Development and Validation of In Vitro Safety Assays for Dietary Supplements and Foods

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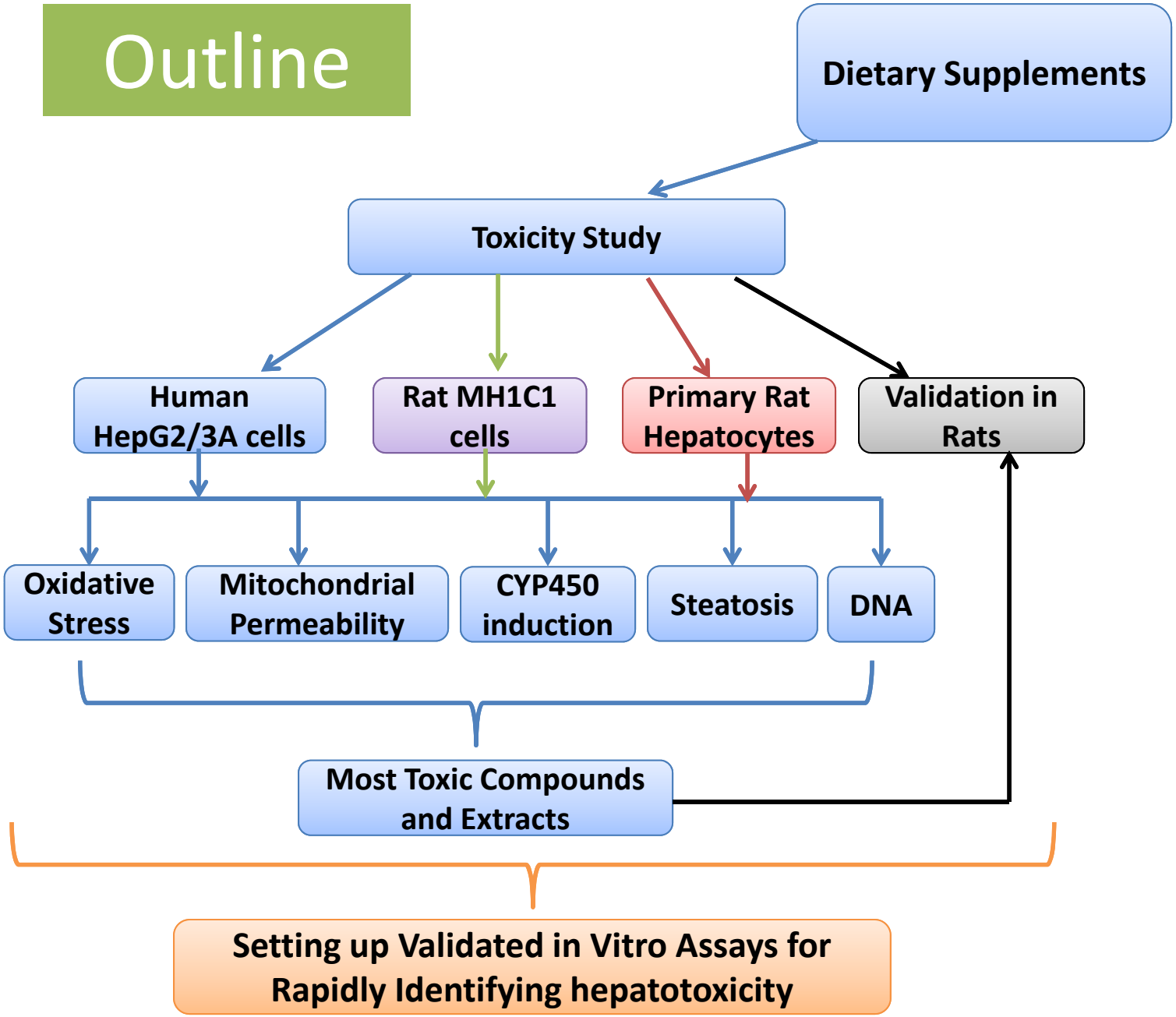
Background

- **Dietary supplement usage is highly prevalent in the United States: 68% of American adults reported use of dietary supplement products.**
- **Most dietary supplements are produced, sold, and consumed without strict regulations.**

Safety Concerns

- **liver injury is one of the most serious safety concerns for drugs, food additives, food contaminants, and dietary supplements.**
- **Potential Food-Drug Interactions mediated by Liver**

Outline

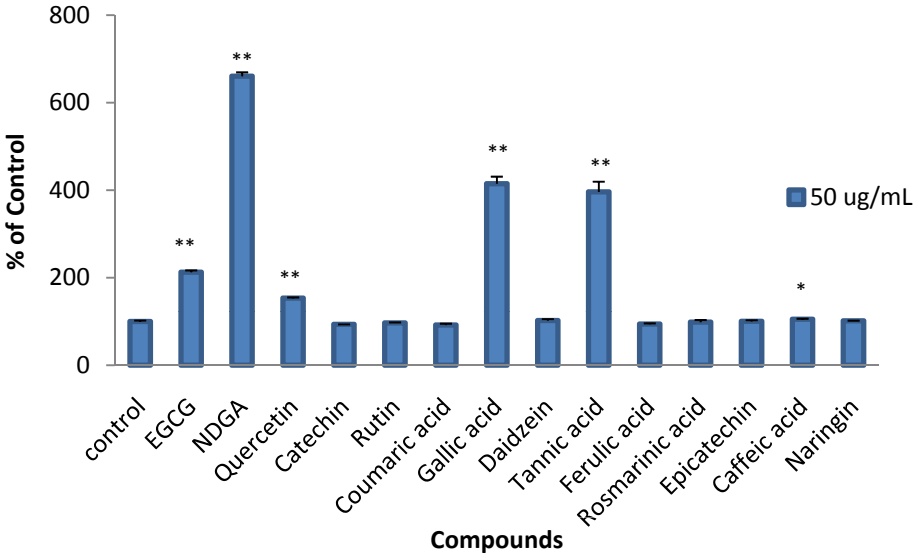

Dietary Supplements
Toxicity Study
Human HepG2/3A cells
Rat MH1C1 cells
Primary Rat Hepatocytes
Validation in Rats
Oxidative Stress
Mitochondrial Permeability
CYP450 induction
Steatosis
DNA
Most Toxic Compounds and Extracts
Setting up Validated in Vitro Assays for Rapidly Identifying hepatotoxicity

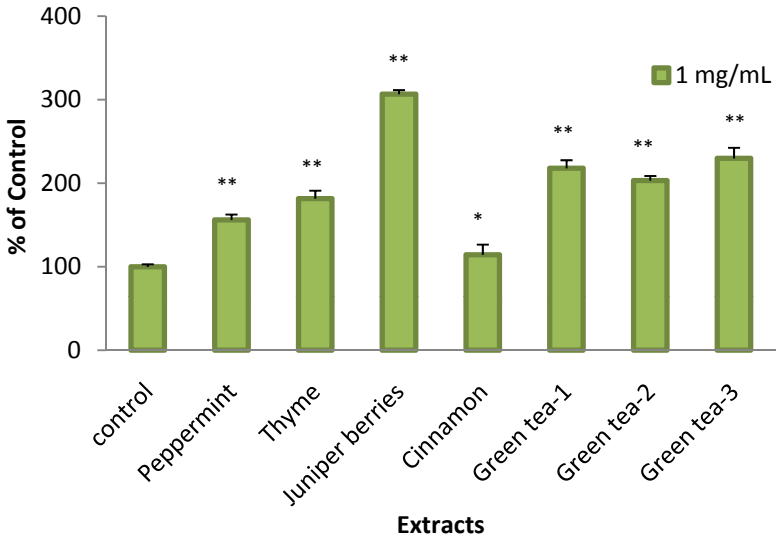
Methods and Materials

Study I

- **Toxicity Assays:** Oxidative stress, Mitochondrial permeability and P-gp regulation, CYP450 1A, 2B/3A induction, Steatosis/phospholipidosis, DNA.
- **Compounds and Extracts tested:** EGCG, NDGA, Quercetin, Catechin, Rutin, *P*-Coumaric acid, Gallic acid, Daidzein, Tannic acid, Ferulic acid, Rosmarinic acid, Epicatechin, Caffeic acid and Naringin; Peppermint leaves, Thyme, Juniper berries, Cinnamon extracts, Green tea extracts.

Study I – Oxidative stress in HepG2/3A cells



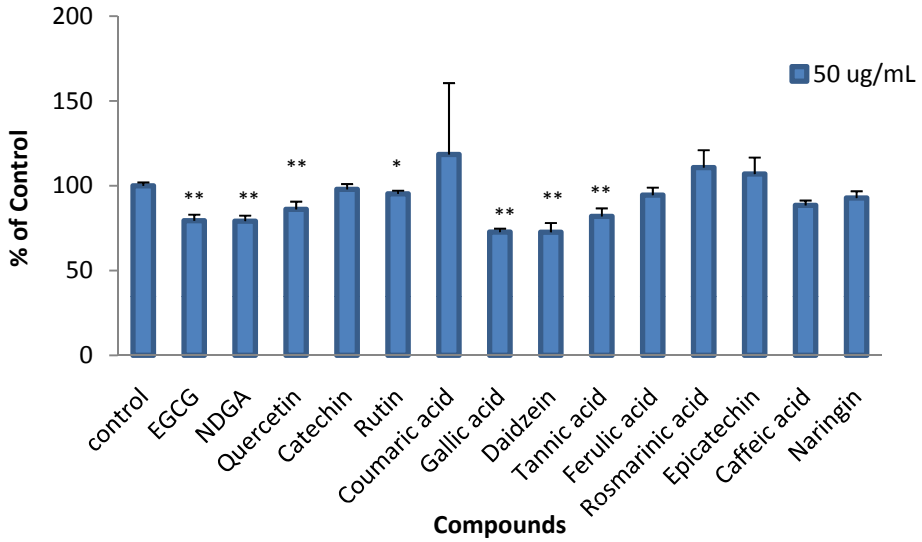


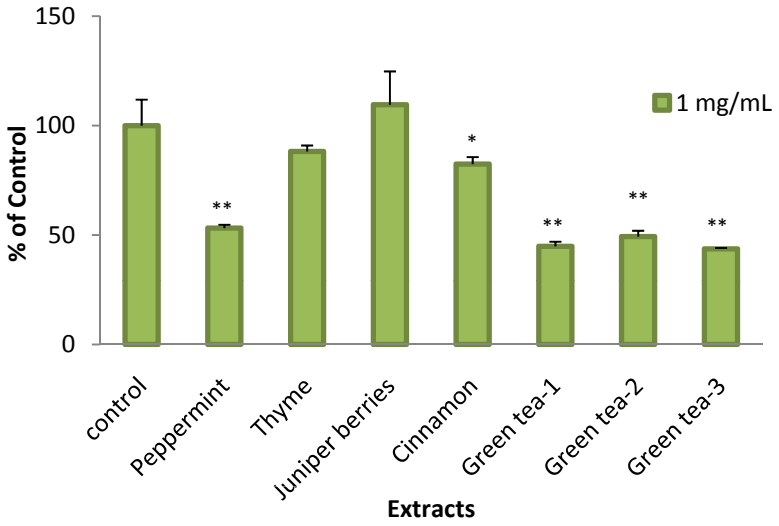
*"**", P<0.01; "*", P<0.05*

EGCG, NDGA, Gallic acid and Tannic acid dramatically (2 - 6 fold) increased oxidative stress at 50 μg/mL, while quercetin increased by ~ 50% compared with the control.

All of the tested extracts exhibited up-regulation effect of oxidative stress on HepG2/3A cells.

Study I – Mitochondrial permeability and/or P-gp regulation



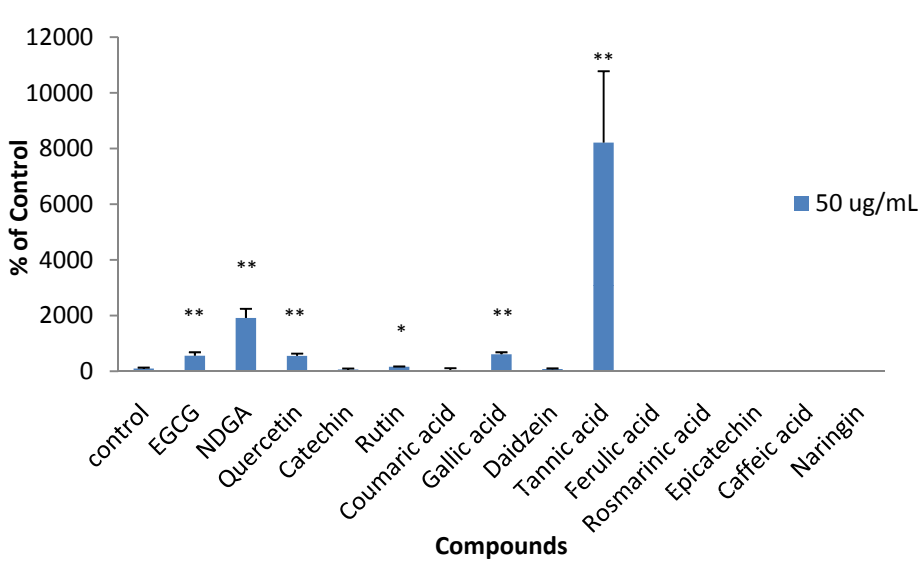


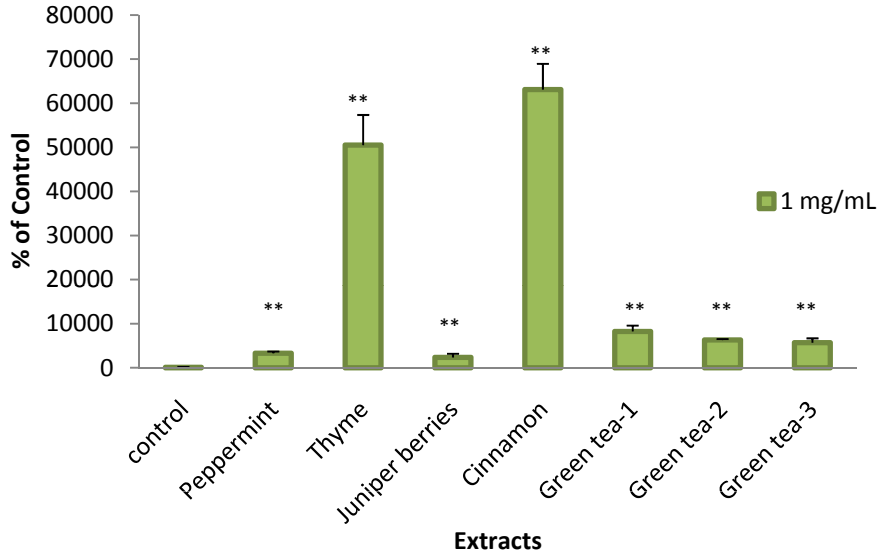
*"**", P<0.01; "*", P<0.05*

EGCG, NDGA, gallic acid, daidzein and tannic acid can decrease Rhodamine 123 uptake by 19%~28%, suggesting their effects on mitochondrial membrane depolarization or induction of P-gp in HepG2/3A cells.

Peppermint, cinnamon and green tea extracts can decrease Rhodamine 123 uptake by ~50%.

Study I – CYP1A induction in HepG2/3A cells

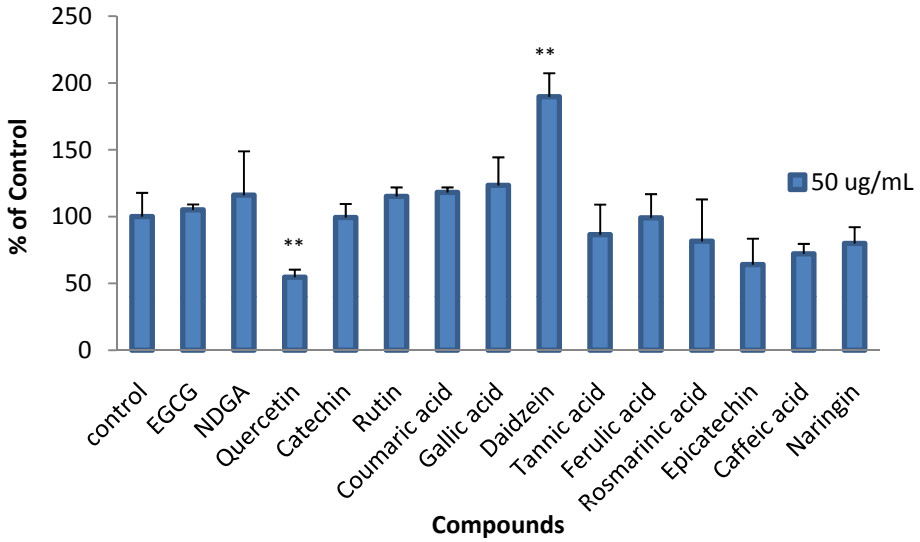


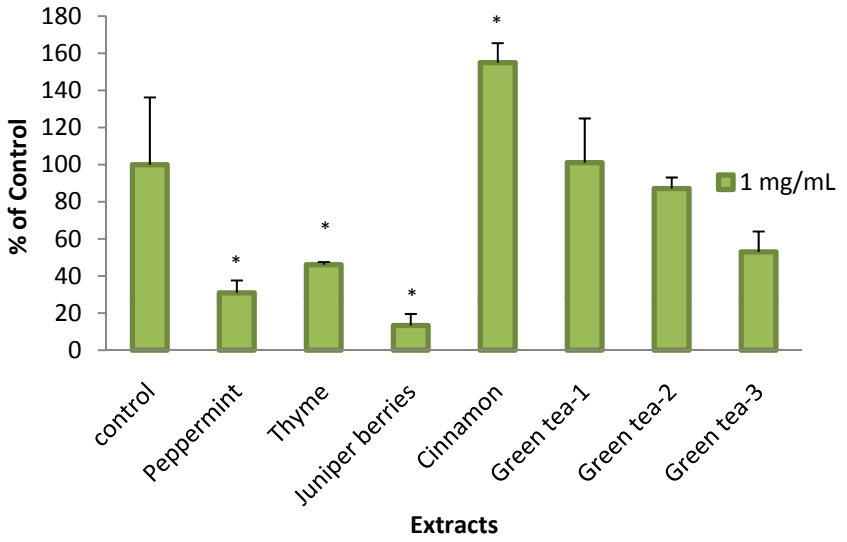


*"**", P<0.01; "*", P<0.05*

Six of fourteen tested compounds can induce CYP1A in the order of tannic acid > NDGA > EGCG > quercetin > gallic acid > rutin, and all of seven tested extracts can dramatically induce CYP1A activity in vitro.

Study I – CYP2B and/or 3A induction in HepG2/3A cells

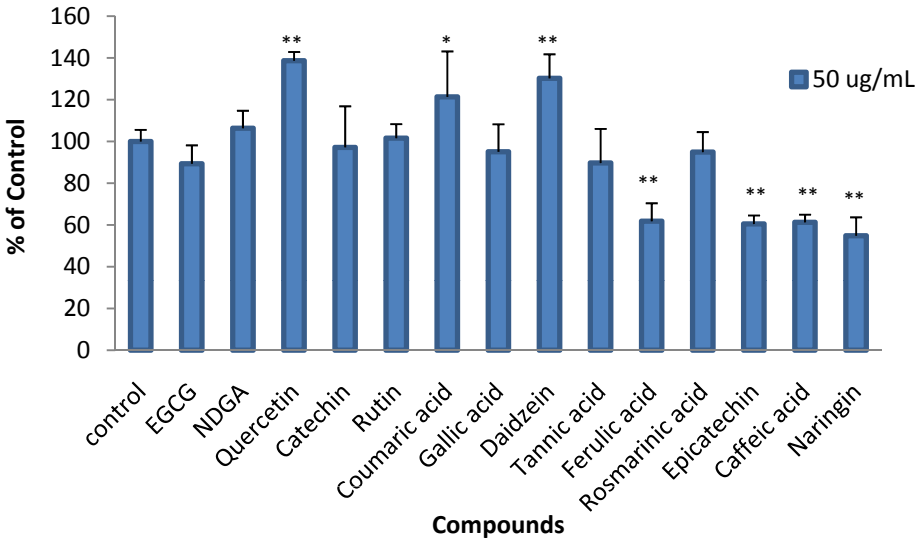


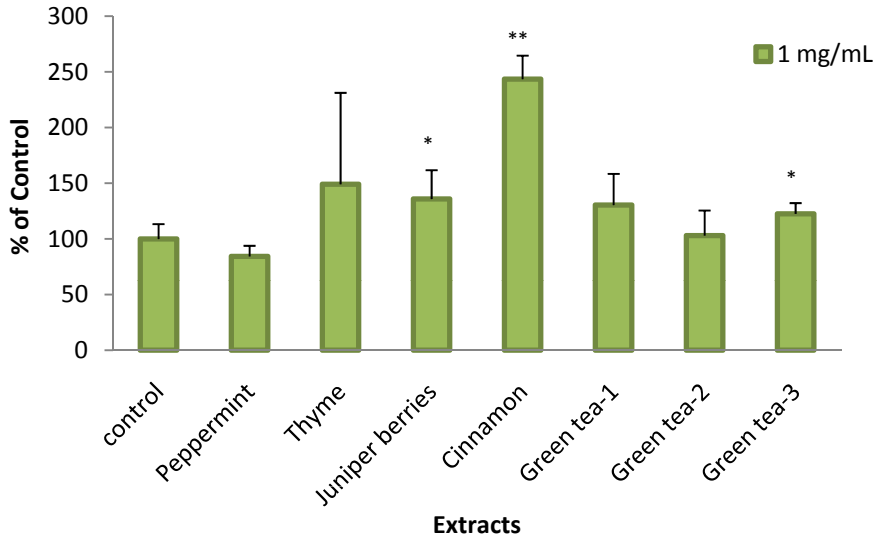


*"**", P<0.01; "*", P<0.05*

Among pure compounds, daidzein can induce CYP2B and/or 3A by 78% compared with control, while quercetin can inhibit CYP2B and/or 3A activity by 54%. For extracts, peppermint, thyme and juniper berries can significantly inhibit CYP2B and/or 3A by 54%-87%, while cinnamon can induce CYP2B and/or 3A by 55%.

Study I – Cellular neutral lipids in HepG2/3A cells

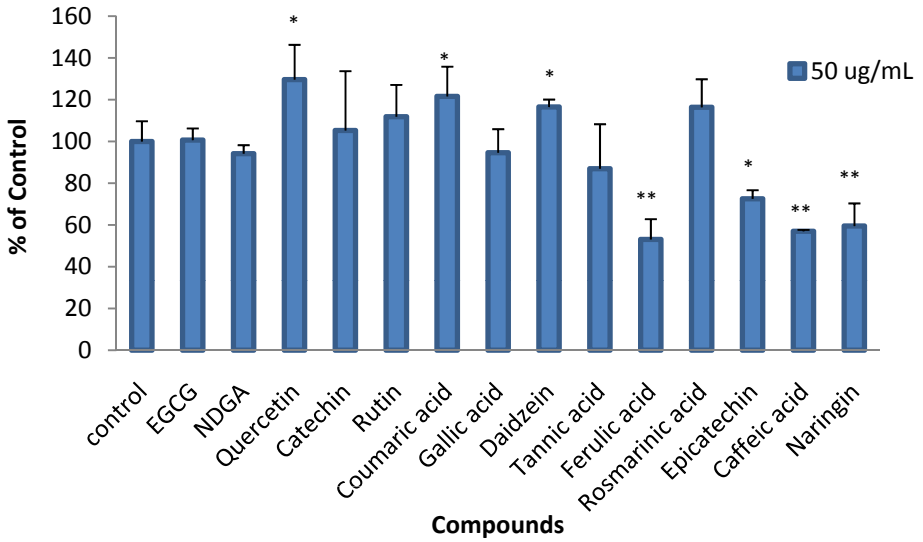


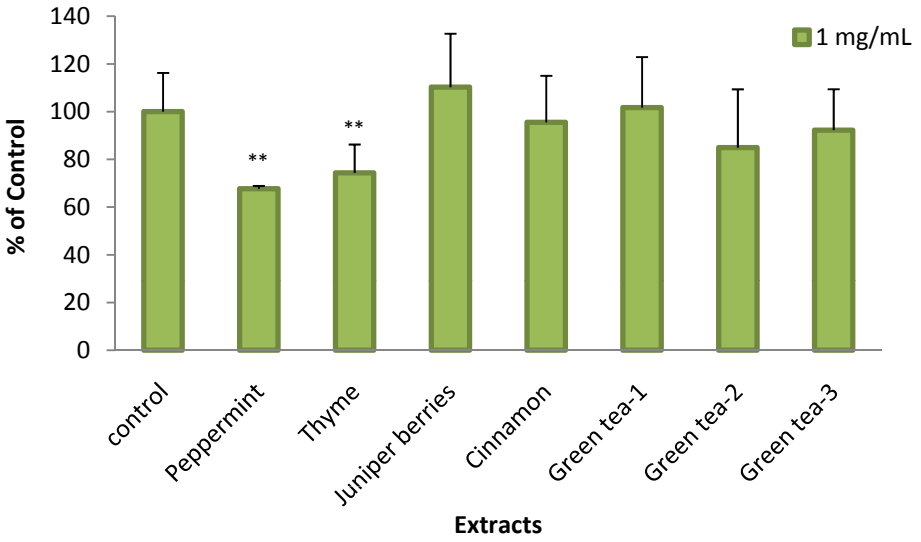


*"**", P<0.01; "*", P<0.05*

Daidzein, quercetin and coumaric acid, as well as juniper berries, cinnamon and green tea-3 extracts increased the accumulation of neutral lipid in HepG2/3A cells, which may lead to a potential steatosis or "fatty liver".

Study I – Cellular polar lipids in HepG2/3A cells

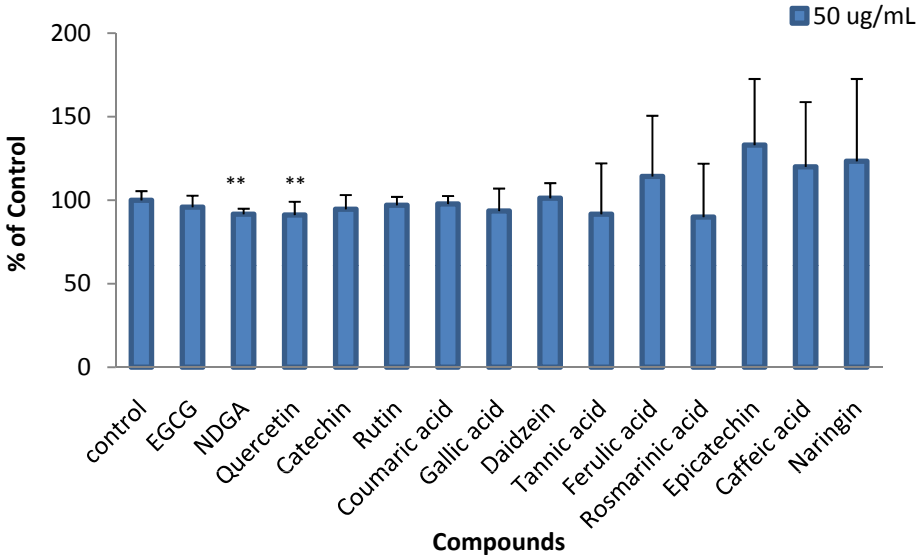


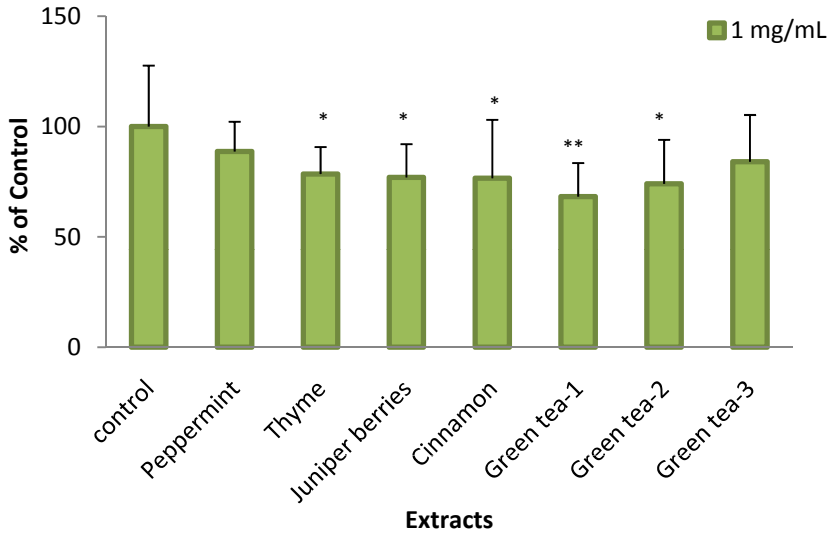


*"**", P<0.01; "*", P<0.05*

Three of fourteen tested compounds (quercetin, coumaric acid and daidzein) can slightly increase polar lipid on HepG2/3A cells membrane, implying potential phospholipidosis.

Study I – Total DNA content on HepG2/3A cells





*"**", P<0.01; "*", P<0.05*

Total DNA contents were decreased by NDGA, quercetin, thyme, juniper berries, cinnamon and green tea -1, -2 treatment.

Study I – summary

Chemical	Endpoint Assays						
	Oxidative Stress	Mitochondrial Permeability/P-gp	CYP1A	CYP2B/ 3A	Neutral Lipid	Polar Lipid	DNA
EGCG	+	+	+				
NDGA	+	+	+				–
Quercetin	+	+	+	–	+	+	–
Catechin	–						
Rutin		+	+				
Coumaric acid	–				+	+	
Gallic acid	+	+	+				
Daidzein		+		+	+	+	
Tannic acid	+	+	+				
Ferulic acid	–				–	–	
Rosmarinic acid							
Epicatechin					–	–	
Caffeic acid	+				–	–	
Naringin					–	–	

Among pure compounds, EGCG, NDGA, quercetin, gallic acid, daidzein and tannic acid display most potent biological effects in HepG2/3A cells.

Study I – summary

Extracts	Endpoint Assays						
	Oxidative Stress	Mitochondrial Permeability/P-gp	CYP1A	CYP2B/ 3A	Neutral Lipid	Polar Lipid	DNA
Peppermint	+	+	+	–		–	
Thyme	+		+	–		–	–
Juniper berries	+		+	–	+		–
Cinnamon	+	+	+	+	+		–
Green tea-1	+	+	+				–
Green tea-2	+	+	+				–
Green tea-3	+	+	+		+		

For dietary supplement extracts, peppermint, thyme, juniper berries, cinnamon and green tea extracts all display potent biological effects in HepG2/3A cells.

Conclusion

In vitro tests may provide a rapid means to identify dietary supplements with potential to cause undesired liver side-effects and supplement-drug interactions.


Thank you