# Project summary

Exposure to chemical substances can lead to permanent or transient morphological and functional damage of organs/tissues/cells in living organisms. No matter specific properties (toxic substances, medicaments, drug of abuse, etc.), chemical agents produce effect(s) in an organism through different pathways.

Thus, many agents achieve their toxicity through oxidative/nitrosative stress (OS/NS) development, leading to either increased formation of free radicals or their inadequate sequestration or reduced reparation of already oxidatively/nitrosatively damaged biomolecules due to reduced/ inhibited antioxidant enzymatic activities. Deteriorated cell morphology, functionality and signalization leads to modified morphology and dysfunctionality of higher anatomical organized units, tissues and organs.

One of the objectives of this project was to test the effects of ethanol, disulfiram (DSF) drug used for aversive therapy of alcoholism and cadmium (Cd) on the liver and reproductive organs on the animal model. The oxidative metabolism of ethanol results in an increase of nicotine adenine dinucleotide (NADH) and nicotine adenine dinucleotide phosphate (NADHP); being transformed into dithiocarbamates metabolites (containing sulfhydryl groups strong nucleophilic properties) DSF has been recognized as a potential reducing and chelating agent, also; while Cd indirectly induces OS by inhibition of many metalloenzymes including antioxidant enzymes (by ion exchanging reactions, with zinc in particular).

An additional goal was to analytically solve the determination of glutathione in its reduced and oxidized forms, in tissue homogenates. The spectrophotometric measurement of these compounds is nonpecific and unreliable. Especially, this is the case when biological matrix contains agents with interfering sulfhydryl groups, as was have in our experimental study.

An animal study was designed to mimic alcoholics and smokers (an increased health risk has been documented for smokers due to Cd exposure from cigarettes) on DSF therapy (implies the absence of alcohol intake). For this kind of testing we used male Wistar rats, randomly divided them in several groups and exposed to ethanol and DSF (by gavage) and Cd (intraperitoneally), individually or in combination, acutely, subacutely and subchronically. It has been shown that all tested agents disturb redox homeostasis of the examined organs. These events are drastic and persistent in Cd exposure (pronounced reproductive and hepatotoxicity induced by inhibition of antioxidant enzymes), whereas post-parallel administration of DSF cannot repair or alleviate already completed damages. Ethanol induces much more less reproductive and hepato- toxicity than Cd. Histopathological cross-sections of these tissues ascertained no significant changes, as well as the case with exposure to DSF. The glutathione cycle is significantly preserved by DSF, which is in favor of its antioxidant potential. Also, functional disorder of these organs was not going to happened by DSF, approving its safe use.

We have established a simple and reproductive chromatographic analytical method (HPLC-UV) for simultaneous measurement of the reduced and oxidized glutathione in tissue homogenates of the examined organs.

Keywords**:** oxidative stress, bioelements, disulfiram, alcohol, cadmium, testes, reproductive toxicity, liver, hepatotoxicity, rats

# Sažetak projekta

Izloženost organizma brojnim agensima može dovesti do trajnih ili prolaznih morfoloških i funkcionalnih oštećenja orgna/tkiva/ćelija. Bez obzira na specifična svojstva (otrovi, lekovi, substance zloupotrebe, i dr.), hemijski agensi ostvaruju efekete posredstvom brojnih mehanizama. Tako, brojni agensi ostvaruju svoju toksičnost posredstvom oksidativnog/nitrozativnog stresa (OS/NS), dovodeći ili do povećanog stvaranja slobodnih radikala, njihovog neadkevatnog uklanjanja ili reparacije oksidativno/nitrozativno oštećenih biomolekula usled redukovane/inhibirane aktivnosti  antioksidatvnih i reparativnih enzima. Narušen morfološko-funkcionalni integritet ćelije i ćelijska signalizacija narušavaju morfologiju i funkciju viših organizacionih nivoa, tkiva i organe.

Jedan od ciljeva ovog projekta je bio da testiramo efekte etanola,  leka disulfirama (DSF) koji se koristi u averzivnoj terapji alkoholizma i kadmijuma (Cd)  na jetru i reproduktvine organe na animalnom modelu. Oksidativni metabolizam etanola ima za posledicu povećan sadržaj nikotin adenin dinukleotida (NADH) i nikotin adenin dinukleotid fosfata (NADHP); transformišući se u ditiokarbamatne metabolite (koji sadrže sulfhidrilne grupe - jak nukleofilni agens) DSF je prepoznat kao mogući redukcioni ali i helatni agens; dok Cd na inidirektan način (jonskom izmenom sa cinkom) inhibira dejstvo mnogih metaloenzima, uključujući i antioksidantne enzime.

Dodatni cilj bio je da se analitički reši merenje glutationa, njegove redukovane i oksidovane forme, u homogenatima tkiva ispitivanih organa. Spektrofotometrijsko merenje ovih jedinjenja je pokazalo veoma veliku nespecifičnost i nepouzdanost. Pogotovo je nepouzdana ova metoda kada se u biloškom matriksu nađu agensi koji sadrže interferirajuće sulfhidlilne grupe, kao što je slučaj u našoj eksperimentalnoj studiji.

Animalna studija je osmišljena na način da imitira preslikava izloženost zavisnika alkoholu i pušenju (postoji povećan rizik po zdravalje pušača usled izloženosti Cd iz cigareta) podvrgnutih terapiji DSF (koja podrazumva odsustvo unosa alkohola). Mužjaci Wistar pacova su u tu svrhu bili podeljeni u više grupa i izlagani indidvidualno ili u kombinaciji akutno, subakutno i subhornično etanolu i DSF (gavažom) i Cd (intraperiotenalno).

Pokazano je da svi testirani agensi remete redoks homeostazu ispitivanih organa. Ovi događaji su drastični i trajni kod izloženosti Cd (izrazita reproduktivna i hepatotoksičnost indukovana inhibicijom antioksidativnih enzima) i post-paralelna primena DSF nema potencijala da popravi niti ublaži ova oštećenja. Etanol dovodi do značajno manje reproduktivne i hepatotoksičnosti. Histopatološki preseci ovih tkiva pokazuju da nema značajnih izmena, kao i kod izloženosti DSF.  Glutationski ciklus je značajno očuvan kod primene DSF, što ide u prilog povećanog antiokdiatvinog potencijala.Takođe, nisu zabeležen ni poremećaj funkcije ovih organa, što je u prilog njegove bezbedne primene.

Postavili smo jednostavnu i reproduktivnu hromatografsku analitičku metodu (HPLC-UV) za simultano merenje redukovane i oksidovane forme glutationa u homogenatima tkiva ispitivanih organa.

Ključne reči**:** oksidativni stres, bioelementi, disulfiram, alkohol, kadmijum, testisi, reproduktivna toksičnost, jetra, hepatotoksičnost, pacovi

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