STUDY THE PROTECTIVE ROLE OF QUERCETIN AGAINST O-ANISIDINE TOXICITY ON SOME HEMATOLOGICAL PARAMETERS OF LABORATORY MALE RATS (*RATTUS NORVEGICUS*).

Wissam Sajid Al-Uboody *Muhammed Ali Al-Diwan**

*Department of Physiology and Medical Physics, College of Medicine, University of Al-Muthanna,. Al-Muthanna, Iraq.

**Department of Physiology, College of Veterinary Medicine, University of Basrah,Basrah,Iraq

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ABSTRACT

The study was designed to assess the protective role of Quercetin against O-anisidine toxicity. 24 male rats (*Rattus norvegicus*) were used and divided into 3 equal groups of 8 male rats each. The first group was the control group in which the animals were fed on a standard ration for 15 days, then they were terminated. The second group was fed on a ration contains 1000mg/kg O-anisidine hydrochloride for 15.The third group was fed on a ration contains 1000mg/kg O-anisidine hydrochloride + 80mg/kg Quercetin dihydrate for 15. The results revealed that the treatment with O-anisidine hydrochloride for 15 days (1rst treated group) caused significant decrease in the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant increase in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with control group. When Quercetin dihydrate was offered as a protective agent in the ration of the 2nd treated group, it showed a significant ameliorating effect by increasing the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant decrease in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with the 1rst treated group. For the blood indices (RDW, MCH, MCHC, MCV, and MPV) there were no significant differences among all the experiment groups except for the mean platelet volume (MPV), where O-anisidine hydrochloride caused significant decrease in the MPV of the 1rst treated group as compared with control and 2nd treated group at ($P \leq 0.05$).

INTRODUCTION

Ortho-anisidine is one of the widely spread family known as aromatic amines (1). (2) mentioned that the principal commercial use of O-anisidine is an intermediate in the manufacture of dyes and pharmaceuticals and as an intermediate in the manufacture of synthetic guaiacol and its derivatives. It has been known that O-anisidine is released from textiles and leather goods colored with azo dyes and a large part of the population may be exposed and as a constituent of cigarette smoke (3). Hemoglobin adducts of O-anisidine were detected in blood samples of persons living in urban or rural areas of Germany (4). (5) mentioned that young children are exposed by oral suckling clothes which are colored with O-anisidine based dyes, and workplace exposure occurs by inhalation in the production and processing of O-anisidine, dermal contact during the formulation of O-anisidine based printing inks, general population in dermal contact with packing materials printed with O-anisidine based pigments; and man exposed indirectly via the environment. Flavonoids are a group of naturally occurring polyphenolic compounds widely distributed as secondary metabolites in plant kingdom (6). Quercetin (3, 5, 7, 3, 4-pentahydroxy flavon), is one of the most prominent dietary antioxidants (7). Quercetin occurs in glycosylated form in French beans, broccoli, and apples and especially in onions (8). Quercetin is the most abundant antioxidant in the nature and has an antioxidant potential four times that of vitamin E (9). Quercetin has been reported to increase the genomic stability in rats and enhance the antioxidative defense system by up regulating antioxidant enzymes (10). 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 (11). Quercetin prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals, protecting against lipid peroxidation and chelating metal ions (12).

MATERIALS AND METHODS

Experimental animals and diets

The experiment was conducted at the animal house of the Veterinary Medicine College–University of Basra, where 24 male rats (*Rattus norvegicus*) of 170–175 grams weights were used. The experiment conditions were unified for all animals, where the room temperature was set between 20–25 C by the use of an air

conditioner, and the daily light period was 12 hours by the use of two fluorescent lamps, and the humidity rate was about 50 %. Food and water were provided daily (*ad libitum*).

Experimental design

The animals were allocated into three groups; each group consists of 8 male rats. The hematological tests were done after 15 days of the experiment. The groups were:

Control group: In this group, 8 male rats were fed with standard ration for 15 days then they were terminated for the necessary tests.

The first treated group (T1) group: This group consisted of 8 male rats which were fed with a ration contains O-Anisidine hydrochloride 1000mg/kg for 15 days, then they were terminated for the necessary tests .

The second treated group (T2) group: This group consisted of 8 male rats which were fed with a ration contains 1000mg/kg O-Anisidine hydrochloride +80 mg/kg Quercetin dihydrate for 15 days, then they were terminated for the necessary tests.

Specimens' collection

After the animals were anaesthetized by holding them apart in a chloroform containing conduit. After their chests were dissected, blood samples were collected via cardiac puncture by using 5ml disposable syringe according to the method of (13). Then the blood was put in an anticoagulant containing tubes.

Hematological parameters

All the hematological parameters in this study were obtained by the use of a highly developed hematology analyzer (Ruby®, Germany made), where 1ml of the noncoagulated blood is aspirated by a special needle equipped within the device and then the analyzed results would appear in a detailed printed report.

Statistical Analysis

In this study, ANOVA Analysis and LSD tests are used according to (SPSS version 18) programme at the ($P \leq 0.05$) to find the significant differences among means for all treatments.

RESULTS AND DISCUSSION

The results revealed that the treatment with O-anisidine hydrochloride for 15 days (T1 group) caused significant decrease in the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant increase in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with control group at ($P \leq 0.05$) tables (1,3). When Quercetin dihydrate was offered as a protective agent in the ration of the (T2 group), it showed a significant ameliorating effect by increasing the R.B.C. count, Hb concentration, P.C.V. percentage, neutrophil and lymphocyte counts and it caused significant decrease in platelets count, total leukocytes, monocytes, eosinophil and basophil counts, as compared with the T1 group at ($P \leq 0.05$) tables (1, 3). For the blood indices (RDW, MCH, MCHC, MCV, and MPV) there were no significant differences among all the experiment groups except for the mean platelet volume (MPV), where O-anisidine hydrochloride caused significant decrease in the MPV of the T1 group as compared with control and T2 group at ($P \leq 0.05$) table (2).

These deleterious effects of O-anisidine on the blood parameters can be explained pending upon either the direct effect of O-anisidine or indirectly by the effects of the metabolites which are resulted from its metabolism in the liver of rat. Where, O-anisidine is oxidized by human, rat and rabbit hepatic microsomes containing cytochromes P450 not only to N-(2-methoxyphenyl)hydroxylamine, but that this compound is a subject of complex redox cycling reactions, forming also o-aminophenol, 2- nitrosoanisole and one additional metabolite, the exact structure of which has not been identified as yet (14). O-Demethylation of 2-nitroanisole to 2-nitrophenol and its hydroxylated products, 2, 5-dihydroxynitrobenzene and 2, 6-dihydroxynitrobenzene, (15). N-Hydroxyarylamines can be further metabolized to N-sulfonyloxyarylamines, N-acetoxyarylamines or N-hydroxyarylamine N-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects (8) of this class of compounds. One of the most effects of these metabolites is the formation of reactive oxygen species (ROS) especially by the metabolite O-aminophenol like superoxide radicals or hydrogen peroxide. ROS can induce oxidative damage to the cell and can form a very stable structure by extracting electrons from other sources (17). ROS are also able to generate other forms of ROS. Superoxide can be dismutated into H2O2 and oxygen. H2O2 has the ability to form

the more damaging _OH, through a combination of the Fenton and Haber-Weiss reactions (18). The ROS which are not neutralized, can target biological molecules such as DNA, lipids, proteins, and carbohydrates, which can result in cell dysfunction or cell death. Red blood cell (RBC) membranes contain lipids rich in unsaturated fatty acids. RBCs are more frequently exposed to oxygen than other body tissue and, thus, are more susceptible to oxidative damage. Erythrocytes are highly susceptible to the oxidative damage due to the high cellular concentration of oxygen and hemoglobin—a potentially powerful promoter for the oxidative processes (19). Invasion of the RBC membrane by peroxidants may lead to cell hemolysis. Moreover, the hemoglobin in RBCs is a strong catalyst which may initiate lipid peroxidation. In addition to lipid peroxidation, oxidants affect vital –SH groups of proteins which are highly active and may be targeted during oxidative stress (20). Platelets, a major player in thrombus formation, are obviously a prime target for oxidants produced or released in the vascular lumen (21). So, the elevated platelets count in the results of our study can be explained by the following mechanisms: Production of hydrogen peroxide seems to promote thromboxane synthesis, and hence platelet aggregation, in response to arachidonic acid stimulation. This conclusion is also supported by data obtained by (22) and (23), who showed that low concentrations of hydrogen peroxide could potentiate the aggregatory response to arachidonic acid or collagen. In summary, the effects of reactive oxygen metabolites on in vitro platelet function are complex. Low levels of HO may promote thromboxane synthesis and aggregation.

Role of other platelet agonists. Another possible mechanism by which oxidants may influence platelet aggregation is through potentiation of the effects of platelet-activating factor _PAF. PAF is an autacoid released by platelets and other cell types _e.g. endothelium, leukocytes, which acts on platelets at extremely low concentrations (24). Superoxide and hydroxyl radicals can rapidly _i.e. within seconds and irreversibly inactivate plasma PAF-acetylhydrolase, the enzyme that catabolizes PAF. Once PAF is formed or released in the blood, inhibition of PAF-acetylhydrolase would enhance concentrations and prolong half-life of this powerful agonist. Thus, it may be speculated that oxygen radicals may indirectly enhance platelet aggregation, through local increases in PAF concentrations secondary to reduced breakdown of PAF. In vivo this hypothesis is indirectly supported by data derived from a study by (25), in which administration of superoxide dismutase was associated with a

significant reduction in PAF-mediated aggregation of platelets resuspended in plasma, consistent with preserved activity of plasma acetylhydrolase.

Quercetin has a high antioxidant potential, the effect that is mainly based on inactivation of reactive oxygen species (ROS). Thus, Quercetin protects the cells against free radicals (26).Quercetin, inhibit glycosylation by 52%. This is an important effect, as flavonoids are stored in RBCs (27). Pure Quercetin inhibits hemolysis, Quercetin decreases hemolysis in a dose-dependent manner. This was also shown by (28). Additionally, flavonoids have inhibitory effects on the functions of platelets and leukocytes. They also protect endothelial cells, and counterbalance the interactions between the blood stream and vascular wall, which may lead to thrombosis. The latter effect is mediated through the effect of flavonoids on human monocyte tissue factor, which itself may trigger blood coagulation (29). The mechanism of action of Quercetin has been attributed largely to the antioxidant properties, which are known to augment GSH and antioxidant enzyme levels and scavenge lipid peroxides. A concept is now emerging of “adaptogenic drugs” - drugs that increases non-specific resistance to variety of stresses. The neutropenia in the results which was caused by O-anisidine treatment came in accordance with the results obtained by (30) who found that oxidative stress caused neutropenia and accelerated neutrophil apoptosis in dogs, and also agreed with the results obtained by (31) who reported that exposure to benzene derivatives causes lymphopenia and neutropenia in rats.

Table (1). Role of Quercetin dihydrate against O-anisidine hydrochloride on blood parameters of rats. Different letters refer to significant differences among groups at (P≤0.05).

PARAMETERS / GROUPS	RBC ($n\times10^6/\mu l$)	Hb (g/dl)	PCV (%)	Platelets ($n\times10^3/\mu l$)
Control	a 8.6 ±1.1	a 14.6 ±1.2	a 44.5 ±3.4	c 542.6 ±20.6
T1	b 6.6 ±0.9	c 10.7 ±1.2	c 33.4 ±3.8	a 876.7 ±19.62
T2	a 7.7 ±1.0	b 12.6 ±1.7	b 38.8 ±2.3	b 586.5 ±8.6
LSD	**1.4**	**1.9**	**5.7**	**24.6**

Table (2). Role of Quercetin dihydrate against O-anisidine hydrochloride on blood indices of rats. Different letters refer to significant differences among groups at (P≤0.05).

PARAMETERS / GROUPS	RDW (%)	MCH (pg)	MCHC (g/dl)	MPV (fl)	MCV (fl)
Control	a 10.5 ±1.0	a 16.6 ±1.05	a 32.7 ±1.8	a 4.24 ±0.22	a 50.8 ±1.7
T1	a 10.3 ±1.0	a 16.4 ±1.2	a 32.1 ±2.2	b 3.77 ±0.8	a 51.0 ±1.3
T2	a 10.5 ±1.0	a 16.5 ±1.0	a 32.6 ±1.6	a 4.06 ±0.24	a 50.7 ±1.1
LSD	**00**	**00**	**00**	**0.28**	**00**

Table (3). Role of Quercetin dihydrate against O-anisidine hydrochloride on leukocytes count of rats. Different letters refer to significant differences among groups at (P≤0.05).

PARAMETERS / GROUPS	WBC ($n\times10^3/\mu l$)	Nutrophil (%)	Lympho (%)	Acidophil (%)	Basophil (%)	Monocyte (%)
Control	c 6.2 ±1.2	a 12.1 ±1.3	a 80.1 ±1.3	b 1 ±0.5	b 0.3 ±0.07	b 4 ±0.8
T1	a 9.3 ±1.1	b 8.3 ±1.1	b 70.4 ±3.0	a 4 ±0.6	a 8.5 ±1.4	a 6.2 ±0.9
T2	b 7.2 ±1.3	a 11.1 ±1.5	a 77.9 ±4.1	b 0.9 ±0.2	b 1.1 ±0.8	b 4.6 ±1.3
LSD	**1.7**	**1.9**	**6.1**	**2.06**	**0.9**	**0.9**

دور الكويرستين الوقائي ضد التأثير السمي للأنيسدين في معايير الدم للجرذان المختبرية (*Rattus Norvegicus*)

وسام ساجد العبودي*؛ محمد علي الديوان**

*فرع الفسلجة والفيزياء الطبية، كلية الطب، جامعة المثنى ،المثنى ،العراق.

**فرع الفسلجة، كلية الطب البيطري، جامعة البصرة،البصره،العراق.

الخلاصة

شملت هذه الدراسة ٢٤ ذكرا من الجرذان المختبرية وتم توزيعها على ثلاث مجموعات بواقع ٨ جرذ ذكر لكل مجموعة. مثلت المجموعة الاولى مجموعة السيطرة وفيها تم تغذية الحيوانات على عليقة نموذجية لمدة ١٥ يوما ثم تم قتلها لأجراء الاختبارات اللازمة عليها. المجموعة الثانية تم تغذيتها على عليقة تحتوي على ١٠٠٠ملغم\كغم هيدروكلوريد ألأورثو أنيسيدين لمدة ١٥ يوما ثم تم قتلها لأجراء الاختبارات اللازمة. أما المجموعة الثالثة فقد تمت تغذيتها على عليقة تحتوي على ١٠٠٠ملغم\كغم هيدروكلوريد ألأنيسيدين + ٨٠ملغم\كغم كويرستين دايهايدريت لمدة ١٥ يوما ثم تم قتلها لأجراء الاختبارات اللازمة .

اظهرت النتائج أن استخدام هيدروكلوريد الاورثو انيسدين لمدة ١٥ يوما (مجموعة المعاملة الاولى) قد تسبب بانخفاض معنوي في عدد كريات الم الحمراء، تركيز الهيموغلوبين، نسبة حجم الخلايا المرصوص، عدد الخلايا البيضاء العدلة والخلايا اللمفاوية وقد تسبب ايضا بارتفاع معنوي في عدد الصفيحات الدموية، العدد الكلي لخلايا

الدم البيضاء، وعدد الخلايا وحيدة النواة، الخلايا الحمضة والخلايا القعدة مقارنة مع مجموعة السيطرة عند مستوى المعنوية ($P\leq0.05$). لقد بينت النتائج انه عندما تم استخدام الكويرستين دايهيدريت كعامل وقائي في مجموعة المعاملة الثانية فأنه قد أظهر تأثيرا محسنا للمعايير المتأثرة بالانيسدين حيث تسبب الكويرستين بارتفاع معنوي في عدد كريات الم الحمراء، تركيز الهيموغلوبين، نسبة حجم الخلايا المرصوص، عدد الخلايا البيضاء العدلة والخلايا اللمفاوية وقد تسبب ايضا بانخفاض معنوي في عدد الصفيحات الدموية، العدد الكلي لخلايا الدم البيضاء، وعدد الخلايا وحيدة النواة، الخلايا الحمضة والخلايا القعدة مقارنة مع مجموعة المعاملة الاولى عند مستوى المعنوية ($P\leq0.05$). بالنسبة لمعايير الدم الحسابية (مدى توزيع كرات الدم الحمراء، معدل هيموغلوبين الكرية، معدل تركيز هيموغلوبين الخلية، معدل حجم الكريات الحمراء، ومعدل حجم صفيحات الدم) فأنه لم تكن هنالك فروقات معنوية بين جميع المعاملات ماعدا معدل حجم صفيحات الدم حيث انه هيدروكلوريد الاورثوانيسدين قد تسبب بانخفاض معنوي في معدل حجم صفيحات الدم لمجموعة المعاملة الاولى مقارنة مع مجموعة المعاملة الثانية ومجموعة السيطرة عند مستوى المعنوية ($P\leq0.05$).

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