IVANE JAVAKHISHVILI TBILISI STATE UNIVERSITY

Faculty of Exact and Natural Sciences

Applied Biosciences and Biotechnology

Assessment of the Effect of Polyphenolic Compounds Extracted from Wine Production Waste on Non-enzymatic Antioxidant Capacity in an Experimental Liver Pathology Model

Authors: Shalva Dorokashvili, Ekaterina Pipia, Mariam Gogiberidze,

Merab Sepashvili, Zurab Kuchukashvili.

Supervisor: Dr. Zurab Kuchukashvili,
Associate Professor,
Department of Biology

Co-Supervisor: Merab Sepashvili,
Doctoral Candidate,
Tbilisi State University

Tbilisi, 2024

Abstract

Today, unhealthy lifestyles are prevalent worldwide, characterized by the consumption of poor-quality foods. This trend has led to a rise in obesity, type 2 diabetes, and various metabolic disorders. One such condition is Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD). Among the strategies for treating this disorder, incorporating antioxidants into the diet has gained attention. Ongoing research around the globe is exploring the potential of antioxidants in treating various pathologies, including MAFLD.

Our research aims to investigate the impact of polyphenolic compounds on the non-enzymatic antioxidant status of rat liver in the context of pathology induced by a high-fat diet and streptozotocin. The research subjects were male, white, non-linear rats, which were fed a high-fat diet and subsequently injected with streptozotocin to induce pathology. We then extracted the animals' livers, homogenized them, and measured the non-enzymatic antioxidant capacity using validated commercial test systems. The results were then compared.

Our research explores a potential treatment for Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD) using grape pomace, a previously untapped resource. This approach not only reduces production costs but also helps protect the environment from pollution.

The study demonstrated that a high-fat diet combined with streptozotocin injection induced a specific pathology. To combat this, we utilized wine production by-products, known for their potent antioxidant effects. We compared their efficacy with that of chemically pure quercetin and found that these by-products show promising potential for treating fatty liver pathologies.

Study Results and Their Discussion

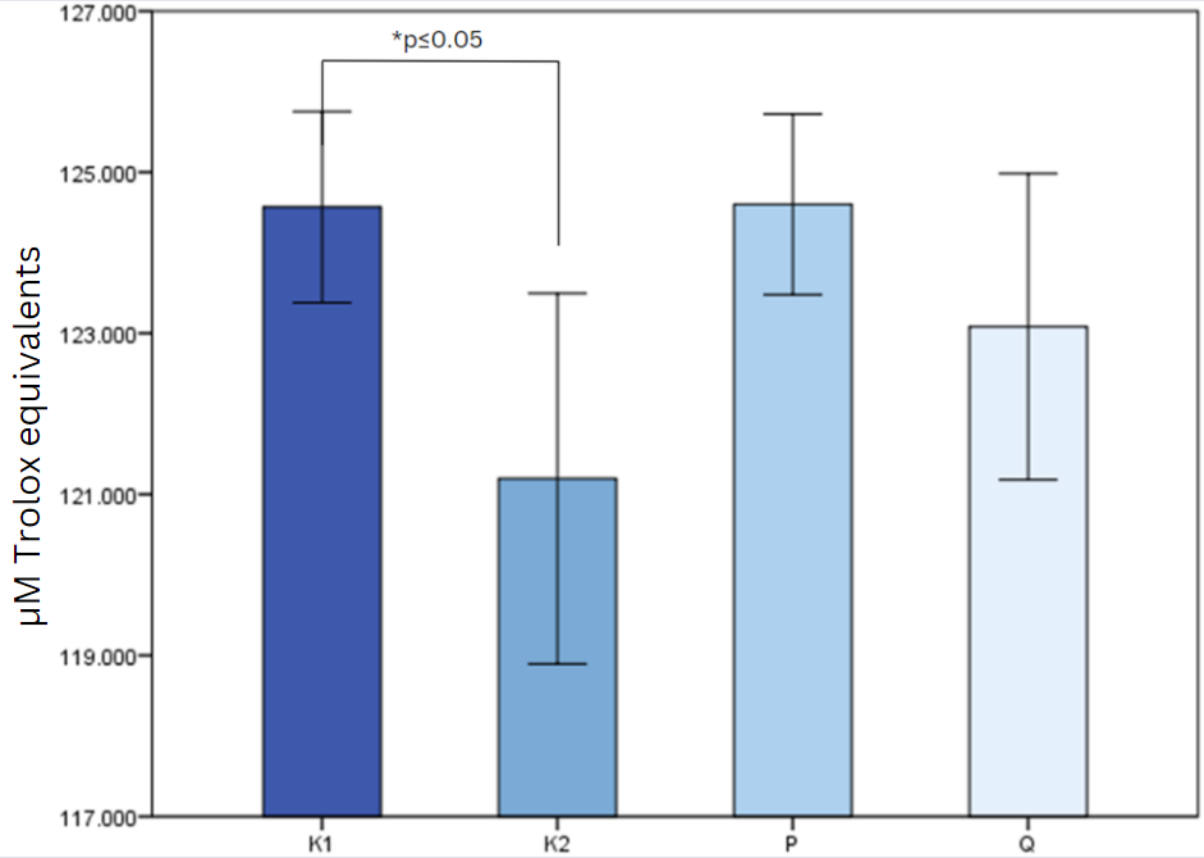

Figure 1 Non-enzymatic antioxidant capacity measured with OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC)

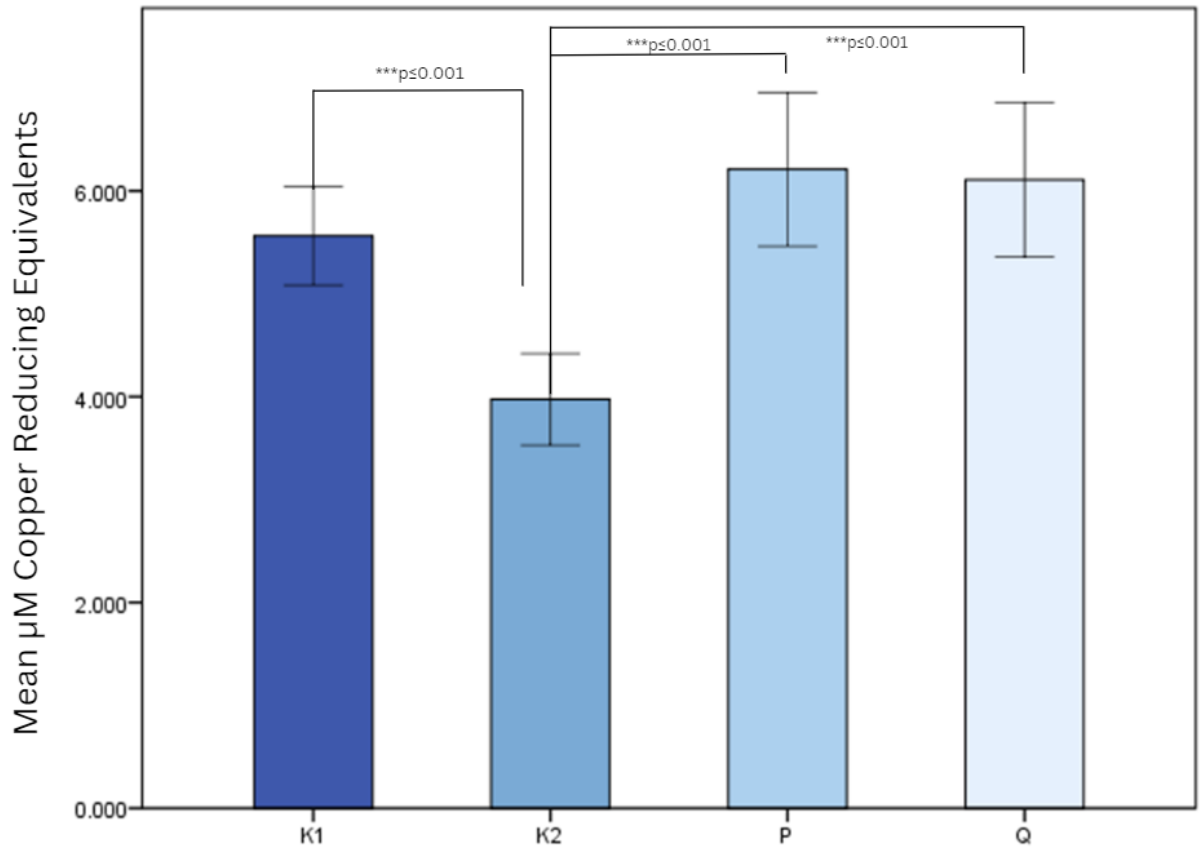

Figure 2 *Non-enzymatic antioxidant capacity measured with OxiSelect™ Total Antioxidant Capacity (TAC)*

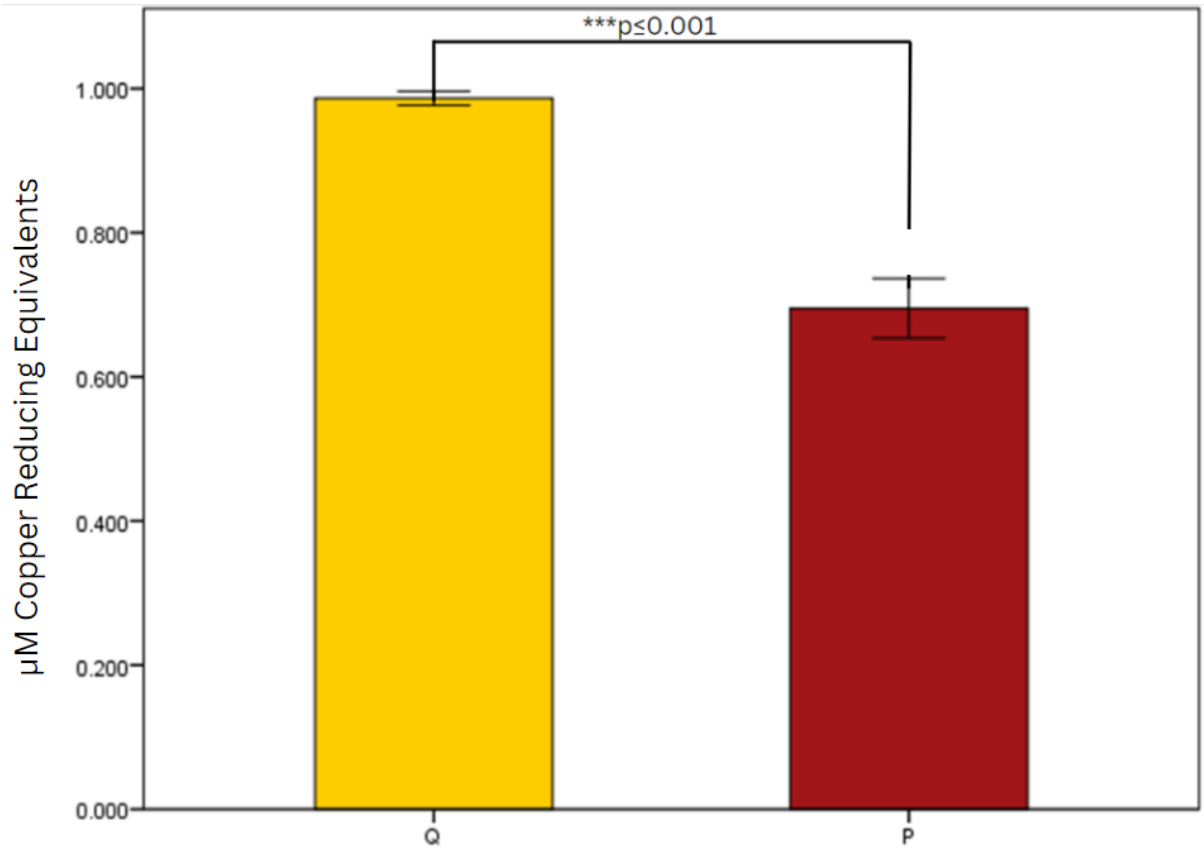

Figure 3 *Antioxidant capaciry measured with OxiSelect™ Total Antioxidant Capacity (TAC). Difference between Quercetin and Polyphenolyc fraction*

Table 1 OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC)'s One-Way Anova

ANOVA

µM Trolox Equivalents/Mg Tissue

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	78.190	3	26.063	3.782	.020
Within Groups	220.544	32	6.892		
Total	298.734	35			

Table 2 OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC)'s TukeyPost Hoc Test

Multiple Comparisons

Dependent Variable: **µM Trolox Equivalents/Mg Tissue**

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K1	K2	3.372332*	1.071759	.018	.46855	6.27612
	F	-.033622	1.268123	1.000	-3.46943	3.40218
	Q	1.486112	1.355680	.694	-2.18692	5.15914
K2	K1	-3.372332*	1.071759	.018	-6.27612	-.46855
	F	-3.405954	1.355680	.077	-7.07898	.26707
	Q	-1.886220	1.437916	.562	-5.78205	2.00962
F	K1	.033622	1.268123	1.000	-3.40218	3.46943
	K2	3.405954	1.355680	.077	-.26707	7.07898
	Q	1.519735	1.589676	.775	-2.78727	5.82674
Q	K1	-1.486112	1.355680	.694	-5.15914	2.18692
	K2	1.886220	1.437916	.562	-2.00962	5.78205
	F	-1.519735	1.589676	.775	-5.82674	2.78727

*. The mean difference is significant at the 0.05 level.

T

able 3 OxiSelect™ Total Antioxidant Capacity (TAC)'s One-Way Anova

ANOVA

µM Copper Reducing Equivalents/Mg Tissue

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.727	3	8.909	13.044	.000
Within Groups	20.489	30	.683		
Total	47.216	33			

Table 4 OxiSelect™ Total Antioxidant Capacity (TAC)'s TukeyPost Hoc Test

Multiple Comparisons

Dependent Variable: µM Copper Reducing Equivalents/Mg Tissue

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
K1	K2	1.590079*	.347614	.000	.64488	2.53528
	F	-.646439	.407881	.402	-1.75551	.46264
	Q	-.544170	.434895	.600	-1.72670	.63836
K2	K1	-1.590079*	.347614	.000	-2.53528	-.64488
	F	-2.236518*	.426765	.000	-3.39694	-1.07610
	Q	-2.134248*	.452653	.000	-3.36506	-.90344
F	K1	.646439	.407881	.402	-.46264	1.75551
	K2	2.236518*	.426765	.000	1.07610	3.39694
	Q	.102270	.500426	.997	-1.25844	1.46298
Q	K1	.544170	.434895	.600	-.63836	1.72670
	K2	2.134248*	.452653	.000	.90344	3.36506
	F	-.102270	.500426	.997	-1.46298	1.25844

*. The mean difference is significant at the 0.05 level.

Table 5 OxiSelect™ Total Antioxidant Capacity (TAC)'s One Way Anova

ANOVA

µM Copper Reducing Equivalents/Mg Tissue

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.127	1	.127	186.309	.000
Within Groups	.003	4	.001		
Total	.130	5			

Summary of Results:

During pathology induced by a high-fat diet and streptozotocin, the non-enzymatic antioxidant capacity of rat liver decreases. However, exposure to polyphenols significantly increases this capacity. The total antioxidant activity of the extracted polyphenol fraction is lower compared to chemically pure quercetin.

The decrease in antioxidant capacity is likely caused by intensified peroxidation processes in the liver, leading to the depletion of the non-enzymatic antioxidant system. Conversely, the increase in capacity due to polyphenol exposure is probably a result of the introduction of exogenous polyphenols, which possess strong antioxidant properties. These polyphenols bolster the non-enzymatic antioxidant system in the context of the induced pathology.

The lower antioxidant ability of the polyphenol fraction compared to quercetin can be attributed to the fact that the extracted fraction is a mixture of polyphenols, not a single, chemically pure compound like quercetin. Consequently, this mixture exhibits both synergistic and antagonistic antioxidant effects.