Prunus mahaleb fruit extract prevents chemically induced colitis and enhances mitochondrial oxidative metabolism via the activation of the Nrf2 pathway.

Alessandra Ferramosca[a], Lucia Treppiccione[b], Mariangela Di Giacomo[a], Vera Rotondi Aufiero[b], Giuseppe Mazzarella[b], Francesco Maurano[b], Carmela Gerardi[c], Mauro Rossi[b], Vincenzo Zara[a], Giovanni Mita[c], Paolo Bergamo[b#]

[a] Department of Environmental and Biological Sciences and Technologies, University of Salento, 73100 - Lecce, Italy

[b] Institute of Food Sciences, CNR, 83100 - Avellino, Italy

[c] Institute of Sciences of Food Production, CNR, 73100 - Lecce, Italy

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Correspondence:

Dr. Paolo Bergamo

Institute of Food Sciences, National Research Council (CNR-ISA),

via Roma 64, 83100 Avellino, Italia.

Phone: +39 0825-299506

FAX +39 0825-299161

e-mail: p.bergamo@isa.cnr.it

Abbreviations: ACC, acetyl-coenzyme A carboxylase, CIC, Citrate Carrier; CPT, carnitine palmitoyl-CoA transferase; DSS, Dextran Sodium Sulphate; FAS Fatty Acid Synthase, GSH, Reduced glutathione; GSSG, Oxidized glutathione; GSR, Glutathione-S-reductase; GST Glutathione-S-transferase, G6PD, Glucose-6-Phosphate Dehydrogenase, HO-1, Heme oxygenase-1, mcfe, mahaleb concentrated fruit extract; NQO1, NAPDH-Quinone Oxidoreductase-1; Nrf2, Nuclear factor erythroid 2-related factor 2; PC, carbonylated proteins; PGC-1α, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS, reactive oxygen species; SOD2, Superoxide dismutase 2, mitochondrial, TG, Triglycerides

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ABSTRACT

Scope. Polyphenols exhibit their antioxidant activity downstream the activation of the Nuclear factor erythroid 2-related factor 2 pathway (Nrf2), but the connection between lipid metabolism and Nrf2 pathway is still unknown. Flavonoids-rich concentrated extract from *Prunus mahaleb* (mahaleb concentrated fruit extract; **mcfe**) may act on oxido-reductive homeostasis and hepatic lipid metabolism via Nrf2.

Methods & Results. **Mcfe** ability to enhance the activity of Nrf2-mediated antioxidant/detoxifying enzymes was investigated in liver and colon of BALB/c mice. After a 4-week supplementation, macroscopic, histological and biochemical signs of colitis were examined in mouse colon pulsed with 5% (w/v) Dextran Sodium Sulphate (DSS). Untreated or DSS-supplemented mice were used as negative or positive control. **Mcfe** effect on liver lipid metabolism and its possible link with the Nrf2 pathway was investigated.

Mcfe intake increased antioxidant defenses in mice colon and its pre-treatment blunted pathological signs of colitis, as compared to positive control. In the liver, the increase in antioxidant defenses was associated with enhanced oxidative metabolism and with higher levels of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and of hemeoxygenase-1 (HO-1), in comparison with negative controls.

Conclusion. Cytoprotective and hypolipidemic effect produced by **mcfe** intake results, at least in part, by the activation of the Nrf2 pathway.

1.0 INTRODUCTION

Plant based foods are rich of antioxidant molecules playing a protective role against the damaging effects triggered by Reactive Oxygen Species (ROS). Among them, polyphenols are the most representative antioxidants in the human diet[1] and exert their activity via different mechanisms,[2] including the improvement of endogenous antioxidant defense through the activation of the redox-sensitive leucine-based zipped transcription nuclear factor (erythroid-derived 2)-like 2 (Nrf2).[3] Under mild electrophile or oxidative stress, Nrf2 prompted the synthesis of phase 2 enzymes that are essential for the maintenance of cell redox homeostasis (Redox status). This factor plays a key role in the production/recycling of the main intracellular antioxidant, reduced glutathione (GSH) (glutathione reductase, GSR; glucose 6 phosphate dehydrogenase, G6PD glutathione peroxidase, GSHPx), or in cellular detoxification (glutathione S-transferase, GST; NAD(P)H:quinone oxidoreductase, NQO1; and hemeoxygenase-1, HO-1).[4] Nrf2 pathway may also improve cytoprotective defenses via the activation of an adaptive response, which is the mechanism whereby the pre-exposure to low ROS levels provides the ability to better resist the damaging effects of further pro-oxidant agents.[5]

Interestingly, beside its role in the modulation of oxido-reductive status (RedOx status), Nrf2 is an important player in the maintenance of mitochondrial homeostasis[6] and it impacts on cellular bioenergetics by controlling substrate availability for respiration and ATP synthesis[7] and the efficiency of mitochondrial fatty acid oxidation[8]. Consequently, it was considered a therapeutic target for liver disorders associated to impaired lipid homeostasis (e.g. Non Alcoholic Fatty Liver Disease)[9]

The liver plays a central role in all the steps of lipid and energy metabolism, and some of the enzymes involved in these pathways are modulated by RedOx status. In this context, dietary polyphenols are able to modulate triglyceride accumulation, by regulating expression and/or activity of proteins associated with lipid homeostasis and metabolism[10]. In addition, it was demonstrated that the plant flavonol quercetin enhances hepatic mitochondrial oxidative metabolism via Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α)[11] - a known modulator of mitochondrial biogenesis/energy production[12] – downstream the activation of the Nrf2/HO-1 pathway.

Prunus mahaleb L. is a neglected species, well adapted to marginal soils mainly used as cherry-rootstock and it produces cherry-like dark purple drupes that have a bitter taste and are rich in potentially beneficial components (anthocyanins, flavonols and coumarins[13]). Antioxidant capacity, pro-apoptotic and anti-inflammatory effects of a mahaleb fruit concentrate extract (**mfce**)[14] were recently demonstrated by *in vitro* cell-based assay,[15] nevertheless **mcfe** ability to improve RedOx status, via this mechanism has not been investigated yet. As polyphenols efficacy as Nrf2 inducer has been recently reviewed[3], therefore we assumed that **mcfe** intake, owing to the presence of potentially beneficial molecules, would improve cytoprotective defenses and mitochondrial function via the activation of the Nrf2 pathway.

To test this hypothesis, **mcfe** ability to improve Nrf2-activated antioxidant/cytoprotective enzymes (phase 2) was assessed in colon tissue of healthy or DSS-treated animals. **Mcfe** effect on hepatic redox homeostasis and on lipid metabolism were examined in liver tissue of healthy BALB/c mice and the involvement of the Nrf2/HO-1/PGC-1α pathway was evaluated.

2.0 MATERIALS and METHODS

2.1 Reagents

Reagents were from Sigma-Aldrich (St. Louis, MO) or Serva (Serva electrophoresis GmbH, Heidelberg, Germany). Antioxidant Assay Kit was purchased by Cayman Chemicals (Ann Arbor, MI, USA). The composition of **mcfe** used has been recently reported[14]. DSS (36–50 KDa) was purchased from (MP Biomedicals, Ontario, USA). The list of primary antibodies and dilutions used for used for immunoblotting analysis are reported in the *Supplemental Table I.*

2.2 Animals and treatments

BALB/c mice, (female n = 24 average body weight 22.8±0.6) were used in the study and they were randomly grouped (n = 5) in 4 experimental groups. The animals, originally obtained from Charles River (Charles River - Research Models and Services, Italy), were from a colony reared in our animal facility in a temperature-controlled room (24 °C), under a 12 h light/dark cycle in pathogen-free conditions, with free access to drinking water and standard chow (Mucedola 4RF21-GLP) (*Supplemental Table II*). All the experimental procedures were carried out in accordance with national and international policies (EU Directive 2010/63) and the experiments were approved by the Institutional Committee and authorized by the Italian Ministry of Health (n. 285/2016-PR).

The first group was used to evaluate the effects produced by **mcfe** intake in mouse liver and colon. A group was daily supplemented (p.o.) with **mcfe** for 4 weeks (5 days/week) and the dose of e used (25 μL p.o; 1300 mg/Kg bw), when converted to a human equivalent dose, corresponded to 6.2 g fresh fruit/day (about 10 drupes). The second group was supplemented with **mcfe** before DSS administration (3% w/v DSS) in the drinking water for 5 days. The volumes of DSS solutions were monitored every day to ensure equal water consumption and the disease activity index (DAI) was determined by scoring the combination of weight loss, diarrhea and hematochezia[16] by a blind investigator to experimental groups. Two final groups (treated with DSS or not receiving any treatment) served as positive (DSS) or negative control (Control), respectively.

2.3 Sample preparation

Blood, liver and colon were collected from animals sacrificed at the end of the experimental period. Serum was prepared and stored at -80 °C. Liver was cut in aliquots, snap frozen in liquid nitrogen and stored at -80 °C until their utilization. Colon was taken on and flushed with ice-cold buffer (50 mM Tris–HCl pH 7.6, 150 mM NaCl; TBS) to remove fecal material, cut longitudinally and opened flat. Tissue aliquots were snap frozen in liquid nitrogen and stored at -80 °C until their utilization for biochemical analyses. Colon samples for histological analysis were cut into pieces, embedded in Optimal cutting temperature compound (OCT- Bio-Optica) and snap frozen in liquid nitrogen.

2.4 Phase 2 enzyme activity and oxidative stress evaluation.

RedOx status and activity of Nrf2-activated enzymes were spectrophotometrically evaluated by using standard procedures (Supplementary material).

2.5 Triglyceride and cholesterol evaluation

Hepatic lipids were extracted from liver samples by using a 1:1 mixture of chloroform and methanol. The extracts were dried under nitrogen flow and re-suspended in a suitable volume of 0.1% Triton X-100 before carrying out the individual lipid assays. Triglyceride (TG) and cholesterol (Chol) levels were then measured using commercial kits[17].

2.6 Lipogenic enzymes and carnitine palmitoyl transferase assay.

The expression of mitochondrial Citrate Carrier (CIC), the first protein involved in the lipogenic pathway, and of CPT I were determined by western-blotting (see Supplementary material). The activities of ACC and FAS were measured in the resulting cytosol as previously described, using a NADH- and NADPH-linked assay, respectively[18]. Total carnitine palmitoyl-CoA transferase (CPT) activity was determined spectrophotometrically at 412 nm in freshly isolated mice liver mitochondria, essentially as described previously[19] CPT I activity was calculated by subtracting the CPT activity that was insensitive to 100 μM malonyl-CoA (CPT II) from the total CPT activity experimentally determined.

2.7 Mitochondrial respiration efficiency assays

Western Blotting and mitochondrial respiration analysis were carried out as detailed in the *Supplementary Material*.

2.8 Histological analysis of colon tissues

Cryostat sections (8 μm) were cut, air dried at room temperature and fixed in 10% neutral buffered formalin. The sections were stained with hematoxylin and eosin (H&E) protocol and mounted with DPX (Sigma-Aldrich) for histological evaluation carried out by two blinded investigators under a Zeiss Axioskop microscope for the evaluation of microscopic scoring of colon pathology, accordingly to a published protocol[20](*Supplemental Table III*).

2.9 Statistics

Data were expressed as mean values ± standard deviation. The program GraphPad Prism 6 (GraphPad Software, San Diego, CA) was used to perform t test or one-way ANOVA, followed by the Tukey post-hoc test. $p<0.05$ was considered significant. The appropriateness of the experimental group size (for a two-sample t-test comparison, 5% significance level and a power of 90%) was calculated[21].

3.0 RESULTS and DISCUSSION

3.1 Mcfe intake improves colonic RedOx status and protect against DSS-induced oxidative stress.

Our first experiments examined the **mcfe** ability to improve Nrf2-activated antioxidant defenses in colon of healthy BALB/c and its protective effects against DSS-induced colitis, generally accompanied by enhanced ROS yield.[22] Undetectable influence of **mcfe** on animal weight gain and on blood antioxidant capacity in healthy mice was evidenced by preliminary analysis (*Supplemental Figure 1*). Since GSH is the most abundant endogenous antioxidant, the **mcfe** effects on redox status (GSH/GSSG ratio) and on the activity of several phase 2 enzymes (GSR, G6PD, NQO1, GST) were measured in colonic tissue of healthy animals and in DSS-treated mice (**Figure 1**). **Mcfe** intake resulted in a significantly higher GSH content ($p=0.006$) as compared to controls (**Figure 1A**) and prevented the DSS-induced decline of GSH and GSH/GSSG ratio ($p=0.043$; $p=0.00013$, respectively); a parallel increase in GSSG concentration was induced by DSS-treatment ($p<0.001$), even after the **mcfe** treatment. Moreover, the activity of antioxidant enzymes (GSR, G6PD) was markedly improved by **mcfe** intake ($p<0.05$) and such enhanced protection blunted the pro-oxidant effect triggered by DSS intake (**Figure 1B**). By contrary, **mcfe** supplementation had only minor effects on G6PD and NQO1 activities in colonic tissue of healthy and of DSS-treated animals, as compared to control animals (**Figure 1C**).

Owing to the redox-dependent activation of Nrf2, the concomitant improvement of antioxidant enzyme activity with the lack of alteration of GSH/GSSG ratio in **mcfe**-treated mice are clearly indicative of its antioxidant ability rather that the result of a compensation mechanism in response to a possible pro-oxidant stimulation. As mentioned above, despite the high content of potential Nrf2-inducers in **mcfe**, the identification of the compound

responsible for the observed biological activities was not within the scopes of the present study. This is the first time that Nrf2-mediated antioxidant activity of **mcfe** have been investigated *in vivo*, and the obtained results are consistent with literature data reporting the ability plant-based Nrf2 activators.[3]

3.2 Mcfe intake blunt several macroscopic and histological signs of colitis.

Nrf2 controls genes encoding phase 2 detoxifying enzymes and antioxidant proteins; the induction of these proteins is crucial for cells in counteracting the adverse effects of exogenous insults. As DSS-treatment mimics in rodents several clinical features observed in of human ulcerative colitis[23], therefore we decided to investigate whether Nrf2-mediated defenses triggered by **mcfe** may have protective effect against several pathological signs of DSS-induced colitis.

Mcfe intake improved DSS-induced macroscopic signs of colitis (i.e. body weight loss and DAI)(**Figure 2A,2B**) and oxidative stress (PC accumulation)(**Figure 2C**). Cryostatic sections of control mouse colon (**Figure 2D**) showed a well-preserved mucosa - with no inflammatory cells infiltrate - defined epithelial layer (black arrow) and normal crypt depth (white arrow). On the contrary, in DSS-treated mice apparent signs of histological damage - severe leukocyte infiltration (circled in white), goblet cell depletion (circled in yellow), crypts hyperplasia (white arrow) and an indistinct epithelial layer (black arrow) - are present (**Figure 2E**). Notably the noxious effects produced by DSS treatment were markedly blunted by **mcfe** intake (reduced leukocyte infiltration associated with recovery of epithelial layer (black arrow), and crypt depth architecture (white arrow)(**Figure 2F**). Accordingly, the histological index was significantly reduced in **mcfe**+DSS mice in comparison with diseased mice (**Figure 2G**).

This is the first time that the protective effect exerted by **mcfe** has been investigated *in vivo*; however, our data are in good accordance with similar studies showing the preventive ability of Nrf2-activators in animal and their cytoprotective efficacy against DSS-induced colitis.[24,25]

In particular, on the basis of the redox-sensitive activation of the Nrf2 pathway, it can be hypothesized that such protective effects may result from the activation of an adaptive

response, which is the mechanism whereby the pre-exposure to low ROS levels provides the ability to better resist the damaging effects of further pro-oxidant agents[5].

3.3 Mcfe intake decreases hepatic triglyceride content through the enhancement of fatty acid oxidation.

Our next studies were aimed at investigating the link between its ability to activate Nrf2-mediated defenses and its modulatory effect on liver lipid metabolism. For analysis of the effects of **mcfe** treatment on lipid metabolism, liver lipid composition was first evaluated. Data in **Figure 3A** show a significant decrease (about 20%) in the content of hepatic TG in **mcfe** group in comparison to the controls ($p<0.05$). The possible molecular mechanism responsible for the decline of hepatic TG measured in **mcfe**-fed mice was next examined. In particular, given the major role played by the mitochondrial CIC in the transport of citrate to cytoplasm[26,27] where this molecule is the primer for fatty acid synthesis, its expression was analyzed by western blotting. Moreover, enzymatic activities of ACC and FAS - to which the CIC physiologically supplies substrates - were measured in liver of untreated and of **mcfe**-treated mice. Data in **Figure 3B**,**3C**, showing that **mcfe**-treated mice exhibit levels of CIC protein and a lipogenic activity comparable to those measured in control animals, clearly indicated that hepatic lipogenesis was not modified by **mcfe** intake. Interestingly, when the activity of CPT I – the rate limiting enzyme in the catabolic pathway of fatty acid oxidation – was measured, its significant increase (at both protein and enzyme activity level) was found in **mcfe**-treated mice (**Figure 3D**,**3E**) in comparison to control animals ($p<0.001$).

This finding demonstrates that lower TG content in liver of **mcfe**-treated mice is likely consequential to the stimulation of fatty acid oxidation. The increased fatty acid oxidation observed in **mcfe**-treated mice produces higher levels of reducing equivalents, which are normally addressed towards the mitochondrial oxidative phosphorylation for ATP production. Although a significant increase in the activity of the mitochondrial complex I ($p<0.001$) was observed, RCR values (an index of respiratory chain coupling) calculated for **mcfe**-treated mice were similar to those of the control group (*Supplemental Figure 2*).

This is the first study investigating the *in vivo* effects produced by **mcfe** intake, however presented results indicating **mcfe** ability to modulate lipid homeostasis/metabolism

is suggestive of the possible utilization of *P. mahaleb* fruit - or of its derived products - in dietary strategies for the treatment of fatty liver disease [28].

3.4 Mcfe intake improves hepatic antioxidant defenses and enhances the Nrf2/PGC-1α pathway.

To determine the effects of **mcfe** treatment on liver redox homeostasis, GSH/GSSG ratio and phase 2 enzyme activity were evaluated in mouse liver. A significant increase of GSH concentration ($p<0.01$) and of the GSH/GSSG ratio ($p<0.01$)(**Figure 4A**), along with an increase in antioxidant enzyme activities (G6PD and GSR) were observed in **mcfe**-treated mice as compared to control animals(**Figure 4B**)($p<0.05$ and $p<0.01$, respectively). On the contrary, the activity of detoxifying enzymes (GST, NQO1) was not influenced by dietary supplementation (data not shown). Significantly higher amount of nuclear Nrf2 were found in nuclear extracts of **mcfe**-treated animals (**Figure 4C**). Remarkably, **mcfe** administration resulted in the significant enhancement of HO-1, manganese superoxide dismutase (SOD2) and PGC-1α protein expression, when compared with controls (**Figure 4D**) ($p<0.05$). These results are consistent with literature data showing the causal positive correlation between Nrf2/HO-1 pathway and PGC-1α expression[11] and with the reported link between PGC-1α levels and CPTI activity.[28] In addition, on the basis of the reported interaction of SOD2 with mitochondrial complex I, increased SOD2 level in **mcfe**-treated mice was not unexpected.[29]

When power analysis was carried out[21] by using the values obtained by analysis of antioxidant enzymes (GSH, GSR, G6PD) and metabolic markers (TG, PCT I, Complex I, Pyruvate+malate activity) it was demonstrated that 5 animals/group is an acceptable sample size for the evaluation of biological effects of **mcfe** (assuming 80% power, 5% significance level and a two-sided test)(*Supplemental Table IV*).

4.0 CONCLUDING REMARKS

Presented results demonstrate, for the first time, that increased activity of Nrf2-mediated antioxidant defenses in colon of **mcfe**-treated mice better protects against toxic effects of chemically-induced colitis (likely via the activation of an adaptive response). More interestingly, improved redox homeostasis prompted by **mcfe** intake modulates liver lipid metabolism likely through the activation of the Nrf2/HO-1/PGC-1α pathway. Studies aimed at

better understanding of mitochondrial involvement in Nrf2-mediated effects elicited by **mcfe** intake are in progress.

Conflict of interest

The authors declare no conflict of interest.

5.0 REFERENCES

1. A. Scalbert, I. T. Johnson, M. Saltmarsh, *Am. J. Clin. Nutr*. **2005**,81,215S.
2. K. D. Croft. *Arch Biochem Biophys.* **2016**, 595,120.
3. Martínez-Huélamo M, Rodríguez-Morató J, Boronat A, de la Torre R. *Antioxidants* (Basel). **2017**, 6, 73.
4. M. K. Kwak, N. Wakabayashi, T.W. Kensler, *Mutation Res.* **2004**, 555,133.
5. W. O. Osburn, T. W. Kensler, *Mutat. Res.* **2008**,659,31.
6. A. T. Dinkova-Kostova, A. Y. Abramov, *Free Radic. Biol. Med*. **2015**, 88,179.
7. K. M. Holmström KM, L. Baird, Y. Zhang, I. Hargreaves, A. Chalasani, J. M. Land, L. Stanyer, M. Yamamoto, A. T. Dinkova-Kostova, A. Y. Abramov. *Biol. Open* **2013**,761.
8. M. H. Ludtmann, P. R. Angelova, Y. Zhang, A. Y. Abramov, A. T. Dinkova-Kostova, *Biochem. J*. **2014**,457,415.
9. L. Li, J. Fu, J. Sun, D. Liu, C. Chen, H. Wang, Y. Hou, J. Xu. *Curr Opin Toxicol.* **2019**,13,35.
10. N. Auberval N, S. Dal, E. Maillard, W. Bietiger, C. Peronet, M. Pinget, V. Schini-Kerth, S. Sigrist. *Eur. J. Nutr*. **2017**,56,1467.
11. C.S. Kim, Y. Kwon, S.Y. Choe, S.M. Hong, H. Yoo, T. Goto, T, Kawada, H.S. Choi, Y. Joe, H.T. Chung, R. Yu. *Nutr Metab (Lond)*. **2015**,12,33.
12. R.C. Scarpulla *Physiol Rev.* **2008**,88,611.

13. F. Blando, C. Albano, Y. Liu, I. Nicoletti, D. Corradini, N. Tommasi, C. Gerardi, G. Mita, D.D. Kitts, *J. Sci. Food Agric.* **2015**,96,2641.

14. C. Gerardi, N. Tommasi, C. Albano, F. Blando, L. Rescio, E. Pinthus, G. Mita, *Eur. Food Res. Technol.* **2015**,241,683.

15. C. Gerardi, S. Frassinetti, L. Caltavuturo, A. Leone, G. Mita, *J. Funct. Foods* **2016**,27,537.

16. S. N. Murthy, H. S. Cooper, H. Shim, R. S. Shah, S. A. Ibrahim, D. J. Sedergran, *Dig. Dis. Sci*. **1993**,38,1722.

17. A. Ferramosca, V. Savy, V. Zara, *Biosci. Biotechnol. Biochem.* **2008**,72,62.

18. A. Ferramosca, V. Savy, L. Conte, S. Colombo, A. W. Einerhand, V. Zara. *J. Lipid Res.* **2006**,47,1994.

19. L. L. Bieber, T. Abraham, T. Helmrath, *Anal. Biochem*. **1972**,50,509.

20. U. Erben, C. Loddenkemper, K. Doerfel, S. Spieckermann, D. Haller, M. M. Heimesaat, M. Zeitz, B. Siegmund, A. A. Kühl, *Int. J. Clin. Exp. Pathol*. **2014**,7,4557.

21. J. Charan, N.D. Kantharia. *J Pharmacol Pharmacother.* **2013**,4,303

22. H. Zhu, Y. R. Li, *Exp. Biol. Med. (Maywood)* **2012**,237,474.

23. M. Perše, A. Cerar, J*. Biomed. Biotechnol*. **2012**,2012,718617.

24. A.K. Pandurangan, Z. Saadatdoust, N.M. Esa, H. Hamzah, A. Ismail. *Biofactors*, **2015**,41,1.

25. K.J. Kim, J.M. Park J.S. Lee, Y.S. Kim, N. Kangwan, Y.M. Han, E.A. Kang, J.M. An, Y.K. Park, K.B. Hahm. *J Physiol Pharmacol*. **2018**,69, 3. doi: 10.26402/jpp.2018.3.03

26. A. Ferramosca, V. Zara. *Adv Nutr*. **2014**,5,217.

27. J. A. Watson, J. M. Lowenstein. *J. Biol. Chem.* **1970**,245,5993.

28. S. Song, Y. Zhang, K. Ma, L. Jackson-Hayes, E.N. Lavrentyev, G.A. Cook, M.B. Elam, E.A. Park. *Biochim Biophys Acta.* **2004**,1679,164.

29. W. Suthammarak, B.H. Somerlot, E. Opheim, M. Sedensky, P.G. Morgan. *Aging Cell.* **2013**,12,1132.

FIGURE CAPTIONS

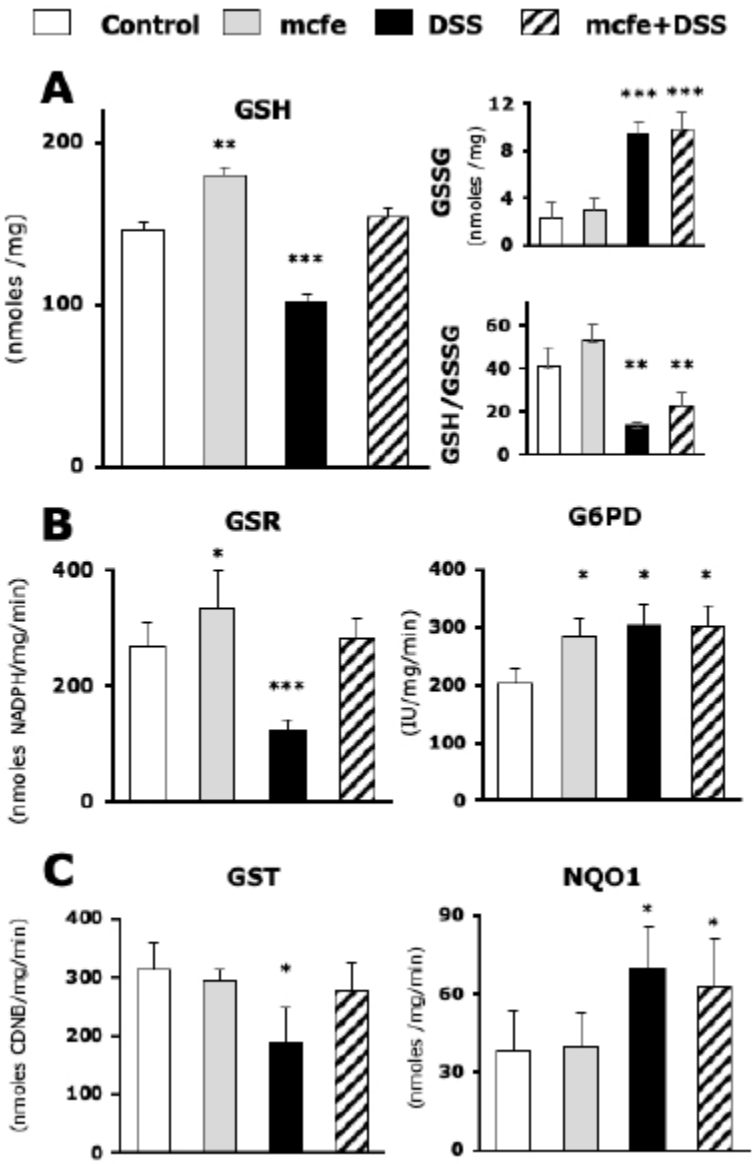

Figure 1. Dietary intake of mcfe improves Nrf2-activated antioxidant defences in colon and prevents redox status alteration in DSS-challenged mice.

GSHtot, GSSG and GSH/GSSG ratio were measured in colon tissue of healthy (control) or DSS-treated mice (DSS) and in mcfe-supplemented mice (mcfe, mcfe+DSS) (**A**). The activity of antioxidant (GSR, G6PD) (**B**) and of detoxifying enzymes (GST, NQO1) (**C**) was shown. Each bar represents the mean values ± SD from triplicate analysis on at least 5 mice/group. Significant differences are indicated (* $p<0.05$, ** $p<0.01$).

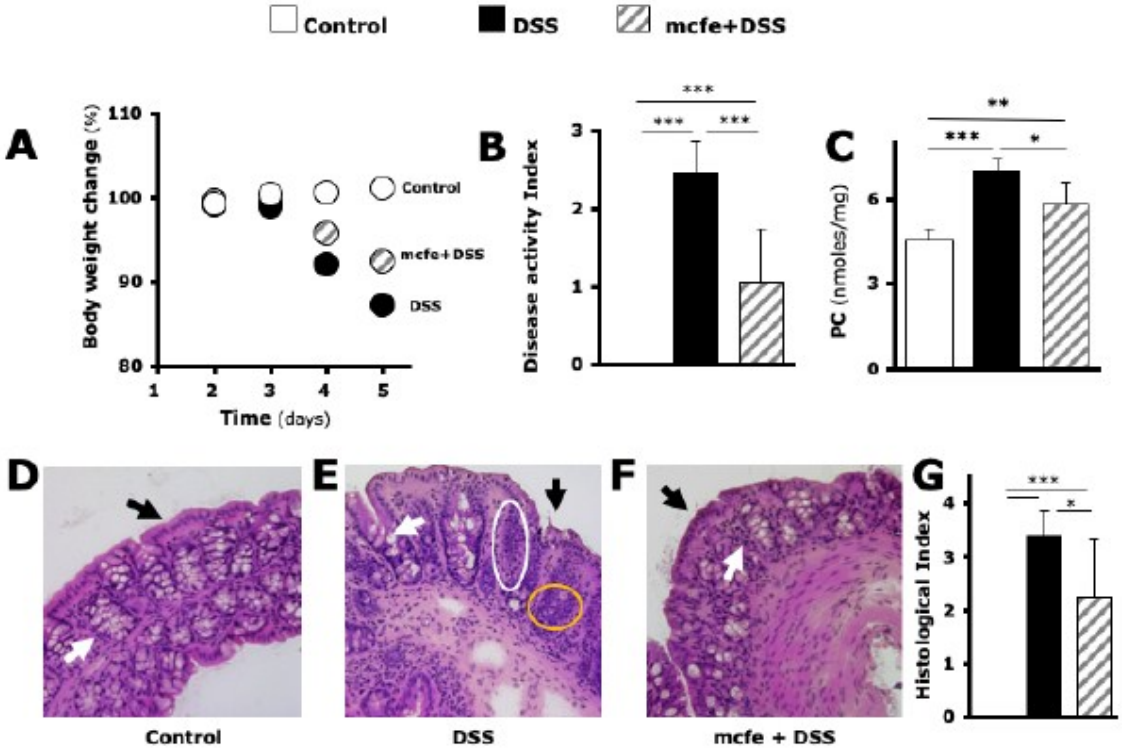

Figure 2. Mcfe pre-treatment protected against macroscopic, oxidative stress and histological alterations triggered by DSS.

Body weight changes (**A**), Disease Activity index (DAI) (**B**), oxidative stress extent (PC accumulation) (**C**) measured in control animals were compared with those in DSS-treated (DSS) and with mice pre-treated with mcfe before DSS challenge. Histological evaluation of colonic epithelium (black arrow) and of crypts architecture (white arrows) in control mice (**D**), in DSS-treated animals without (DSS) -infiltrated Leukocyte (circled in white), goblet cell depletion (circled in yellow) (**E**) or with mcfe pre-treatment are shown (mcfe+DSS) (**F**) (Original magnification 20X). Histological score values calculated in the different experimental groups (**G**). Each bar represents the mean values ± SD from at least duplicate analysis on individual samples. Significant differences from animal groups are indicated (* $p<0.05$, ** $p<0.01$, *** $p<.0.005$).

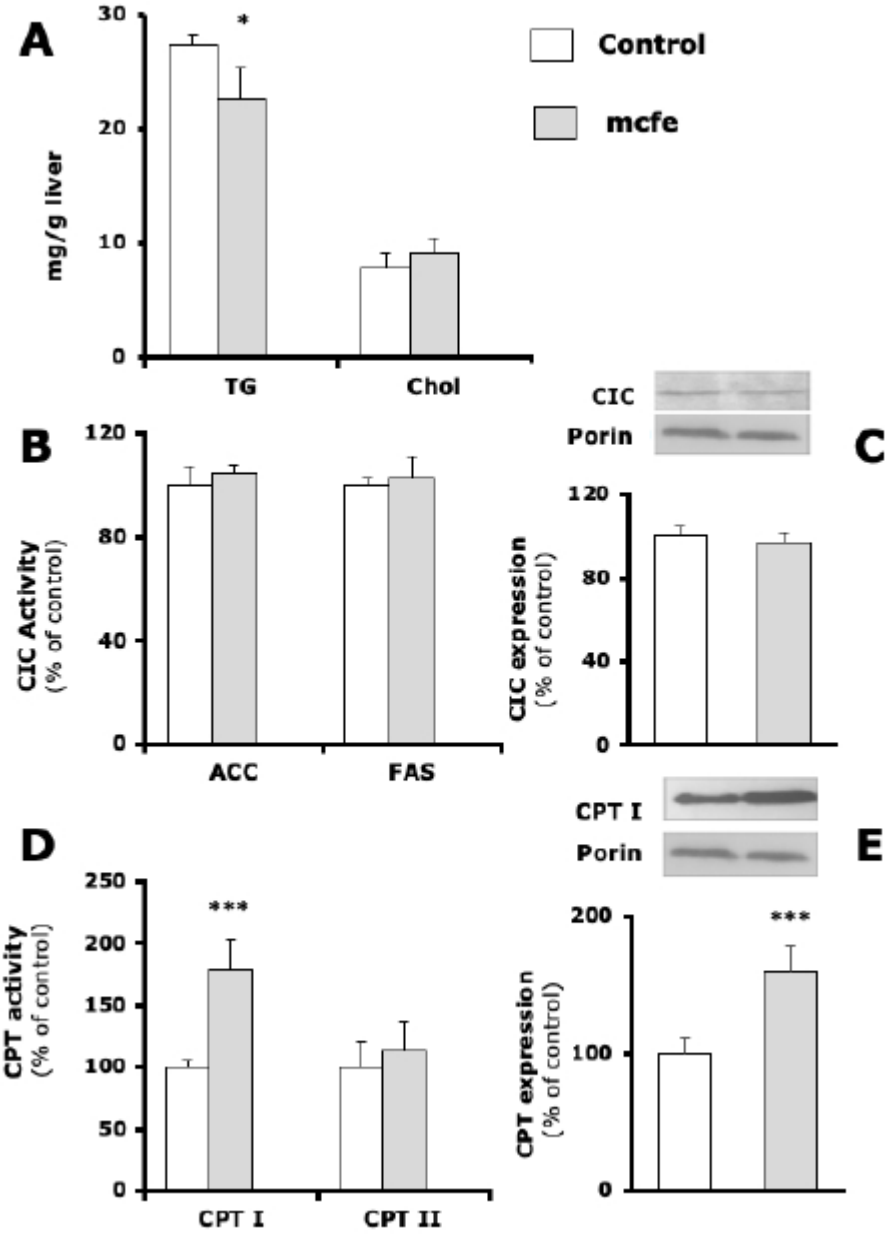

Figure 3. Decreased triglyceride content in liver of mcfe-treated mice associates to enhanced fatty acid oxidation.

TG and Chol level (**A**), ACC, FAS (**B**) and CPT I, II activities (**D**) were measured in liver samples of BALB/c mice supplemented with mcfe and in untreated control animals. Typical Western blots showing CIC and CPT I level are shown. Protein levels were normalized to the control protein (Porin) and expressed as percentage of control animals (**C**, **E**). Significant differences from control group are indicated (* $p<0.05$, *** $p<0.001$).

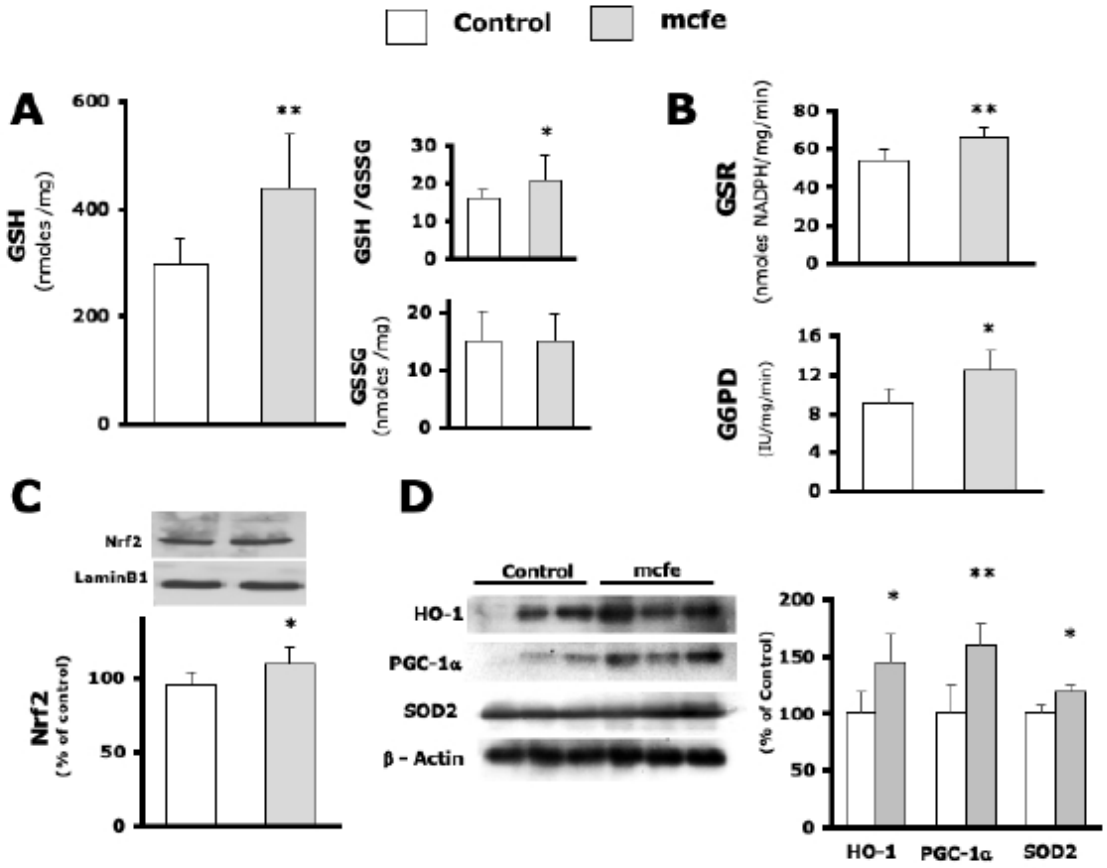

Figure 4. Nrf2 activation in liver of mcfe-treated mice associated with enhanced HO-1, SOD2 and PGC-1α expression.

GSH, GSSG and GSH/GSSG ratio (**A**) measured in liver of mcfe-treated and in control animals. The activity of antioxidant (GSR, G6PD) (**B**) were measured in cytoplasmic extracts. Each bar represents the mean values ± SD from duplicate analysis on at least 5 mice/group. Typical Western blots showing nuclear level of Nrf2 (**C**) and HO-1, PGC-1α and SOD2 levels in cytoplasmic extracts are shown (**D**). Protein levels were normalized to the control protein (LaminB1 or β-actin) and expressed as percentage of control animals. Significant differences from control group are indicated (* $p<0.05$, ** $p<0.01$)

Acknowledgments

P.B. and AF. designed the research, analyzed data and wrote the manuscript. L.T, M.D.G., V.R.A. and C.G. conducted the research and analyzed data. F.M. was responsible for animal management. G.Ma, G.Mi., C.G., M.R., and V.Z. provided input on the study design, analysis interpretation and revised the manuscript. All the authors read and approved the final manuscript.

Dietary supplementation with **mcfe**, polyphenol-rich extract from Mahaleb fruit, improves oxidoreductive homeostasis of mouse colonic tissue downstream the activation the Nuclear erythroid related factor2 (Nrf2) pathway. The activation of Nrf2-mediated antioxidant phase 2 enzymes (G6PD, GSR) is likely involved in its preventive ability against DSS-induced colitis. **Mcfe** intake increases the activity/expression of Nrf2-mediated defenses (G6PD, GSR, SOD2, HO-1) in mouse liver and it enhances hepatic metabolism (reduces fat synthesis and increases β-oxidation) likely via the HO-1/PGC-1a pathway. On the basis of the known crosstalk between Nrf2 and lipid metabolism, functional abilities of **mcfe** might be accounted to its role as Nrf2 inducer