Evaluation of protective effect of Brassica oleracea against hepatic steatosis in rats

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Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of liver function impairment and may appear as as a simple steatosis until the advanced stage of non-alcoholic steatohepatitis (NASH), characterized by increased inflammation, fibrosis, oxidative stress and marked liver damage. NAFLD seems to be related to lifestyle, nutrition, obesity, but also to the presence of some metabolic diseases (diabetes). In order to develop a model of human steatosis, in the presence of diabetes in a rat model, a protocol combining high fat diet (insulin resistance) and streptozotocin (inhibition of pancreas β cell function) was developed. There are many studies correlating the intake of plant-based foods and the human health status, due to the presence of phytochemicals with antioxidant, anticarcinogenic, and anti-inflammatory effects. In particular Brassicaceae comprise a complex mixture of bioactive compounds. The aim of this work was to investigate the possible protective and antisteatotic effects of Kavolì® (Brassica oleracea var. Acephala produced by particular agronomic techniques and harvested at a young stage) in rats with NAFLD. Twenty Wistar male rats were divided in two groups: the control group (CTR, n = 5) fed a standard diet (11% energy from fat), the other group (n=15) fed a high fat diet (55% fat-based, 2% cholesterol) was treated with streptozotocin (40 mg/kg i.p.). The animals that developed hyperglycemia (> 250 mg/dl) were divided into two groups: one who continued to receive the high fat diet (HFD, n = 8) and the other that received simultaneously high fat diet and an aqueous extract of Kavolì® (1g /kg b.w.) (HFD + KAV, n = 7), for 4 weeks by gavage. At the end of the experiment, the animals were sacrificed and samples of blood and liver were taken. Blood parameters, hepatic markers of oxidative stress, activities of drug metabolism enzymes and lipid content were measured. Moreover, histological analysis (hematoxylin-eosin coloration) were performed in liver sections and gene expression was investigated. Total cholesterol, glycemia, triglycerides, bilirubin, ALT and AST were significantly increased in rats of HFD group compared to control ones; in animals treated with Kavolì® ALT values were significantly decreased. Insulin values were significantly decreased in the HFD group compared with the CTR group as well as in the HFD + KAV group. The presence of steatosis was confirmed by the lipid quantification in liver tissue, which was markedly increased in HFD group and significantly reduced in rats treated with Kavolì®. Histological analysis on liver tissue confirmed the presence of steatosis. Glutathione was significantly decreased in HFD group but it was not restored in HDF+ KAV rats. Carbonylated proteins were significantly increased in the group of rats treated with HFD diet and significantly reduced in the HDF+ KAV group and the same trend was shown for MDA values. ECOD activity was induced in the DIPL rats. CYP2E1 marker activities (aniline hydroxylase and p-nitrophenol hydroxylase) significantly increased in HFD group compared to the control one but there was no restoration of values in the HDF + KAV group. Hemeoxygenase-1 activity increased in HFD rats and decreased in Kavolì® treated animals while DT-diaphorase was not influenced. Moreover, the expression of the genes of the Hemeoxygenase-1 and DT-diaphorase showed the same trend. Real time PCR of proinflammatory cytokines TNFα and IL-6 were analyzed; results about TNFα gene

expression showed a significant increase in the HFD group and a significant decrease in rats treated with the Kavolì® extract. It is possible to conclude that the administration of Kavolì® showed antioxidant and antiinflammatory properties in liver tissues in rats characterized by high levels of oxidative stress, inflammatory infiltration caused by high fat diet. Kavolì® also decreased steatosis caused by HFD diet.