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Effect of combined quercetin and resveratrol administration on oxidative-nitrosative stress markers in the liver of rats under conditions of surgical trauma following prolonged stress

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Aim. This study aimed to investigate the effects of combined quercetin and resveratrol administration on markers of oxidative and nitrosative stress in the liver of rats subjected to surgical trauma following prolonged stress exposure.

Materials and methods. The study was conducted on 35 adult male Wistar rats, which were randomly assigned to five experimental groups: intact animals (control, Group 1); rats subjected to single prolonged stress (SPS) followed by laparotomy (Group 2); animals exposed to SPS and laparotomy and treated with a water-soluble form of quercetin (20 mg/kg body weight, Group 3), resveratrol (5 mg/kg body weight, Group 4), and a combination of quercetin and resveratrol (Group 5). Hepatic oxidative-nitrosative stress was assessed by measuring superoxide anion radical ($O_2^{\bullet-}$) production, total nitric oxide synthase (NOS) activity, the activities of constitutive and inducible NOS isoforms (cNOS and iNOS), and the concentration of peroxynitrites of alkali and alkaline-earth metals.

Results. Combined exposure to SPS and surgical trauma induced a marked oxidative-nitrosative imbalance in the liver, manifested by excessive superoxide and peroxynitrite production, mitochondrial redox dysfunction, iNOS overactivation, and profound cNOS uncoupling. Monotherapy with either water-soluble quercetin or resveratrol partially alleviated these stress-induced alterations by reducing $O_2^{\bullet-}$ generation, suppressing iNOS activity, and improving cNOS coupling, confirming their hepatoprotective effects under conditions of combined psycho-emotional and surgical stress. In contrast, combined quercetin-resveratrol administration provided substantially greater protection, resulting in near-complete normalization of mitochondrial $O_2^{\bullet-}$ production, full restoration of cNOS activity and coupling, and a marked reduction in hepatic peroxynitrite accumulation.

Conclusions. These findings indicate a synergistic interaction between the two polyphenols, quercetin and resveratrol in restoring hepatic redox and nitric oxide homeostasis.

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Вплив поєднаного застосування кверцетину та ресвератролу на показники оксидативно-нітрозативного стресу в печінці щурів за умов хірургічної травми після тривалого стресу

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Мета роботи – вивчити вплив поєднаного застосування кверцетину та ресвератролу на маркери оксидативно-нітрозативного стресу в печінці щурів, яким змодельовано хірургічну травму після тривалого стресового впливу.

Матеріали і методи. Дослідження здійснили на 35 дорослих самцях щурів лінії Wistar, яких рандомізовано на 5 експериментальних груп: інтактні тварини (контроль, група 1); щури, які зазнали одноразового тривалого стресу (Single Prolonged Stress, SPS) та яким здійснили лапаротомію (група 2); тварини, яким на фоні SPS і лапаротомії вводили водорозчинну форму кверцетину (20 мг/кг маси тіла, група 3), ресвератрол (5 мг/кг маси тіла, група 4) та комбінацію кверцетину та ресвератролу (група 5). Оксидативно-нітрозативний стрес у печінці оцінювали за показниками продукції супероксидного аніон-радикала ($O_2^{\bullet-}$), загальної активності синтази оксиду азоту (NOS), активності конститутивних та індуцибельних ізоформ NOS (cNOS та iNOS), а також за концентрацією пероксинітритів лужних і лужноземельних металів.

Результати. Поєднаний вплив SPS і хірургічної травми спричиняв виражений оксидативно-нітрозативний дисбаланс у печінці, що виявляли за надмірною продукцією супероксиду та пероксинітритів, порушенням мітохондріального редокс-гомеостазу, гіперактивацією iNOS і глибоким порушенням функціонального зв'язку cNOS. Монотерапія водорозчинним кверцетином або ресвератролом почасти послаблювала ці стрес-індуковані порушення шляхом зниження генерації $O_2^{\bullet-}$, пригнічення активності iNOS та відновлення функціонального зв'язку cNOS, що підтверджує їхні гепатопротекторні властивості за умов поєднаного психоемоційного та хірургічного стресу. Комбіноване застосування кверцетину та ресвератролу сприяє досягненню вираженішого захисного ефекту порівняно з окремим використанням сполук. У разі комбінованого застосування зафіксовано майже повну нормалізацію мітохондріальної продукції $O_2^{•-}$, відновлення активності та функціонального зв'язку cNOS, а також значне зменшення накопичення пероксинітритів у печінці.

Висновки. Підтверджено синергічну взаємодію двох поліфенолів кверцетину та ресвератролу щодо відновлення редокс-балансу та гомеостазу оксиду азоту в печінці.

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Prolonged psychological stress represents a major pathogenic factor contributing to the development of systemic inflammatory and metabolic disorders, particularly when combined with physical trauma. In the context of modern armed conflicts and severe injuries, post-traumatic stress disorder (PTSD) and PTSD-like conditions are increasingly recognized not only as neuropsychiatric entities, but also as systemic pathological states characterized by dysregulation of the hypothalamic-pituitary-adrenal axis, persistent inflammation, and redox imbalance [1,2]. These alterations markedly increase the vulnerability of peripheral organs to secondary injury, especially under conditions of surgical trauma [3].

The liver plays a central role in the regulation of metabolic homeostasis, detoxification, and systemic inflammatory responses. Experimental and clinical evidence indicates that liver injury of various etiologies is accompanied by excessive production of reactive oxygen and nitrogen species (ROS / RNS) in hepatic tissue, leading to oxidative-nitrosative stress, mitochondrial dysfunction, and hepatocellular damage [4]. Activation of pro-oxidant enzymatic systems, including mitochondrial electron transport chain components, NADPH oxidases, and inducible nitric oxide synthase (NOS), together with impaired antioxidant defenses, promote lipid peroxidation, protein nitration, and disruption of hepatic redox homeostasis [5].

Polyphenolic compounds have attracted considerable attention as potential modulators of stress-induced oxidative and inflammatory pathways due to their multitarget biological activity and favorable safety profiles. Quercetin, a widely studied flavonoid, exhibits potent antioxidant and anti-inflammatory properties, including direct scavenging of free radicals, inhibition of pro-inflammatory signaling, and modulation of nitric oxide synthase activity [6]. Resveratrol, a stilbene polyphenol, is known to activate Sirtuin 1 (SIRT1)- and AMP-activated protein kinase (AMPK)-dependent signaling pathways, inhibit activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), improve mitochondrial function, and enhance endogenous antioxidant defense systems [7]. Importantly, quercetin and resveratrol act through partially distinct yet complementary mechanisms, suggesting that their combined administration may confer synergistic protection against oxidative-nitrosative injury.

Despite extensive evidence supporting the individual hepatoprotective effects of quercetin and resveratrol, data on their combined influence under conditions of severe systemic stress remain limited. In particular, the effects of combined quercetin and resveratrol administration on hepatic oxidative-nitrosative stress following surgical trauma in the setting of prolonged stress have not been sufficiently explored. Understanding whether this combination can more effectively attenuate ROS / RNS overproduction and restore hepatic redox balance is of significant relevance for the development of pathogenetically grounded strategies aimed at protecting the liver under stress-associated surgical conditions.

Aim

The aim of the present study was to investigate the effects of combined quercetin and resveratrol administration on markers of oxidative and nitrosative stress in the liver of rats subjected to surgical trauma following prolonged stress exposure.

Materials and methods

Experimental design. The study was conducted on 35 adult male Wistar rats weighing 210–230 g, randomly allocated into five experimental groups (n = 7 per group):

– Group 1 (Control) – intact animals without stress exposure or surgical intervention;

– Group 2 (SPS + Laparotomy) – rats subjected to single prolonged stress (SPS) followed by laparotomy;

– Group 3 (SPS + Laparotomy + Quercetin) – animals exposed to SPS and subsequent laparotomy and treated with a water-soluble form of quercetin (Corvitin; PJSC SIC "Borshchahivskiy Chemical-Pharmaceutical Plant", Ukraine) at a dose of 200 mg/kg, corresponding to 20 mg/kg of quercetin [8];

– Group 4 (SPS + Laparotomy + Resveratrol) – rats exposed to SPS and subsequent laparotomy and treated with resveratrol (Shaanxi Jiahe Phytochem Co., China) at a dose of 5 mg/kg body weight [5].

– Group 5 (SPS + Laparotomy + Quercetin + Resveratrol) – animals exposed to SPS and subsequent laparotomy and treated with a combination of water-soluble quercetin (20 mg/kg) and resveratrol (5 mg/kg body weight).

Rats were maintained under standard vivarium conditions, including an ambient temperature of 22 ± 2 °C, relative humidity of 30–60 %, and a 12:12 h light / dark cycle. All animals had unrestricted access to standard laboratory chow and tap water. Every effort was made to minimize animal discomfort and to use the minimum number of animals required to achieve statistical validity.

All experimental procedures were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. The study protocol was reviewed and approved by the Bioethics and Ethics Committee of Poltava State Medical University (Protocol No. 246, dated February 09, 2026).

Beginning 24 hours after surgery, animals received once daily intragastric administration of the test formulations at a dosing volume of 5 mL/kg for seven consecutive days. After completion of the treatment period, rats were anesthetized with thiopental sodium and euthanized in accordance with institutional ethical standards. Liver tissue was rapidly excised, and a 10 % tissue homogenate was prepared by homogenizing samples in ice-cold 0.1 M Tris-HCl buffer (pH 7.4) at a 1:10 weight-to-volume ratio. All procedures were performed under cold conditions to preserve enzymatic activity and redox-sensitive parameters.

SPS was induced according to a validated protocol. Briefly, rats were immobilized in a dorsal position on a metal platform using surgical tape restraints for 2 hours. This procedure was followed by a forced swim test in a Plexiglas cylinder filled with water maintained at 24 °C. Subsequently, animals were exposed to sevoflurane vapor (Sevoran, AbbVie S.r.l., Italy) until loss of consciousness. After completion of the SPS procedure, rats were housed in pairs under standard conditions and left undisturbed for seven days. This experimental paradigm is widely recognized as a reliable model of post-traumatic stress disorder, reproducing key behavioral, neuroendocrine, and physiological features observed in humans [9].

Experimental surgical trauma was induced by midline laparotomy under intraperitoneal thiopental sodium anesthesia (50 mg/kg; Kyivmedpreparat, Ukraine). After antiseptic preparation of the abdominal skin, a 1-cm midline incision was performed, followed by blunt dissection through the muscular and peritoneal layers. A segment of the small intestine was gently exteriorized and manually palpated for 10 seconds to simulate surgical manipulation, after which it was repositioned into the abdominal cavity. The incision was closed in anatomical layers using polyglycolide suture material and an atraumatic needle (Biopolymer, Ukraine), as previously described [3].

Production of the superoxide anion radical (O_2•⁻) in hepatic homogenates was assessed spectrophotometrically using a ULAB 101 spectrophotometer (China), based on the reduction of nitroblue tetrazolium (NBT; IUPAC: 2,2'-bis(4-nitrophenyl)-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-diphenylene) ditetrazolium chloride; Sigma-Aldrich, USA) to diformazan. Experimental conditions were optimized to selectively evaluate the contribution of distinct cellular sources of reactive oxygen species [10]. Mitochondrial electron transport chain-dependent superoxide generation was assessed using β-nicotinamide adenine dinucleotide, reduced disodium salt hydrate (NADH, ≥97 %; Sigma-Aldrich, USA) as an electron donor. To evaluate superoxide production associated with the endoplasmic reticulum and NOS activity, β-nicotinamide adenine dinucleotide phosphate, reduced tetrasodium salt hydrate (NADPH, ≥97 %; Sigma-Aldrich, USA) was used. Lipopolysaccharide from *Salmonella typhi* (Sigma-Aldrich, USA) served as an inducer of phagocytic NADPH oxidase activity (EC 1.6.3.1).

Total NOS activity was determined by measuring nitric oxide (NO) generation following a 30-minute incubation of 10 % hepatic homogenates in a reaction medium containing 0.1 M Tris-HCl buffer (pH 7.4), L-arginine (320 mM; Sigma-Aldrich, USA), and NADPH (1 mM) [10]. Constitutive NOS (cNOS) activity was assessed in parallel samples supplemented with aminoguanidine hydrochloride (1 % w/v; 98 %; Sigma-Aldrich, USA), a selective inhibitor of inducible NOS (iNOS), according to established protocols. Inducible NOS (iNOS) activity was calculated as the difference between total NOS and cNOS activities. Protein concentrations were measured using the Biuret method and used to normalize enzyme activities. NOS coupling efficiency was evaluated by calculating the cNOS coupling index, defined as the ratio of cNOS activity to NADPH-dependent superoxide production rate, reflecting the balance between nitric oxide and superoxide generation by NOS isoforms.

Peroxynitrite concentrations, expressed as peroxynitrites of alkali and alkaline-earth metals, were determined spectrophotometrically based on their oxidative reaction with potassium iodide (≥99.0 %; Sigma-Aldrich, USA) in 0.2 M phosphate buffer (pH 7.0), according to a standardized method [10].

Statistical analyses were performed using Microsoft Excel with the Real Statistics 2019 add-in. Data normality was assessed using the Shapiro-Wilk test. Results are presented as the mean ± standard error of the mean (SEM). For datasets with a normal distribution, group comparisons were conducted using one-way analysis of variance (ANOVA), followed by pairwise comparisons with Student's t-test for independent samples and Tukey's honestly significant difference (HSD) post hoc test. To control for type I error associated with multiple comparisons, the Dunn–Šidák correction was applied. Differences were considered statistically significant at $p < 0.05$.

Results

Combined exposure to SPS and surgical trauma led to a pronounced increase in O_2•⁻ generation in rat liver tissue, reflecting robust activation of multiple oxidative pathways *(Table 1)*. In Group 2, O_2•⁻ production associated with endoplasmic reticulum-linked systems and NOS was elevated by 69.7 % compared with intact controls ($p < 0.001$). Mitochondrial electron transport chain-derived O_2•⁻ formation increased by 72.3 % ($p < 0.001$), while activity of phagocytic NADPH oxidase nearly doubled, rising by 97.6 % relative to control values ($p < 0.001$). Collectively, these results indicate extensive activation of both intracellular and membrane-bound ROS-generating systems in response to the combined psychological and surgical stress load.

Administration of the water-soluble form of quercetin (Group 3) markedly attenuated stress-induced oxidative alterations in liver tissue. Specifically, O_2•⁻ production associated with endoplasmic reticulum-linked systems and NOS in liver homogenates was reduced by 39.7 % compared with Group 2 values ($p < 0.001$). Mitochondrial electron transport chain-derived O_2•⁻ generation declined by 14.0 % ($p < 0.001$), approaching control levels. Quercetin treatment was found to significantly suppress the phagocytic NADPH oxidase-dependent component of O_2•⁻ production by 22.2 % relative to the untreated stress group ($p < 0.001$).

Resveratrol administration (Group 4) also exerted a pronounced antioxidant effect, although of slightly lower magnitude. In this group, O_2•⁻ generation from endoplasmic reticulum-associated systems and NOS decreased by 34.5 % compared with Group 2 ($p < 0.001$), while mitochondrial electron transport chain-dependent O_2•⁻ production was reduced by 11.3 % ($p < 0.001$). Furthermore, resveratrol significantly attenuated phagocytic NADPH oxidase-mediated superoxide O_2•⁻ formation by 17.0 % relative to the untreated SPS + surgery group ($p < 0.001$).

Table 1. Effect of polyphenols on the superoxide anion radical production (nmol/s per g) in the liver of rats exposed to single prolonged stress and subsequent laparotomy, M ± SEM

Sources of superoxide anion radical generation	Control rats, n = 7	SPS + subsequent laparotomy, n = 7	Administration of polyphenols after induction of SPS and laparotomy		
			Water-soluble form of quercetin, n = 7	Resveratrol, n = 7	Water-soluble form of quercetin + resveratrol, n = 7
Endoplasmic reticulum and NOS	17.59 ± 0.33	29.85 ± 0.28*	17.99 ± 1.01**	19.56 ± 0.52*,**	18.16 ± 0.28**,#
Mitochondria	19.58 ± 0.41	33.74 ± 0.20*	29.02 ± 1.07*,**	29.92 ± 0.56*,**	19.96 ± 0.42**,&,#
Phagocytic NADPH oxidase	2.05 ± 0.04	4.05 ± 0.04*	3.15 ± 0.18*,**	3.36 ± 0.07*,**	2.21 ± 0.04*,**,&,#

:** $p < 0.05$ compared to findings in the control group; *:** $p < 0.05$ compared to findings in the rats of Group 2; **&:** $p < 0.05$ compared to findings in the rats of Group 3; **#:** $p < 0.05$ compared to findings in the rats of Group 4.

Administration of the combined polyphenol regimen comprising water-soluble quercetin and resveratrol (Group 5) resulted in the most pronounced suppression of stress-induced $O_2{\bullet}^-$ overproduction in hepatic tissue. Specifically, $O_2{\bullet}^-$ generation associated with endoplasmic reticulum-linked redox systems and NOS was reduced by 39.2 % compared with the SPS + laparotomy group (Group 2) and by 24.2 % relative to resveratrol monotherapy (Group 4) (both $p < 0.001$), with values no longer differing significantly from those of intact controls.

Mitochondrial electron transport chain-derived $O_2{\bullet}^-$ production was nearly completely normalized following the combined treatment. This parameter did not differ significantly from control values and was markedly lower than those observed after quercetin or resveratrol monotherapy, by 31.2 % and 33.3 %, respectively (both $p < 0.001$), indicating a superior efficacy of the combined intervention in restoring mitochondrial redox homeostasis.

Similarly, phagocytic NADPH oxidase-dependent $O_2{\bullet}^-$ generation was markedly suppressed following combined polyphenol administration. The resulting values closely approximated those of the control group and were 45.4 % lower than in the SPS + laparotomy group, as well as 29.8 % and 34.2 % lower than in the quercetin- and resveratrol-treated groups, respectively (all $p < 0.001$).

Overall, the combined administration of water-soluble quercetin and resveratrol demonstrated a synergistic antioxidant effect, providing more effective suppression of superoxide production from multiple cellular sources than either compound alone. These findings indicate enhanced protection against oxidative stress in hepatic tissue under conditions of combined psychological and surgical stress.

The combined application of SPS and surgical injury led to marked activation of NOS pathways in rat liver tissue, consistent with the development of pronounced nitrosative stress *(Table 2)*. In Group 2, total NOS activity was elevated by 127 % compared to control animals ($p < 0.001$), indicating a substantial increase in overall NO generation. This effect was primarily driven by a robust induction of iNOS, whose activity exceeded control values by 175 % ($p < 0.001$). Conversely, cNOS activity was profoundly reduced, showing a 77.8 % decrease compared to controls ($p < 0.001$).

As a result of these opposing alterations, the cNOS coupling index, an indicator of efficient electron transfer and physiological NO synthesis, decreased 6.8-fold compared with control values ($p < 0.001$), reflecting a pronounced shift toward NOS uncoupling and pro-oxidant enzyme behavior. In parallel, hepatic levels of peroxynitrites of alkali and alkaline-earth metals, which represent stable end products of $O_2{\bullet}^-$–NO interactions, increased by 65.4 % compared with control values ($p < 0.001$), providing further evidence of excessive ROS formation under conditions of combined psychological and surgical stress.

Administration of the water-soluble form of quercetin (Group 3) markedly alleviated SPS- and surgery-induced disturbances in hepatic NO metabolism. Total NOS activity declined by 40.5 % compared with Group 2 ($p < 0.001$), primarily due to a pronounced reduction in iNOS activity (-43.4 %, $p < 0.001$) accompanied by a partial restoration of cNOS activity (+118 %, $p < 0.02$). Consistent with these changes, the cNOS coupling index increased by 233 % relative to untreated stressed animals ($p < 0.001$), indicating substantial restoration of NOS coupling and redox efficiency. In parallel, hepatic peroxynitrite levels decreased by 18.0 % compared to Group 2 ($p < 0.001$), reflecting attenuation of nitrosative stress and improved redox homeostasis in liver tissue.

A comparable protective pattern was observed following resveratrol administration (Group 4). Total NOS activity was reduced by 43.4 % relative to Group 2 ($p < 0.001$), driven by significant suppression of iNOS activity (-46.5 %, $p < 0.001$) and partial recovery of cNOS function (+118 %, $p < 0.05$). The cNOS coupling index increased 3-fold compared with untreated SPS-exposed rats ($p < 0.01$), indicating improved enzymatic coupling and NO bioavailability. Concurrently, peroxynitrite content in hepatic tissue declined by 17.7 % versus Group 2 ($p < 0.001$), confirming effective mitigation of nitrosative stress.

Administration of the combined polyphenol treatment consisting of water-soluble quercetin and resveratrol (Group 5) produced the most pronounced normalization of NO metabolism in hepatic tissue following SPS and laparotomy *(Table 2)*. Total NOS activity in this group did not differ significantly from control values and was 54.7 % lower than in untreated SPS-exposed rats ($p < 0.001$). Importantly, total NOS activity was also significantly reduced compared with animals receiving either quercetin or resveratrol alone by 23.9 % ($p < 0.01$) and 19.9 % ($p < 0.02$), respectively, indicating a superior regulatory effect of the combined intervention.

Table 2. Effect of polyphenols on the reactive nitrogen species formation in the liver of rats exposed to single prolonged stress and subsequent laparotomy (M ± SEM)

Parameter, units of measurement	Control rats, n = 7	SPS + subsequent laparotomy, n = 7	Administration of polyphenols after induction of SPS and laparotomy		
			Water-soluble form of quercetin, n = 7	Resveratrol, n = 7	Water-soluble form of quercetin + resveratrol, n = 7
Total NOS activity, µmol (NO_{2-}) / min per g of protein	3.82 ± 0.28	8.70 ± 0.23*	5.18 ± 0.21*,**	4.92 ± 0.22*,**	3.94 ± 0.27**,&,#
cNOS activity, µmol (NO_{2-}) / min per g of protein	0.72 ± 0.06	0.16 ± 0.04*	0.35 ± 0.05*,**	0.35 ± 0.06*,**	0.73 ± 0.15**,&,#
iNOS activity, µmol (NO_{2-}) / min per g of protein	3.10 ± 0.24	8.54 ± 0.20*	4.83 ± 0.19*,**	4.57 ± 0.21*,**	3.21 ± 0.16**,&,#
cNOS coupling index	0.041 ± 0.003	0.006 ± 0.001*	0.020 ± 0.003*,**	0.018 ± 0.003*,**	0.040 ± 0.008**,&,#
Peroxynitrites concentration, µmol/g	1.91 ± 0.06	3.16 ± 0.04*	2.59 ± 0.06*,**	2.60 ± 0.06*,**	1.96 ± 0.04**,&,#

:** p < 0.05 compared to findings in the control group; *:** p < 0.05 compared to findings in the rats of Group 2; **&:** p < 0.05 compared to findings in the rats of Group 3; **#:** p < 0.05 compared to findings in the rats of Group 4.

This normalization was accompanied by a complete restoration of cNOS activity, which was statistically indistinguishable from control levels and 108 % higher than the values observed in both monotherapy groups (p < 0.05). In parallel, iNOS activity was markedly suppressed, reaching levels close to those in control rats and remaining significantly lower than in Groups 3 and 4 by 33.5 % and 29.8 %, respectively (both p < 0.001).

As a consequence of these coordinated effects, the cNOS coupling index was fully restored, matching control values and significantly exceeding those observed following individual polyphenol administration (p < 0.001), indicating effective restoration of NOS coupling and physiological NO synthesis.

Consistent with the improvement in redox homeostasis, hepatic peroxynitrite concentrations in Group 5 declined to levels comparable with control rats and were 38.0 % lower than in the SPS + laparotomy group (p < 0.001). Moreover, peroxynitrite levels were significantly reduced compared with quercetin or resveratrol monotherapy by 24.3 % and 24.6 %, respectively (both p < 0.001).

Collectively, these findings demonstrate that combined quercetin and resveratrol administration confers superior protection against nitrosative stress, effectively restoring NOS homeostasis and limiting reactive nitrogen species accumulation in the liver under conditions of prolonged psychological stress and surgical trauma.

Discussion

The present study demonstrates that the combination of prolonged psychological stress and surgical trauma induces a profound oxidative-nitrosative imbalance in rat liver tissue, characterized by excessive ROS / RNS generation, mitochondrial dysfunction, and marked dysregulation of NOS activity. These findings reinforce the concept that PTSD-like stress states, when superimposed on physical injury, act as potent amplifiers of systemic redox pathology, extending far beyond the central nervous system and significantly affecting metabolically active peripheral organs such as the liver [11,12].

Our results show that SPS followed by laparotomy markedly activates multiple hepatic sources of $O_2{\bullet^-}$ production, including mitochondrial electron transport chain components, endoplasmic reticulum-associated redox systems, NOS, and phagocytic NADPH oxidase. This observation is consistent with our previous findings showing that psychological stress and surgical injury promote mitochondrial ROS leakage and activate NADPH oxidase, whereas their combination elicits a synergistic response that results in sustained oxidative stress in rat cardiac tissue [13]. Excessive ROS generation at the mitochondrial level is particularly detrimental, as it disrupts ATP synthesis, promotes mitochondrial permeability transition, and sensitizes hepatocytes to inflammatory damage [14].

In parallel with oxidative stress, the present study revealed pronounced activation of nitrosative pathways, evidenced by a strong induction of inducible NOS, suppression of constitutive NOS activity, severe uncoupling of NOS isoforms, and accumulation of peroxynitrites. Such a shift toward iNOS-dominant NO production and NOS uncoupling is a recognized hallmark of stress- and inflammation-driven liver injury [15]. Peroxynitrite formation, resulting from the rapid reaction between NO and $O_2{\bullet^-}$, further exacerbates cellular damage through protein nitration, lipid peroxidation, and inactivation of mitochondrial enzymes [16]. The observed reduction in the cNOS coupling index in stressed and surgically injured rats indicates a transition of NOS from a physiological signaling enzyme to a pro-oxidant source, thereby reinforcing the oxidative-nitrosative vicious cycle.

Administration of water-soluble quercetin or resveratrol alone significantly attenuated both oxidative and nitrosative stress markers, confirming their well-documented hepatoprotective properties. Quercetin reduced $O_2{\bullet^-}$ production from multiple intracellular sources and partially restored NOS coupling, likely through suppression of pro-inflammatory signaling and direct radical-scavenging activity [6,8]. Resveratrol exerted comparable effects, which may be attributed to its ability to enhance mitochondrial function, activate AMP-activated protein kinase and sirtuin-1 pathways, and upregulate endogenous antioxidant defenses [7]. These findings are in line with previous studies demonstrating that each compound independently mitigates oxidative stress and improves NO bioavailability in models of metabolic and inflammatory liver injury [5,17,18].

Importantly, the combined administration of quercetin and resveratrol exerted the most pronounced hepatoprotective effect, clearly exceeding the efficacy of either polyphenol used alone. This combined intervention almost completely normalized mitochondrial $O_2{•}^-$ generation, fully restored cNOS activity and its coupling, and reduced hepatic peroxynitrite accumulation to levels indistinguishable from those of intact animals. Such a pattern indicates a synergistic rather than merely additive interaction between quercetin and resveratrol in limiting oxidative-nitrosative stress under conditions of prolonged psychological stress and surgical trauma.

The enhanced efficacy of the combined treatment may be explained by the synergistic actions of the two polyphenols. Quercetin predominantly suppresses pro-oxidant and pro-inflammatory pathways and directly scavenges reactive oxygen species, whereas resveratrol improves mitochondrial function, optimizes redox signaling, and stimulates endogenous antioxidant defenses [6,7]. Acting in concert, these mechanisms more effectively interrupt the self-amplifying ROS / RNS cascade and promote stabilization of hepatic redox homeostasis following stress-induced injury.

Consistent with this interpretation, previous experimental studies in metabolic and inflammatory disease models have demonstrated that combined quercetin and resveratrol therapy provides superior protection against oxidative damage, metabolic dysregulation, and tissue injury compared with monotherapy. In experimental models of diabetes, cotreatment with quercetin and resveratrol more effectively reduced hyperglycemia, dyslipidemia, oxidative stress, and tissue injury while preserving hepatic metabolic enzyme activity and pancreatic β-cell integrity, highlighting a clear synergistic interaction between these polyphenols [19]. Together with our findings, these data support the concept that combined polyphenol administration represents a rational and pathogenetically grounded strategy for mitigating hepatic oxidative-nitrosative stress associated with severe psychogenic stress and surgical trauma.

The observed normalization of NOS coupling under combined treatment is of particular importance. Restoration of cNOS function not only limits $O_2{•}^-$ generation from uncoupled cNOS but also preserves physiological NO signaling, which is essential for hepatic microcirculation, mitochondrial respiration, and cytoprotection [20]. By simultaneously suppressing iNOS overactivation and restoring cNOS-derived NO synthesis, the quercetin-resveratrol combination effectively shifts NO metabolism from a cytotoxic to a regulatory profile.

From a translational perspective, these findings are highly relevant. Liver dysfunction is a common complication in patients experiencing severe psychological stress and trauma, including those undergoing surgery after combat-related or civilian injuries. The present results suggest that combined polyphenol-based interventions may offer a promising adjunctive strategy for limiting stress-associated hepatic injury by targeting both oxidative and nitrosative pathways. Importantly, the use of a water-soluble quercetin formulation likely enhanced bioavailability and tissue distribution, further contributing to the observed efficacy [21].

Several limitations should be acknowledged. The study did not directly assess molecular signaling pathways such as NF-κB, Nrf2, SIRT1, or AMPK, which are known targets of quercetin and resveratrol. Therefore, mechanistic interpretations regarding transcriptional regulation remain inferential and based on established literature. In addition, only a single dose and treatment duration were evaluated; future studies should explore dose-response relationships and longer-term outcomes. Nevertheless, the consistent normalization of multiple independent redox and NOS-related parameters strongly supports the robustness of the observed protective effects.

Conclusions

Combined administration of quercetin and resveratrol provides superior protection compared with either compound alone, leading to near-complete normalization of mitochondrial $O_2{•}^-$ production, full restoration of cNOS activity and coupling, and marked reduction of peroxynitrite accumulation. These findings indicate a synergistic interaction between the two polyphenols in restoring hepatic redox and nitric oxide homeostasis.

Prospects for further research. Future studies should focus on direct molecular validation of the signaling pathways involved (e. g., NF-κB, Nrf2, SIRT1, and AMPK), evaluation of dose-response relationships and long-term outcomes, and assessment of functional hepatic parameters. Investigating the effects of combined quercetin and resveratrol therapy in clinically relevant models and exploring its translational potential in stress- and trauma-associated liver dysfunction are also warranted.

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References

1. Karatzias T, Shevlin M, Ben-Ezra M, McElroy E, Redican E, Vang ML, et al. War exposure, posttraumatic stress disorder, and complex posttraumatic stress disorder among parents living in Ukraine during the Russian war. Acta Psychiatr Scand. 2023;147(3):276-85. doi: 10.1111/acps.13529
2. Boiko DI, Shyrai PO, Mats OV, Karpik ZI, Rahman MH, Khan AA, et al. Mental health and sleep disturbances among Ukrainian refugees in the context of Russian-Ukrainian war: A preliminary result from online-survey. Sleep Med. 2024;113:342-8. doi: 10.1016/j.sleep.2023.12.004
3. Taran OV, Solovyova NV, Kostenko VO. [Effect of laparatomy and lipopolysaccharide-induced systemic inflammatory response on metabolic disorders in rats]. Fiziologichnyi Zhurnal. 2022;68(3):35-43. Ukrainian. doi: 10.15407/fz68.03.035
4. Mooli R, Mukhi D, Ramakrishnan SK. Oxidative Stress and Redox Signaling in the Pathophysiology of Liver Diseases. Compr Physiol. 2022;12(2):3167-92. doi: 10.1002/cphy.c200021
5. Frenkel Y, Cherno V, Kostenko H, Kostenko V. Resveratrol attenuates the development of nitro-oxidative stress in the liver of rats under a round-the-clock lighting and high-carbohydrate-lipid diet. Romanian Journal of Diabetes, Nutrition and Metabolic Diseases. 2023;30(1):48-54. Available from: https://www.rjdnmd.org/index.php/RJDNMD/article/view/1217/780
6. Boo HJ, Yoon D, Choi Y, Kim Y, Cha JS, Yoo J. Quercetin: Molecular Insights into Its Biological Roles. Biomolecules. 2025;15(3):313. doi: 10.3390/biom15030313
7. Ren ZQ, Zheng SY, Sun Z, Luo Y, Wang YT, Yi P, et al. Resveratrol: Molecular Mechanisms, Health Benefits, and Potential Adverse Effects. MedComm (2020). 2025;6(6):e70252. doi: 10.1002/mco2.70252
8. Frenkel YD, Zyuzin VO, Cherno VS, Kostenko VO. [Effect of epigallocatechin-3-gallate and quercetin on the production of reactive oxygen and nitrogen species in liver of rats exposed to round-the-clock light and kept on carbohydratelipid diet]. Fiziologichnyi Zhurnal. 2022;68(1):20-7. Ukrainian. doi: 10.15407/fz68.01.020
9. Smail MA, Cotella EM, Martelle SE, Chambers JB, Parikh RK, Moore CE, et al. Regulation of behavioral responses to single prolonged stress in male and female rats: Role of PACAP. Neurobiol Stress. 2025;36:100727. doi: 10.1016/j.ynstr.2025.100727
10. Akimov OY, Kostenko VO. Oksydatyvno-nitrozatyvnyi stres ta metody yoho doslidzhennia [Oxidative-nitrosative stress and methods of its research]. Lviv: Mahnoliia; 2025. Ukrainian.
11. Chorniy S, Denefil O, Miroshnyk V. Features of liver structural organization in posttraumatic stress disorder. Journal of Education, Health and Sport. 2024;70:55689. doi: 10.12775/JEHS.2024.70.55689
12. Lawrence S, Scofield RH. Post traumatic stress disorder associated hypothalamic-pituitary-adrenal axis dysregulation and physical illness. Brain Behav Immun Health. 2024;41:100849. doi: 10.1016/j.bbih.2024.100849
13. Ryabushko RM, Kostenko VO. [Effects of NF-κB and Nrf2 modulators on reactive oxygen and nitrogen species production in rat heart following surgical trauma under prolonged stress]. Fiziologichnyi Zhurnal. 2025;71(2):51-7. Ukrainian. doi: 10.15407/fz71.02.051
14. Jaeschke H, Ramachandran A. Central Mechanisms of Acetaminophen Hepatotoxicity: Mitochondrial Dysfunction by Protein Adducts and Oxidant Stress. Drug Metab Dispos. 2024;52(8):712-21. doi: 10.1124/dmd.123.001279
15. Pérez-Torres I, Manzano-Pech L, Rubio-Ruíz ME, Soto ME, Guarner-Lans V. Nitrosative Stress and Its Association with Cardiometabolic Disorders. Molecules. 2020;25(11):2555. doi: 10.3390/molecules25112555
16. Prolo C, Piacenza L, Radi R. Peroxynitrite: a multifaceted oxidizing and nitrating metabolite. Curr Opin Chem Biol. 2024;80:102459. doi: 10.1016/j.cbpa.2024.102459
17. Opryshko V, Prokhach A, Akimov O, Riabushko M, Kostenko H, Kostenko V, et al. Desmodium styracifolium: Botanical and ethnopharmacological insights, phytochemical investigations, and prospects in pharmacology and pharmacotherapy. Heliyon. 2024;10(3):e25058. doi: 10.1016/j.heliyon.2024.e25058
18. Jin D, Jin S, Zhou T, Sheng G, Gao P, Li G. Effects of Quercetin on Metabolic Dysfunction-Associated Steatotic Liver Disease: A Systematic Review and Meta-Analysis. Food Sci Nutr. 2025;13(12):e71358. doi: 10.1002/fsn3.71358
19. Yang DK, Kang HS. Anti-Diabetic Effect of Cotreatment with Quercetin and Resveratrol in Streptozotocin-Induced Diabetic Rats. Biomol Ther (Seoul). 2018;26(2):130-8. doi: 10.4062/biomolther.2017.254
20. Andrabi SM, Sharma NS, Karan A, Shahriar S, Cordon B, Ma B, et al. Nitric Oxide: Physiological Functions, Delivery, and Biomedical Applications. Adv Sci (Weinh). 2023;10(30):e2303259. doi: 10.1002/advs.202303259
21. Liu L, Barber E, Kellow NJ, Williamson G. Improving quercetin bioavailability: A systematic review and meta-analysis of human intervention studies. Food Chem. 2025;477:143630. doi: 10.1016/j.foodchem.2025.143630