia due to chemical and physical agents. In Beutler E, Coller BS, Lichtman MA, Kipps TJ, Seligsohn U (eds): Williams Hematology, 6th edition, New York , pp. 629–632. .
20. **Ballinger, A. (2007).** Gastroenterology and anemia. Medicine, 35: 142- 146.
21. **Evgeny V. Buravlev , Oksana G. Shevchenko, Irina Y. Chukicheva , Alexandr V. Kutchin(2017):** Synthesis and membrane-protective properties of aminomethyl derivatives of quercetin at the C-8 position,chemical papers springr link . pp 1–8| Cite as
22. **Ivana Milackova, Marta SoltesovaPrnova, Magdalena Majekova, Ruzena Sotnikova, Michal Stasko, Lucia Kovacikova, Sreeparna Banerjee,**

Miroslav Veverka& Milan Stefek (2015). Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Bratislava, Slovakia, and Milan.stefek:2-Chloro-1,4-naphthoquinone derivative of quercetin as an inhibitor of aldose reductase and anti-inflammatory agent ,journal of enzyme inhibition and medical chemistry ,30:1,107-113.

23. **Elshater, A.A.,Salman, M.M.A.;Moahmed ,S.;(2013):** The hepato- ameliorating effect ofSolanum nigrumagainstCCL4induced liver toxicity in Albino rats. Egypt Acad J BiologSci ., 5(10): 59-66.
24. **Maher A.Amer, Mohamed A.EL-missiry, AyaAbdrab EL-nabi(2015):** The Role of Ficuscarica Leaf Extract in Modulation of the experimentally induced Hepatotoxic Damage in Male Rats, International Journal of Advanced Research , Volume 3, Issue 12, 572 – 585.
25. **Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO.(2007).** Prevention of CCl4-Induced Liver Damage by Ginger, Garlic and Vitamin E. Pak J Biol Sci.; 10: 617-621.
26. **Weir DM, Stewart J. (1999).** Immunology. 8th Ed. London: Churchill Livingstone.
27. **Abuharfeil, N.; Sarsour, E. and Hassuneh, M. (2001).** The effect of sodium nitrite on some parameters of the immune system. *Food ChemToxicol.* 39 (2):119-24.
28. **Sule, O.J.; Elekwa ,I.; Ayalogu, EO.;(2012):**Effect of Acalyphawilkesianamuell arg. on haematologicalparameters in wistar albino rats. Int J Biol. Med Res., 3(1): 1234-1237.
29. **Mada, S. B.; Inuwa, H. M.; Abarshi, M. M.; Mohammed, H. A. and Aliya, A. (2014):** Hepatoprotectiveeffect of Momordicacharantia extract against CCL4 induced liver damage in rats. British Journal ofPharmaceutic Research.,4(3): 368-380.
30. M.G.Ortega, A.C.Saragusti, J.L.Cabrera, G.A.Chiabrando, (2010): Quercetin tetraacetyl derivative inhibits LPS-induced nitric oxide synthase (iNOS)

expression in J774A.1 cells. Archives of Biochemistry and BiophysicsVolume 498, Issue 2, 15 June 2010, Pages 105-110