Mahonia Aquifolium Flowers Extract Effects in Acute Experimental Inflammation

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Abstract

Natural products were proved to have inhibitory effect on the nitro-oxidative stress. The aim of the study was to evaluate the effect of *Mahonia aquifolium* (MA) flowers extract upon nitro-oxidative stress in acute experimental inflammation. The extract was prepared by repercolation method. Acute experimental inflammation was induced with turpentine oil (0,6ml/kg b.w. i.m.). MA extract was given for 7 days. Were used 6 groups (n=5) of male Wistar rats: Groups 1-3 were with acute inflammation and treated with MA dilutions (100%, 50%, 25%); Group 4 was acute inflammation control; Group 5 was negative control; Group 6 was acute inflammation treated with diclofenac (10mg/kg b.w. p.o). In day 8 nitro-oxidative stress was evaluated by measuring serum nitrites and nitrates (NOx), Total oxidative stress (TOS), Total antioxidant capacity (TAC), Oxidative stress index (OSI), Malondialdehyde (MDA) and Thiols (SH). MA reduced OSI and TOS, increased SH, and had no important effect on TAC, NO and MDA. Compared to MA, Diclofenac was a stronger inhibitor of TOS and OSI, and had a smaller effect on SH. *Mahonia aquifolium* flowers extract had inhibitory effect on the oxidative stress, without influencing NO and lypoperoxides production, the effect being smaller than that of Diclofenac.

Keywords: inflammation, Mahonia, oxidative stress,

Introduction

Plant compounds are known to have antioxidant, anticancer and antimutagenic activity (Oneschuk and Younus, 2008) mainly due to the phenolic compounds content. Recent studies have established the importance of diet based phenolic agents in many diseases (Wong et al., 2009). Natural antioxidants from plants can be found in all plant parts (Shahidi and Ambigaipalan, 2015).

An antioxidant compound can be defined as a substance which, when present in low concentrations compared with those of an oxidable substrate, prevents pro-oxidant initiated oxidation of a substrate. Pro-oxidants are substances which can damage lipids, proteins and DNA and are synonyms with reactive oxygen species (ROS) (PRIOR and CAO, 2001).

Phenolic compounds have both antioxidant and pro-oxidant properties. The antioxidant ability is due to the reactive oxidant species (ROS) scavenging activities, with protection of intracellular structures against oxidative stress (Zhu et al., 2015). Thereby, phenolic compounds are beneficial in cancer, diabetes, cardiovascular and cerebrovascular diseases and osteoporosis (Marta et al., 2002) (Wang et al., 1996)strawberry had the highest ORAC activity (micromoles of Trolox equivalents per gram. Furthermore, they draw the attention due to the potential in preventing cancer with minimal toxicity and side effects (Wong et al., 2009) by acting as pro-oxidants that stimulate apoptosis and inhibit tumour growth (Zhu et al., 2015).

Plant derived compounds are able to stimulate both specific and non specific immunity, (Saha et al., 2014) Phenolic compounds have been found to prevent pathogen invasion, and favours the wound healing (Zhang and Tsao, 2016). Much importance was given to alkaloid berberine which seem to posses immunoregulatory activity (Neag et al., 2018).

Mahonia species were used in traditional medicine and have many pharmacological activities (Berberidaceae, 2015) like antibiotics, antioxidants, antimutagenic and motility inhibitors (Letašiová et al., 2006). In America, *Mahonia aquifolium* (MA) was used to treat fever, diarrhoea, dyspepsia, rheumatism and diseases affecting the kidney and liver (Goetz and Ghedira, 2014) and nowadays, MA extracts have demonstrated antioxidant, antibacterial, antifungal and anti-inflammatory properties (Wong et al., 2009) (Pyrkosz-Biardzka et al., 2014). The alkaloids from MA seem to be responsible for the anti-inflammatory and keratinocytes proliferation inhibition (He and Mu, 2015) which explains why MA has a good efficacy in psoriasis and atopic dermatitis (Gulliver and Donsky, 2005)global assessment, psoriasis history questionnaire, Dermatology Life Quality Index, and Psoriasis Disability Index. The results indicate statistically significant improvement in PASI score and Dermatology Life Quality Index after 4 weeks of treatment. This response continued 1 month after the end of treatment. Study 2 was a clinical trial of 32 patients with mild to moderate bilateral psoriasis treated up to 6 months. One side of the body received Mahonia and the other standard psoriatic treatment (eg, Dovonex cream.

Given all these information, the aim of this study was to evaluate whether MA flower extracts had antioxidant effects in rat experimental acute inflammation.

Material and methods

Plant material

Mahonia aquifolium (Pursh) Nutt flowers were obtained from the A. Borza Botanical Garden "Babes-Bolyai" University of Cluj-Napoca, Romania between April and June 2018. The ethanolic flowers extract 1: 1 (w:v) was obtained by using a modified Squibb's repercolation method in the Mycology Laboratory of Babes-Bolyai University, Cluj-Napoca, Romania (Parvu et al., 2014)1-diphenyl-2-picrylhydrazyl bleaching method (6.72\u00b10.44 g/mg DPPH.

Experimental design

For the study, 30 Wistar Bratislawa rats weighting 200-250g were used. The animals were purchased from Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca. Animal facility and were kept at 20^{o}C under standard lighting and humidity conditions. The rats were fed daily with special rat granules and had *ad libitum* water access. All the procedures and treatments performed in the laboratory were approved by the Institutional Animal Ethical Committee (IAEC) of the Iuliu Haţieganu University of Medicine and Pharmacy Cluj-Napoca, Romania (nr. 18/13.12.2016). The animals were randomly assigned to 6 groups (n=5). The tested MA flowers alcoholic extracts were administrated orally by gavage (1mL/animal) in three dilutions (100%, 50%, 25%) for seven days. The study groups were: Group 1 = Acute inflammation + 100% MA; Group 2 = Acute inflammation + 50% MA; Group 3 = Acute inflammation + 25% MA; Group 4 = Acute inflammation + Tap water (1mL/animal);Group 5 = Negative control group + Tap water (1mL/animal); Group 6 = Acute inflammation + Diclofenac (10mg/kg b.w. p.o) as an anti-inflammatory control was

also used. Acute experimental inflammation was induced by turpentine oil (0,6ml/kg b.w. i.m.) (Pillon et al., 2017) (Ramm and Mally, 2013)it appears that drug-independent risk factors that increase reactive metabolite formation or alter cellular stress and immune response may be critical determinants in the response to an otherwise non-toxic drug. Thus, we were interested to determine the impact of various drug-independent stress factors - lipopolysaccharide (LPS. In day 8 the blood was harvested by retro-orbital puncture under anaesthesia induced with 60 mg/kg b.w. ketamine and 15 mg/kg b.w. xylazine (Francischi et al., 2017) we have shown that injection of carrageenan, a standard proinflammatory stimulus, into the cheek (intra-oral injection. Serum was stored in –80°C until use. All the animals were used only once and they were killed by cervical dislocation immediately after the assay.

Antioxidant effect evaluation

To evaluate the *in vitro* antioxidant effects of MA flower extracts a 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was used (Andreicuţ et al., 2018).

To evaluate the *in vivo* antioxidant effects, serum total oxidant status (TOS), total antioxidant capacity (TAC), oxidative stress index (OSI), nitrites and nitrates (NOx), malondialdehyde (MDA) and total thiols (SH) were measured (Toiu et al., 2018).

Statistical analysis

Results were given as mean and standard deviation and the statistical analysis was performed using SPSS 16 (Inc, Chicago, IL, USA).

One way analysis of variance (ANOVA) test and Bonferroni-Holm posthoc test were used for group comparison, considering significant p values under 0.05.

Results and Discussions

The tests performed in the present study revealed antioxidant effects of the MA in acute experimental inflammation.

Oxidative stress is an unbalance between ROS and antioxidant defence (Ozben, 2007). ROS represent the oxygen derived molecules which are able to accept electrons and also oxidize other molecules (Sullivan and Chandel, 2014). These organic or inorganic compounds (Letašiová et al., 2006)(Ozben, 2007) can be free radicals (superoxide anion- O_2^-, hydroxyl radical OH^-, nitric oxide- NO, hydroperoxyl- HOO^-, peroxyl- ROO- and alkoxy radical RO^-) or non-radicals (hydrogen peroxide- H_2O_2, organic hydroperoxides- ROOH, hypochlorous acid- HOCl) (Storz, 2005). Lipids, proteins and DNA are targets of ROS damage (Reuter et al., 2010). Lipid peroxidation will change the membrane permeability and fluidity with secondary modifications in cellular integrity (Barrera, 2012), while protein oxidation will affect the enzymatic activity which may lead to cellular apoptosis or necrosis (Barrera, 2012). DNA damage by ROS is associated with errors in transcription, transduction, replication and genomic instability. All these processes may predispose to carcinogenesis (Valko et al., 2006) (Halliwell, 2007)(Bartsch and Nair, 2006) (Catoi et al., 2014).

Chronic inflammation is the substrate for degenerative diseases (Zhang and Tsao, 2016) and oxidative stress caused by ROS has a crucial role in those diseases (Shahidi and Ambigaipalan, 2015)(Pârvu et al., 2014). Flavonoids, phenolic acids, lignans, tocopherols and phospholipids are known to be natural antioxidant compounds (Shahidi and Ambigaipalan, 2015). There are a few mechanisms through which flavonoids reduce inflammation: they act as antioxidants (Shahidi and Ambigaipalan, 2015), they block the proinflammatory transduction pathways and interfere with oxidative stress pathways (Zhang and Tsao, 2016).

Among the alkaloids isolated from MA, berberine, jatrorhizine, magnoflorine, berbamine, oxychantine, armoline, baluchistine were found to posess antioxidant activity (Shaffique et al., 2018)(Bezáková et al., 1996). Jatrorhizine and magnoflorine are hidroxil alkaloids and they can act as strong peroxyl radicals collectors due to the content of a free phenolic group in the skeleton (Račková et al., 2004). Berbamine and oxychantine are strong inhibitors of lipooxigenase (Bezáková et al., 1996).

Our previous work showed that MA flower extract contains 3 phenolic acids (chlorogenic acid, p-coumaric acid, ferulic acid) and 2 flavonoids (isoquercetin, rutin) (Andreicut et al., 2018).

Clorogenic acid has antioxidant (Han et al., 2017) and anti-inflammatory properties through modulating the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Lou et al., 2016b). The antioxidant properties provide hepatoprotection and neuroprotection

through apoptosis and ROS reduction (Lou et al., 2016a) with beneficial effects in depression and epilepsy (Wu et al., 2016) (Aseervatham et al., 2016). Clorogenic acid is also a gastroprotector and it reduces lipid peroxidation and enhances NADH dehydrogenase activity through the antioxidant activity (Zhou et al., 2016). In allergic inflammation clorogenic acid suppresses eosinophil activation (Tsang et al., 2016). This phenolic acid has antiangiogenic effects through inhibition of Vascular endothelial growth factor (VEGF) and reduction of angiogenic markers (angiopoietin-2, matrix metalloproteinase-2 and focal adhesion kinase) (Lin et al., 2017)migration, invasion and tube formation in human umbilical vein endothelial cells (HUVECs).

P-coumaric acid has antioxidant (Lee et al., 2009) and anti-inflammatory properties (Luceri et al., 2007), it has free radical scavenging activity (Abdel-Wahab et al., 2003), prevents lipid peroxidation, (Lee et al., 2009) and has cardioprtective activity in the toxicity induced by Doxorubicin (Abdel-Wahab et al., 2003). P-coumaric acid reduces LDL cholesterol levels (Zang et al., 2000) and it is an antidiabetic molecule and suppresses adipogenesis via increased phosphorylation of 5' adenosine monophosphate-activated protein kinase (AMPK) (Janicke et al., 2005). It has also antithrombotic, (Luceri et al., 2007), and immunostimulatory activity (Pragasam et al., 2013). P-coumaric acid can be considered an antitumor molecule through cell cycle arrest in different phases (Janicke et al., 2005).

Ferulic acid has antioxidant (Sultana et al., 2005) anti-inflammatory and antiviral effects (Mathew and Abraham, 2004). It prevents protein oxidation (Kanski et al., 2002) and it reduces the levels of interleukin 8 (Il-8) (Mathew and Abraham, 2004). Thus, it has neuroprotective, (Sultana et al., 2005) cardio and hepatoprotective effects (Mathew and Abraham, 2004). This phenolic compound has beneficial effects in diabetes mellitus by reducing glycaemia, normalization of the associated dislipidemia, preventing further complications (Balasubashini et al., 2003)(Balasubashini et al., 2004). The antitumor effects of ferulic acid were observed in 4-nitroquinoline 1-oxide tongue carcinogenesis, skin tumour, pulmonary cancers in mice, (Morioka et al., 2005) and in the two stage initiation-promotion model in mouse skin (Huang et al., 1988).

Isoquercetin has antioxidant (Jung et al., 2010) with seven compounds (myricitrin, isoquercitrin, hypoletin-7-O-\u03b2-d-xylopyranoside, quercitrin, kaempferin, kaempferol, and amentoflavone anti-inflammatory (Rogerio et al., 2007) and antifungal activities (Yun et al., 2015). In this context, isoquercetin has the ability to scavenge ROS, peroxyl, peroxinitrate and hidroxil radicals (Katerina Valentová, Jirí Vrba, Martina Bancírová, Jitka Ulrichová, 2014). The antioxidant activity offers hepatoprotection, nephroprotection (Li et al., 2011) and neuroprotection (Jung et al., 2010)with seven compounds (myricitrin, isoquercitrin, hypoletin-7-O-\u03b2-d-xylopyranoside, quercitrin, kaempferin, kaempferol, and amentoflavone. The anti-inflammatory activities were observed in a murine model of asthma where isoquercetin reduced leukotriene induced bronchoconstriction (Rogerio et al., 2007) and in acute toxic hepatitis induced with acetaminophen (Xie et al., 2016)20, or 50 mg/kg. Furthermore, this flavonoid posses antitumor capacity in glioblastoma (Amado et al., 2009), pancreas (Chen et al., 2015) and colon carcinoma (Amado et al., 2014).

Rutin is a flavonoid with many pharmaceutical effects: antioxidant (Casa et al., 2000), anti-inflammatory, antiviral, antibacterial, antitumor, antialergic, antithrombotic (Janbaz et al., 2002), hypolipidic, cytoprotective, (Yang et al., 2008) and antiulcer (Casa et al., 2000). Rutin reduces the toxicity caused by paracetamol and CCl4 in the liver, (Janbaz et al., 2002) and in Cisplatin treatment, the antioxidant activity explains the nephroprotective effects (Alhoshani et al., 2017) the present study aimed to study the possible protective effect of rutin against nephrotoxicity induced by cisplatin in rats. METHODS Forty male Wistar albino rats were randomly divided into 4 groups. Rats of group 1 control group intraperitoneal (i.p.. In cardiovascular system, rutin improves the ischemic markers (TNF alpha, CRP, BNP), (Saklani et al., 2016) and it acts also as a blood pressure reducer by lowering nitrotyrosine immunorectivity (Ganeshpurkar and Saluja, 2017). The antioxidant potential of rutin was observed in Streptozotcin induced diabetes mellitus where it reduced the glycaemia values (Kamalakkannan and Prince, 2006).

All these phitochemical and pharmacological data suggested antioxidant effects of MA.

Various methods were described for measuring TOS (Andreicuţ et al., 2018).

TOS analysis showed that inflammation caused an important increase ($p<0.01$) of ROS production and diclofenac induced a significant reduction ($p<0.001$). Compared with the inflammation group, MA caused a reduction of TOS, MA50% having the best effect ($p=0.001$), while MA100% ($p=0.01$) and M25% ($p<0.01$) effects were smaller. MA effects were also smaller than those of diclofenac ($p<0.001$) (figure 1).

In a review published by nutritionists, TAC was defined as "cumulative action of all the antioxidants present in plasma and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants" (Sies, 2007). Thus, TAC represents the effect of all the antioxidants chain-breaking including uric acid and thiol groups (Ghiselli et al., 2000)thus providing an integrated parameter rather than the simple sum of measurable antioxidants. The capacity of known and unknown antioxidants and their synergistic interaction is therefore assessed, thus giving an insight into the delicate balance in vivo between oxidants and antioxidants. Measuring plasma AC may help in the evaluation of physiological, environmental, and nutritional factors of the redox status in humans. Determining plasma AC may help to identify conditions affecting oxidative status in vivo (e.g., exposure to reactive oxygen species and antioxidant supplementation. Therefore TAC can be useful in clinical status monitoring for patients with diabetes, cystic fibrosis, diabetes, phenylketonuria (Janaszewska and Bartosz, 2002).

Experimental inflammation ($p>0.05$) and diclofenac ($p>0.05$) had no effects on TAC. MA had also no important influence on TAC (figure 2).

OSI represents the ratio between TOS to TAC, (Varol et al., 2013). Inflammation increased OSI ($p<0.01$) and diclofenac reduced OSI ($p<0.001$). All MA dilutions were good inhibitors of OSI but diclofenac was better (figure 3).

NO is an oxidation product of l-arginine, mediated by NADPH and catalysed by NOS (Lo Faro et al., 2014). There are three isoforms of NOS: neuronal NOS (nNOS/ NOS1), inducible NOS (iNOS/ NOS2) and endothelial NOS (eNOS/ NOS3) (Fukumura et al., 2006). Highly reactive, NO is a vital effector involved in many biological processes, like smooth muscle relaxation with subsequent blood pressure regulation, immune defence, angiogenesis, apoptosis and neurotransmission (Valko et al., 2006) (Sahni et al., 2018)it is redox active and capable of generating reactive oxygen species (ROS (Cătoi et al., 2013).

Inflammation increased NOx synthesis ($p<0.001$) and diclofenac had no significant effect. All the MA dilutions had no important effect upon NOx synthesis as well (figure 4).

Lipid peroxidation is the result of nonenzymatic auto oxidation of polyunsaturated fatty acids and is involved in ageing, cancer, atherosclerosis, rheumatoid arthritis, inflammation, lupus, diabe-

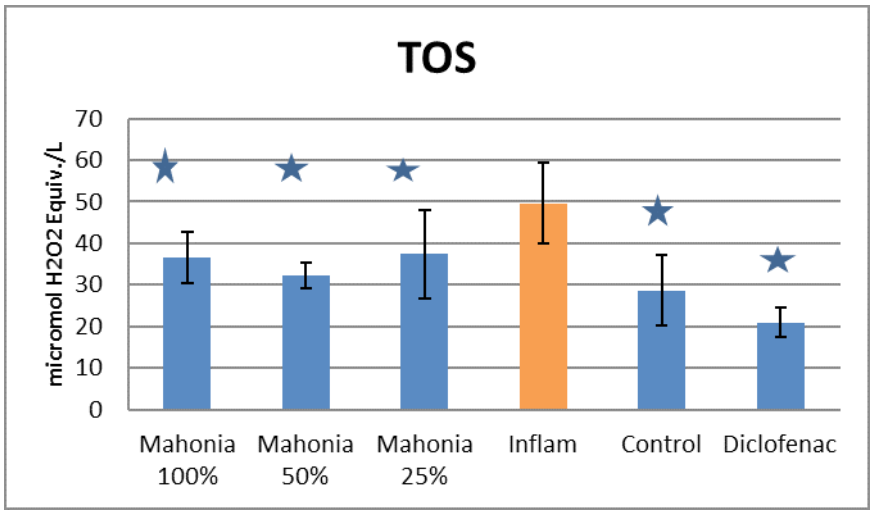

Figure 1. Total oxidative status of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

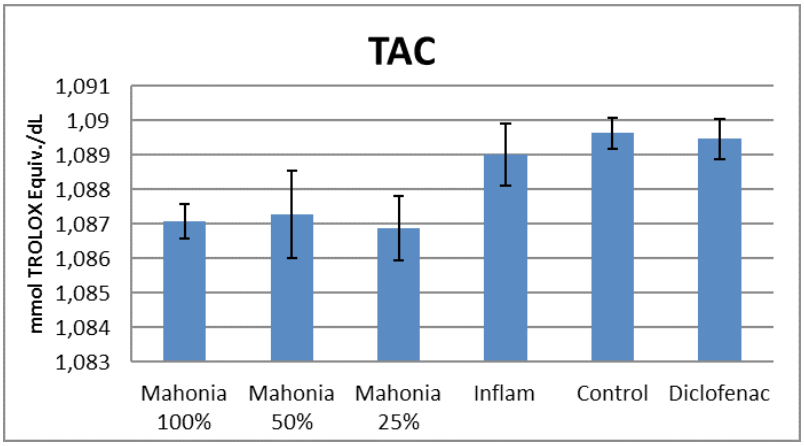

Figure 2. Total antioxidant capacity of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

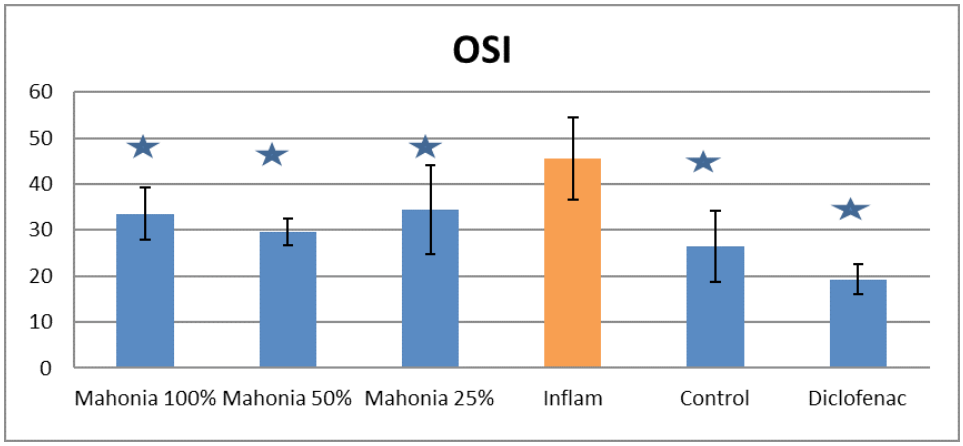

Figure 3. Oxidative stress index of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

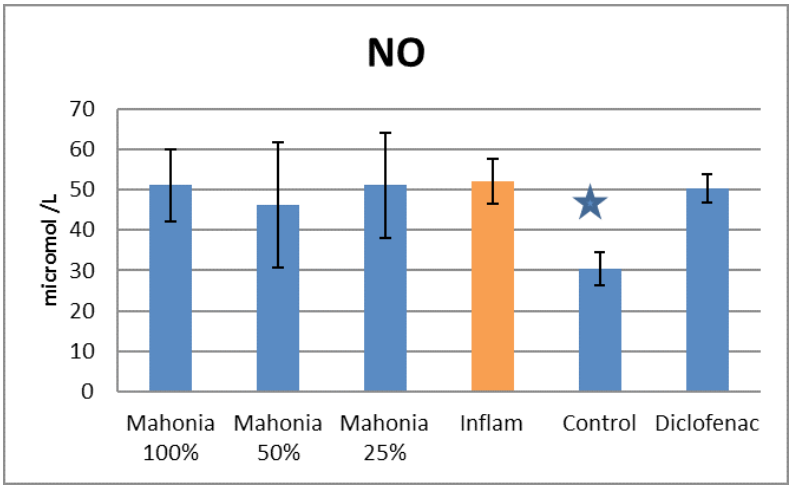

Figure 4. Nitrites and nitrates of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

tes mellitus and reperfusion injury (Gönenç et al., 2001). MDA is one of the major products formed after lipid hydroperoxides breakdown and has mutagenic and cytotoxic effects. MDA is a good marker of the presence of ROS induced lesions (Mateos et al., 2005).

Inflammation determined an important increase in MDA production ($p<0.001$) and diclofenac caused an significant reduction ($p<0.001$). All MA dilutions had no significant inhibitory effect ($p>0.05$) upon MDA production (figure 5).

Total thiols are the major plasma antioxidants in the human body. They are organic compounds containing a sulfhydryl group and albumin represents more than 50% from the total plasma proteins (Prakash et al., 2009). An unbalance of total thiols was found in many diseases, like rheumatoid arthritis, chronic heart disease, cancer, diabetes, Parkinson's and Alzheimer's diseases (Wei Chen, Yong Zhao, Teresa Seefeldt, 2008).

SH was reduced by inflammation ($p=0.05$) and diclofenac had no significant effects. All MA dilutions had a good stimulatory activity on SH, MA100% ($p=0.001$) being better than MA50% ($p<0.01$) and MA25% ($p<0.01$) (figure 6).

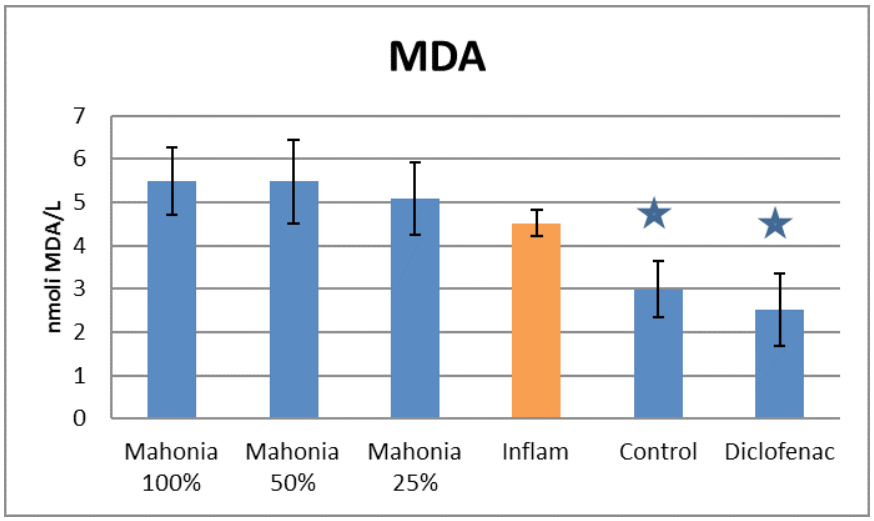

Figure 5. Malondialdehyde of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

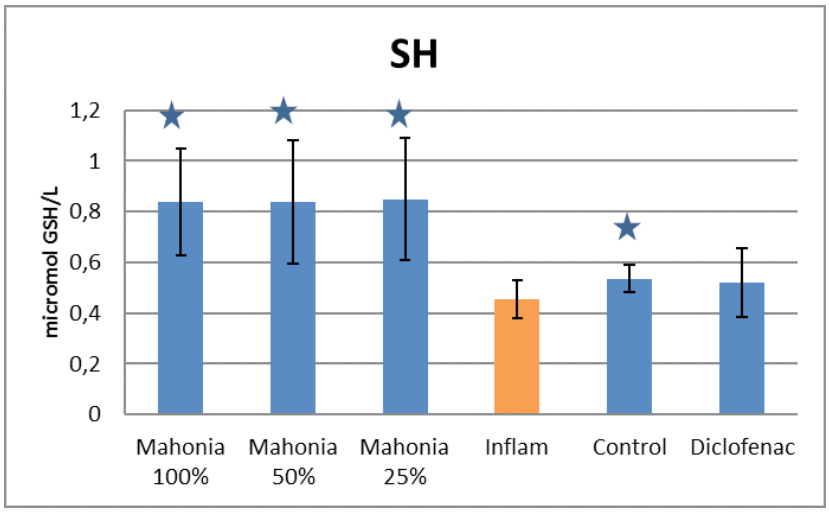

Figure 6. Thiols of the study groups.
Mahonia = *Mahonia aquifolium* flowers ethanolic extract.

Conclusions

The present results and the previous phytochemical analysis helped us to conclude:

- *M. aquifolium* flower alcoholic extract had anti-inflamatory effect by reducing oxidative stress.
- *M. aquifolium* flower alcoholic extract had antioxidant properties by reducing OSI and TOS, and increasing SH.
- *M. aquifolium* flower alcoholic extract had no important effect upon NO and MDA synthesis.
- The best antioxidant effect was obtained with *M. aquifolium* flower alcoholic extract 50%.
- *M. aquifolium* flower alcoholic extract antioxidant effects were smaller than those of diclofenac.

Conflict of interest

None of the authors has any conflict of interest that could affect the performance of the work or the interpretation of the data.

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