RESEARCHES REGARDING THE OBTAINING OF VEGETAL PRINCIPLES WITH POSSIBLE ANTIDOTE PROPERTIES IN LEAD POISONING. NOTE 2. ANTIDOTE EVALUATION OF QUERCETOL IN ACUTE LEAD ACETATE POISONING ON MICE

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Abstract

Our previous research studies on crustaceans (*Euxinia maeotica*) and molluscs (*Mytillus galloprovincialis*) acute lead acetate poisoned and treated with quercetol (3, 3', 4', 5,7-pentahidroxiflavone) showed antidotal and antioxidant properties for quercetol (superoxidedismutase and catalase – oxidative stress enzymes activites decreased). The present study investigated the antidotal effect of quercetol on white mice acute lead acetate poisoned. For this purpose we determined the antidotal potential of a single molar dose of quercetol (70μmoles/Kg b.w.) administered *per os* while lead acetate doses (toxic substance) were administered *i.v.*, each 1 hour variated in geometric progression. The mice were divided in 6 experimental groups of 20 animals each (10 males and 10 females), and the animals were kept under observation 72 hours from the start of the treatment, until the mortality occured. We established: protection index (I_P), number of toxic molecules bioinactivated by a quercetol molecule and the regression equation of the antidotal effect, depending on lead acetate doses.

The I_P for a single dose of 70μmoles/Kg b.w. quercetol was 1.43, the number of toxic micromoles bioinactivated by a quercetol micromole was 2.18, the correlation coefficient (r) between the effect and the dose is 0.997, the fitting error is 0.113. The regression analysis for quercetol antidote effect varying with lead acetate dose shows that this one is considerably determined by the toxic dose variation. The small doses of quercetol used in the experiment did not influence the toxic action by its own toxicity. As a conclusion, quercetol acts as an antidote in lead acetate acute poisoning.

Rezumat

Deoarece unele cercetări anterioare efectuate pe crustacee (*Euxinia maeotica*) şi moluşte (*Mytillus galloprovincialis*) intoxicate acut cu acetat de plumb şi tratate cu quercetol (3, 3',4', 5, 7-pentahidroxoflavonă) au evidențiat proprietăți de antidot şi de antioxidant ale acestuia (au scăzut activitatea enzimelor de stres oxidativ – superoxiddismutază şi catalază), ne-am orientat spre verificarea efectului antidot al quercetolului şi pe şoareci intoxicați acut cu Pb^{2+}.

În acest scop s-a determinat potențialul antidotic al unei doze molare unice de quercetol (70 μmol/Kg b.w.), administrat *p. o*., în condițiile în care au variat în progresie geometrică dozele de acetat de plumb (substanța toxică) administrat *i.v*., la interval de 1 oră. Şoarecii au fost repartizați în 6 loturi omogene de 20 animale (10 masculi şi 10 femele). Perioada de observație a fost de 72 ore de la momentul aplicării tratamentului până la înregistrarea mortalității. S-au stabilit: indicele de protecție a quercetolului (I_P), numărul moleculelor de toxic bioinactivate de o moleculă de quercetol şi ecuația de regresie a efectului antidot funcție de doza de acetat plumb.

I_P pentru o doză unică de 70 μmol/Kg b.w. quercetol a fost de 1.43, numărul de μmoli de toxic bioinactivați de 1μmol quercetol a fost de 2.18, nivelul de corelație între efect şi doză (r) este de 0.997, iar eroarea de fitare 0.113. Din analiza regresiei efectului antidot al quercetolului funcție de doza de acetat de plumb, rezultă că acesta este determinat semnificativ de variația dozei de substanță toxică. Cantitățile mici de quercetol folosite în experiment nu au influențat acțiunea toxicului prin propria toxicitate. În concluzie, quercetolul are efect antidot în intoxicația acută cu acetat de plumb.

Keywords: lead acetate acute poisoning, antidote, protection index, bioinactivation

Introduction

Quercetol (3, 3', 4', 5 ,7-pentahidroxiflavone) selection as a vegetal principle with possible antidote properties is based on multiple experimental observations: it forms stable complexes with plurivalent metal ions (Cu^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Al^{+3}) [1], *in vitro* verification of these complexes formation and stability [2, 3, 4], *in vivo* decrease of oxidative stress (superoxiddismutase, catalase) in crustaceans (*Euxinia maeotica*) and molluscs (*Mytillus galloprovincialis*) poisoned with lead acetate [5, 7], annihilation of some toxic lead acetate poisoning effects [8], free radicals captation (inhibition of membrane lipids peroxidation, protection against oxidative stress – antioxidant activity), and even neuroprotective effect [9, 10], when the plasmatic concentration of homovanilic acid increases [11, 12].

The objectives of this research consist of *in vivo* evaluation of the antidote effect of quercetol on mice poisoned with lead acetate.

The experiment pursued determination of:

- the antidote potential at a single dose of the tested substance (quercetol) and 3 doses of toxic (lead acetate);
- the protection index (I_P) in lead acetate poisoning;
- number of toxic molecules experimentally bioinactivated by a molecule of antidote (quercetol).

Materials and methods

Substances and animals

The lead acetate poisoning was induced to healthy white mice by *i.v.* administration of lead acetate $(CH_3COO)_2Pb$ x $3H_2O$ (Sigma), in a

sterile manitol 5% solution in 40 ml/Kg b.w. volume. Because quercetol is not soluble in water it was administered *p.o.* as a suspension, in a 2% aqueous tween 80 dispersion. The concentration of quercetol in suspension was 1.75 μmoles/ml.

The obtained quercetol suspension was administered intragastric, in a unique volume of 40 ml/kg b.w., corresponding to 70 μmoles/kg b.w. (constant dose, small enough, that would not interfere with the effect through its own toxicity). The mice were divided in 6 experimental groups, each of 20 animals, homogenous regarding the body weight (18 – 25 g), and sex ratio (10 males and 10 females) that received different amounts of lead acetate (table I). The first 3 experimental groups received quercetol 70 μmoles/kg b.w.

The mice were kept under laboratory bioclimatic conditions, free access to deionised water *ad libidum*, standard food and out of insecticides contact.

All procedures were performed in accordance with the ECC Directive 86/609/EEC of 24 November 1986 and Ordinance 37 of the Romanian Government from 2 February 2002, regarding research bioethics.

Antidote effect parameters

The observations were made for 72 hours, from the start of the treatment. Mortality was noted after 0. 5, 1, 2, 24, 48 and 72 hours.

We determined: the antidote potential for a single quercetol dose (test substance), and 3 doses of lead acetate (toxic substance), the protection index (I_p), the number of bioinactivated toxic molecules, the interpendence between the intensity of the antidote effect (*regression equation or estimation equation*).

The antidote potential of quercetol is expressed as sum of minus log of DL_{50} (moles/kg b.w.) of toxic and tested substance as antidote (quercetol).

I_P (the protection index) is expressed as a ratio of sum of DL_{50} (moles/kg b.w.) for toxic (lead acetate) and antidote (quercetol) and DL_{50} (moles/kg b.w.) of the toxic (lead acetate). From the difference between sum of DL_{50} (moles/kg b.w.) of toxic and tested substance as antidote (quercetol) and DL_{50} (moles/kg b.w.) of toxic (lead acetate) we obtained a number of molecules that will be reported to bioinactivate 1 μmole of antidote.

For the evaluation of the antidote effect of quercetol, we used six lead acetate doses (316.3; 354.3; 396.8; 448.2; 502; 562.2; μmoles/Kg b.w.) which were submitted to these conditions: 1. they were chosen in geometric progression in order to be sure that a ratio of 2 successive doses is constant, and the antidote effect remains within the dose – effect linearity interval; 2.

the smallest dose assured a survival percentage of 70 – 95% from the 20 individuals animals from the experimental group; 3. the highest dose assured a survival percentage of 5 – 30% of the individuals from a 20 individuals experimental group.

In the effect – dose correlation analysis the intensity of the antidote effect is considered a *dependent variable* and the toxic dose represents the *independent variable* (prediction). For the determination of the antidote effect there are involved the inactivated toxic doses. The interdependence relationship between the intensity of the antidote effect and each dose of bioinactivated toxic (*regression equation*) was evaluated using a bidimensional mathematic model for a quantitative evaluation. The results of the correlation analysis of the antidote effect varying with the toxic dose were expressed using the following statistical parameters: 1. linear regression correlation coefficient (r); the determination coefficient ($D_{Y/X}$); 3. the determination of fitted coefficient after the freedom degree number ($D_{Y/X}$ adjusted); 4. standard error of estimation; for measurement of experimental values disperssion of antidote effect from the regression line; 5. F parameter (Fisher test) as a total test of a determination relation between effect and the independent variable from the estimation model (value of ratio of average variance of regression and residual average variance of the antidote effect) [13, 15].

The correlation coefficients and the estimation equation are statistical methods used to connect the pharmacological effect and the investigated variable (the individual response of the animals). The biologic response, appreciated by presence or absence of a biological event (death or survival at the toxic dose administration), was quantal. As a measure of an average antidote effect on the experimental group, we took the values of *probit* corresponding to the percentage of positive answers (registered death). The independent varible (*prediction*) used to calculate the dose – effect correlation was the value of the natural logarithm of toxic doses, expressed in milimoles. If there is no dependence between the antidote effect and the prediction variable, or the dependence is curved the simple linear correlation coefficient is nonsignificant, different to zero.

Statistical analyses

The regression analysis parameters of the antidote effect varying with lead acetate dose, determined with specific methodologies from a quantative analysis are: the regression coefficient b_{y-x}, standard error, "t" test probability (p) (Student), the confidence interval of the two coefficients (95%).

Results and discussion

The results of the pharmacotoxicological researches of quercetol in lead acetate poisoning (table I) show that the protection index is $I_p = 1.43$, and the bioinactivation ratio lead acetate/quercetol is 2.18. The regression equation of the antidote effect of quercetol varying with the toxic dose (lead acetate) annihilated is found in table II and figure 1, and the toxic effect regression of lead acetate varying with dose are represented in table II and figure 2.

Table I

Experimental data upon antidotal potential of quercetol in acute lead acetate poisoning

Experimental group	Quercetol dose µmoles/ kg b.w.	Lead acetate dose µmoles/kg b.w.	Effect % (mortality)	Toxic potential (Lead acetate)	Protection index I_P	Bioinactivation index $Pb(CH_3COO)_2$ / quercetol
1	70	448.2	10	3.297	1.43	2.18
2		502	40			
3		562.2	85			
4	-	316.3	20	3.454	-	-
5		354.3	55			
6		396.8	80			

Table II

Regression equations of quercetol antidote effect varying with lead acetate dose and toxic effect of lead acetate

Tested substance	Regression equation
Quercetol + Lead acetate	Y = -65.310 +26.010 x
Lead acetate	Y = -38.444 +17.061 x

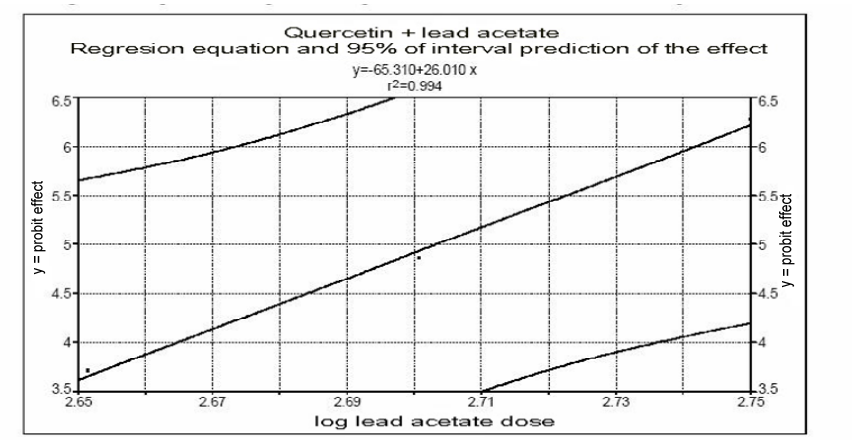

Figure 1

The regression equation of quercetol antidote effect varying with the lead acetate doses

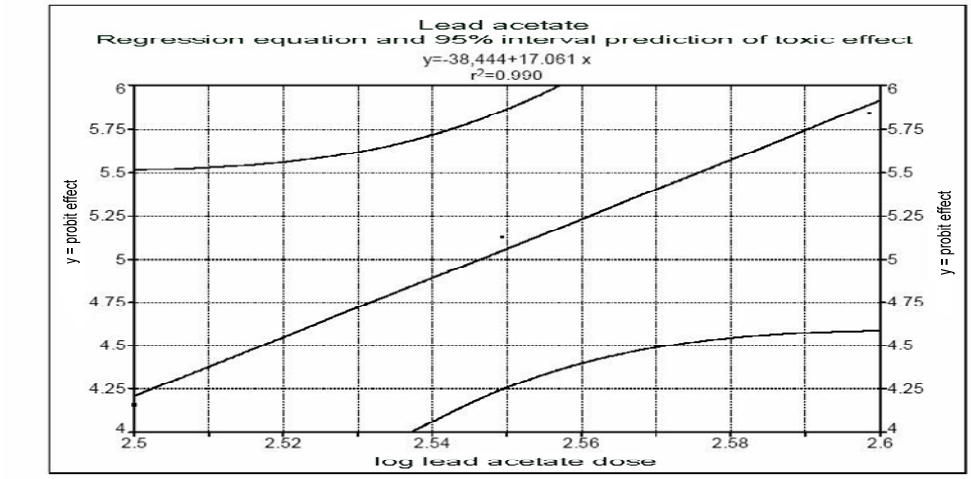

Figure 2
The regression equation of lead acetate toxic effect varying with the doses

The results of the correlation parameters for quercetol antidote effect varying with the toxic dose (lead acetate) are presented in table III.

Table III
Correlation analysis parameters for quercetol

Substance	Correlation coefficient $r_{y/x}$	Determination coefficient $D_{y/x}$;	$D_{y/X}$ fitted after degrees of freedom	Standard error of fitting	F Test
Quercetol	0.997	0.994	0.989	0.113	196.9

From table III data analysis, we can see that quercetol antidote effect is significant, because:

- the correlation coefficient r (0.997) shows a good correlation between the analysed parameters;
- the determination coefficient $D_{Y/X}$ (0.994) value proves that total variation of antidote effect is due to the independent variable (toxic dose);
- the fitted coefficient after the freedom degree number ($D_{Y/X}$ adjusted) is 0.989, sustains the accuracy of antidote effect determination;
- standard error of fitting (0.113) reflects the accuracy of the experimental determinations, the experimental values disperssion of the antidote effect from the regression line are small;
- the total test of determination of relation between effect and lead acetate dose logarithm using the estimation equation, the F value calculated is bigger than the critical value, shows that the antidote effect is significant for quercetol.

Antidote regression effect varying with lead acetate dose (table IV) reveals that:

- the regression coefficient is significant, proving that the experimental researches were well conducted;
- the small value of the standard error proves that the experimental data used for protection index determination assured a good accuracy level.

Table IV

Regression equation of antidote effect varying with lead acetate dose

Tested substance	Regression Parameters y = a + bx	Standard Error	"t"test value	Confidence interval 95%
Quercetol	a = -65.310	5.005	-13.046	-128
	b = 26.010	1.853	14.033	2.426

$_{0900}t_{critic}=6.314$

Conclusions

Based on quercetol protection index value (I_p = 1.43), number of μmoles of toxic substance bioinactivated by a 1 μmole of tested substance (2.18), and the correlation between the antidote effect of tested substance with the lead acetate doses administrated (r = 0.997, $D_{Y/X}$ = 0.994), we can conclude that the tested substance has an antidote effect in lead acetate poisoning.

References

1. Istudor V. - Farmacognozie, Fitochimie, Fitoterapie, Editura Medicală, Bucureşti, 1998, vol.1, 152 – 153.
2. Istudor V., Nacea V., Uivaroşi V., Roncea F. – Cercetări preliminare privind obținerea unui eventual antidot în intoxicațiile cu metale grele în *volumul de Rezumate ale celei de a X-a Sesiuni de Comunicări Ştiinţifice de la Universitatea „Ovidius“ Constanța*, Facultatea de Medicină şi Farmacie, 6 - 8 aprilie 2000, 269 – 270.
3. Istudor V., Sava C., Roncea F. - Cercetări preliminare privind obținerea unor antidoturi în intoxicațiile cu metalele grele în *volumul de Rezumate ale celei de a X-a Sesiuni de comunicări ştiinţifice de la Universitatea „Ovidius“ Constanța*, Facultatea de Medicină şi Farmacie din 6-7 aprilie 2001, 154.
4. Roncea F., Negreanu-Pirjol T., Bratu M., Istudor V., Dobrinas S., Birghilă S. -Use of flavonoid structures as antitoxic agents against heavy metals contamination; obtaining and characterization of complexes metal-flavonoids, Second International Symposium on Trace Elements and Minerals in Medicine and Biology, Munchen, 2004, abstract book, 151.
5. Roncea F., Istudor V., Bratu M. M. Pîrjol T. - Rolul protector al rutozidei asupra moluştei *Mytilus galloprovincialis* intoxicată cu plumb, *Farmacia,* 2003*,* 40(2), 55- 60.

6. Roncea F., Istudor, V., Bratu M. M., Pîrjol T. - Rolul antitoxic al quercetolului asupra moluştei *Mytilus galloprovincialis* intoxicată cu plumb, *Revista de Medicină şi Farmacie*, 2004, 50, Supliment II, Târgu – Mureş, 271 - 274.
7. Roncea F., Bratu M. M., Istudor V., Coprean D., Schiopu S., Tănase E. - Researches regarding obtaining of a possible antidote in heavy metals poisoning. NOTE 1. Preliminary *in vivo* data upon quercetol effect in lead acetate poisoning, *Farmacia,* 2008*,* 56 (4), 409 – 418
8. Amundsen T., Naess I. A., Hammerstrom J., Brudevold R., Bjerve K. S. – *Tidskr Nor Laegeforen*, Lead poisoning -an overview*,* 2002, 122 (15), 1473 - 6.
9. Heo H. ., Lee C.Y. - Protective effects of quercetin and vitamin C against oxidative stress-induced neurodegeneration, *J Agric Food Chem.*, 2004, 52 (25), 7514 - 7.
10. Saija A., Tomaino A., Trombetta D., Pellegrino M. L., Tita B., Messina C., Bonina F. P., Rocco C., Nicolosi G., Castelli F. - 'In vitro' antioxidant and photoprotective properties and interaction with model membranes of three new quercetin esters, *Eur J Pharm Biopharm.*, 2003, 56(2), 167 - 74.
11. Madani A., Bălălău D., Mitrea N., Ilie M. - Saturnismul. Mecanisme de acţiune toxică a compuşilor cu plumb, metode de diagnostic şi tratament, *Farmacia*, 2004, 52 (3), 52 - 62.
12. Weldin J., Kack R., Dugaw K., Kapur R. - Quercetin, an over the counter supplement, causes neuroblastoma-like elevation of plasma homovanilic acid, *Pediatric and Developmental Pathology,* 2003, 6, 125 – 129.
13. Miron S. D., Mircioiu C,. Ionescu M, Mircioiu I., F. Rădulescu F**.** - Formulation and evaluation of a preventive antidote in organophosphorous poisoning, *Farmacia*, 2006, 54 (2), 63 – 71.
14. Simionovici M., Cârstea Al., Vlădescu C. – Cercetarea farmacologică şi prospectarea medicamentelor, Ed. Medicală, Bucureşti, 1983, 128 – 129.
15. XXXX - Farmacopeea Română, ediția a X-a, Ed. Medicală, Bucureşti, 1993, 1125 – 1154.

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