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Thai Kratom Extracts Ameliorate MAFLD through Multi-Target Mechanism in FFA-induced HepG2 Cells

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SUPPLEMENTARY MATERIAL

Supplementary Method

Ethanol-Induced Lipid Accumulation

To investigate the impact of test compounds on ethanol-induced lipid accumulation, HepG2 cells ($4x10^5$ cells/well) were plated onto coverslips 24 hours before co-treatment with 1% ethanol (EtOH) and either red or green kratom extracts (10, 50, 100 µg/mL). Following the 24-h co-treatment, cells were stained with Oil Red O and lipid droplet formation was assessed via imaging using a standard protocol.

Supplementary Table S1: List of antibodies for Western blot analysis.

Name	P-Site	Function	Supplier	Cat no.

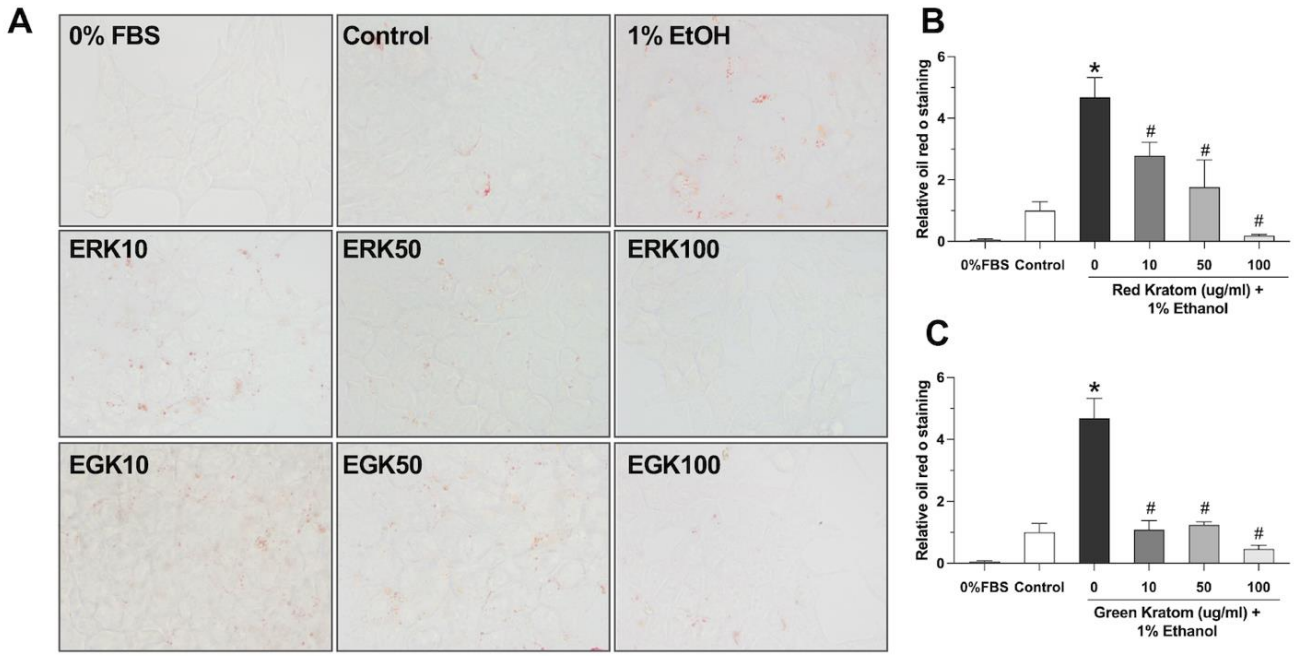
ACC		Catalyzation of the first step in fatty acid	Cell Signaling Danvers, USA	3662
FAS		Biosynthesis of fatty acids or metabolism	CST.	3189
Phospho-AMPK	Ser47	Regulates energy balance & metabolism	CST	5759
AMPK		Regulator of lipid metabolism	CST	2532
Phospho-p38MAPK	Thr180	Mediates stress, inflammation responses	CST	9211
P38MAPK		Molecule stress and inflammatory protein	CST	9212
Phospho-IRS-1	Ser302	A key mediator of insulin signaling	CST	2381
IRS-1		Mediator of insulin on glucose uptake	CST	2382
Phospho-AKT	Ser473	Promotes growth and survival	CST.	9275 S
AKT		Regulates growth, survival, metabolism	CST.	9272
Phospho-GSK3α	Ser641	Inhibits glycogen synthase activity	CST	9331
GSK3α		Regulates glycogen metabolism, signaling	CST	9338
Phospho-GS	Ser845	Activates glycogen synthase activity	CST	3891
Glycogen synthase		Synthesizes glycogen from glucose	CST	3893

Supplementary Table S2: Human primer sequences used for qPCR.

Gene	**Access number**	**Primer Forward (5′-3′)**	**Reverse Primer (5′–3′)**

TLR 4	NM_13855 4	CCCTGAGGCATTTAGGCAGCTA	AGGTAGAGAGGTGGCTTAGGC
c-JUN	NM_00222 8	CCTTGAAAGCTCAGAACTCGGAG	TGCTGCGTTAGCATGAGTTGGC
CCL2	NM_00298 2	AGAATCACCAGCAGCAAGTGTCC	TCCTGAACCCACTTCTGCTTGG
CCL21	NM_00298 9	AGCAGGAACCAAGCTTAGGCTG	GGTGTCTTGTCCAGATGCTGCA

Supplementary Figure



Supplementary Fig. S1 Kratom extracts mitigate ethanol-induced lipid accumulation in HepG2 cells. (A) Representative Oil Red O-stained images showing lipid accumulation induced by 1%

ethanol (EtOH) and the effects of co-treatment with varying concentrations of red kratom extract (ERK) or green kratom extract (EGK) in HepG2 cells. Quantification of Oil Red O staining intensity after treatment with red kratom extract (B) or green kratom extract (C). Data are presented as mean ± SEM of three independent experiments (n=3). Statistical significance was determined using one-way ANOVA followed by Turkey's post-hoc test: $^{*}p < 0.05$ compared to the control group, $^{\#}p < 0.05$ compared to the ethanol-treated group. A 0% FBS (fetal bovine serum) control was included to assess the effect of serum starvation on lipid accumulation.