

An Hydroalcoholic Chamomile Extract Modulates Inflammatory and Immune Response in HT29 Cells and Isolated Rat Colon

Questa è la versione Post print del seguente articolo:

Original

An Hydroalcoholic Chamomile Extract Modulates Inflammatory and Immune Response in HT29 Cells and Isolated Rat Colon / Menghini, L.; Ferrante, Maria Carmela; Leporini, L.; Recinella, L.; Chiavaroli, A.; Leone, Silvio; Pintore, G.; Vacca, M.; Orlando, G.; Brunetti, L.. - In: PHYTOTHERAPY RESEARCH. - ISSN 0951-418X. - 30:9(2016), pp. 1513-1518. [10.1002/ptr.5655]

Availability:

This version is available at: 11388/224497 since: 2022-06-07T11:02:36Z

Publisher:

Published

DOI:10.1002/ptr.5655

Terms of use:

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

Publisher copyright

note finali coverpage

(Article begins on next page)



**AN IDROALCHOLIC CHAMOMILE EXTRACT MODULATES
INFLAMMATORY AND IMMUNE RESPONSE IN ISOLATED RAT
COLON CHALLENGED WITH LPS**

Journal:	<i>Phytotherapy Research</i>
Manuscript ID	PTR-16-0318
Wiley - Manuscript type:	Full Paper
Date Submitted by the Author:	09-Mar-2016
Complete List of Authors:	Menghini, Luigi; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Ferrante, Claudio; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Leporini, Lidia; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Recinella, Lucia; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Chiavaroli, Annalisa; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Leone, Sheila; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy PINTORE, GIORGIO; university, chemistry and paharmacy Vacca, Michele; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Orlando, Giustino; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy Brunetti, Luigi; University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy
Keyword:	Colon, Immunomodulation, Chamomile, Inflammation, Oxidative Stress

SCHOLARONE™
Manuscripts

1
2
3 **AN IDROALCHOLIC CHAMOMILE EXTRACT MODULATES INFLAMMATORY AND**
4 **IMMUNE RESPONSE IN ISOLATED RAT COLON CHALLENGED WITH LPS**
5

6 Menghini Luigi^{1*}, Ferrante Claudio¹, Leporini Lidia¹, Recinella Lucia¹, Chiavaroli Annalisa¹,
7 Leone Sheila¹, Pintore Giorgio², Vacca Michele¹, Orlando Giustino¹, Brunetti Luigi¹
8

9 ¹ Dipartimento di Farmacia, Università “G. D’Annunzio” Chieti-Pescara, Via dei Vestini 31, 66100
10 Chieti, Italy;
11

12 ² Dipartimento di Chimica e Farmacia, Università di Sassari, Via Muroni 23, 07100 Sassari, Italy;
13
14

15
16 *Mailing address:*

17 Prof. Luigi Menghini,
18 Dipartimento di Farmacia,
19 Università “G. D’Annunzio” Chieti-Pescara,
20 Via dei Vestini 31,
21 66100 Chieti, Italy;
22 Tel.: +39 0871 3554655
23 Fax: +39 0871 3554912
24 e-mail: lmenghini@unich.it
25
26
27
28
29
30
31

32 *Key words: Colon, Immunomodulation, Chamomile, Inflammation, Oxidative Stress*
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Chamomile modulate colonic inflammation

ABSTRACT

Inflammatory bowel diseases (IBDs) are chronic disorders characterized by disruption and ulceration of the colonic mucosa or of any part of the digestive tract (Crohn's disease). Antioxidant/anti-inflammatory herbal extract supplementation could represent an innovative approach to contrast IBDs. Clinical trials demonstrated the efficacy of natural formulas, containing chamomile, in patients with gastrointestinal disorders. This is consistent, albeit in part, with the antioxidant and anti-inflammatory properties of chamomile. The aim of the present study was to explore the possible protective role of a chamomile extract, on human colorectal adenocarcinoma HT29 cell line, and isolated rat colon specimens treated with lipopolysaccharide (LPS) to induce an inflammatory stimulus, a well established model of acute ulcerative colitis. In this context, the activities of different biomarkers of inflammation and lipid peroxidation such as ROS, myeloperoxidase (MPO), serotonin (5-HT), prostaglandin (PG)E₂, 8-iso-prostaglandin (8-iso-PGF)_{2α}, tumor necrosis factor (TNF)α and interleukin (IL)-6 were assessed. We found that chamomile extract was as effective as sulfasalazine (5 mM) in reducing the production of MPO, 5-HT, IL-6, TNFα, PGE₂ and 8-iso-PGF_{2α}, after inflammatory stimulus. The observed modulatory effects support a rationale use of chamomile supplementation as a promising pharmacological tool for the prevention and management of ulcerative colitis in humans.

INTRODUCTION

Inflammatory bowel diseases (IBDs) are chronic disorders characterized by disruption and ulceration of the colonic mucosa (ulcerative colitis) or of any part of the digestive tract (Crohn's disease). Although IBDs etiology is still a matter of debate, oxidative stress seems to play a pivotal role (Rezaie et al., 2007; Achitei et al., 2013; Koutroubakis 2004). On the other hand, antioxidant/anti-inflammatory herbal extract supplementation could represent an innovative approach to contrast IBDs symptoms (Chung et al 2007, Lenoir et al., 2012).

Chamomile has long been used as a medicinal plant in the management of gastrointestinal disorders (McKay et al, 2006). The rationale for the traditional use has been recently corroborated by multiple clinical trials (Langhorst et al., 2013; Albrecht et al., 2014). This is consistent, albeit in part, with the antioxidant and anti-inflammatory properties of chamomile (McKay et al, 2006; Drummond et al., 2013).

The aim of the present study was to explore the possible protective role of chamomile on human colorectal adenocarcinoma HT29 cell line, and isolated rat colon specimens treated with lipopolysaccharide (LPS) to induce an inflammatory stimulus, a well established model of acute ulcerative colitis. (Bahar et al., 2012). In this context, the activities of different biomarkers of colon inflammation and lipid peroxidation such as ROS, myeloperoxidase (MPO), serotonin (5-HT), prostaglandin (PG)E₂, 8-iso-prostaglandin (8-iso-PG)F_{2α} were assessed (Nagib et al., 2013; Motavallian et al., 2013; Regmi et al., 2014). Finally, we evaluated the immune response modulatory effects of chamomile, by measuring the mRNA levels of cytokines playing a key role in colon epithelium damage, such as tumor necrosis factor (TNF)α and interleukin (IL)-6 (Feghali et al., 1997; Lee et al., 2010).

MATERIAL AND METHODS

PLANT EXTRACT

Chamomile extract were kindly furnished by Aboca S.p.A., It consists of freeze-dried extract obtained from ligulate flowers collected from cultivated plants of *Chamomilla recutita* (L.) Rauschert. Solvent, temperature, plant-solvent weight ratio and extraction process are optimized for active principles recovery and stability, and the resulting extract is characterized by high flavonoid content. Phytochemical composition of final extracts is described in table I.

(Please insert table I)

IN VITRO STUDIES

Cell culture and viability test

HT29 cells were cultured in DMEM (Euroclone) supplemented with 10% (v/v) heat-inactivated fetal bovine serum and 1.2% (v/v) penicillin G/streptomycin in 75 cm² tissue culture flask (n = 5 individual culture flasks for each condition). The cultured cells were maintained in humidified incubator with 5% CO₂ at 37 °C. For cell differentiation, HT29 cell suspensions at a density of 1 × 10⁶ cells/ml were treated with various doses (10, 50, and 100 ng/ml) of phorbol myristate acetate (PMA, Fluka) for 24 h or 48 h (induction phase). Thereafter, the PMA-treated cells were washed twice with ice-cold pH 7.4 phosphate buffer solution (PBS) to remove PMA and non-adherent cells, whereas the adherent cells were further maintained for 48 h (recovery phase). Morphology of cells

1
2
3 was examined under an inverted phase-contrast microscope (Sintiprungrat et al., 2010). To assess
4 the basal cytotoxicity of chamomile, a viability test was performed on 96 microwell plates, using 3-
5 (4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) test. Macrophages were
6 incubated with extracts (ranging concentration 10-1000 µg/ml) for 24 h. About 10 µL of MTT (5
7 mg/mL) was added to each well and incubated for 3 h. The formazan dye formed was extracted
8 with dimethyl sulfoxide and absorbance recorded as previously described (Menghini et al., 2011).
9 Effects on cell viability were evaluated in comparison to untreated control group.
10
11

12 ROS generation

13
14 ROS generation was assessed using a ROS-sensitive fluorescence indicator, DCFH-DA. When
15 DCFH-DA is introduced to viable cells, it can penetrate the cell and become deacetylated by
16 intracellular esterases to form 2',7'-dichlorodihydrofluorescein (DCFH), which can react
17 quantitatively with ROS within the cell, and be converted to 2',7'-dichlorofluorescein (DCF), which
18 is detected by a fluorescence spectrophotometer. To determine intracellular effects on ROS
19 production, synaptosomes were seeded in a black 96-well plate (1.5 x 10⁴ cells/well) in medium
20 containing scalar concentration of extracts. Immediately after seeding, the synaptosomes were
21 stimulated for 1 h with H₂O₂ (1 mM). After the cells were incubated with DCFH-DA (20 µM) for
22 30 min, the fluorescence intensity was measured at an excitation wavelength of 485 nm and an
23 emission wavelength of 530 nm, using a fluorescence microplate reader.
24
25
26
27

28 EX VIVO STUDIES

29
30 24 male adult Sprague-Dawley rats (200-250 g) were housed in plexiglas cages (40 cm × 25 cm ×
31 15 cm), one rat per cage, in climatized colony rooms (22±1°C; 60% humidity), on a 12 h/12 h
32 light/dark cycle (light phase: 07:00 – 19:00 h), with free access to tap water and food, 24 h/day
33 throughout the study, with no fasting periods. Rats were fed a standard laboratory diet (3.5% fat,
34 63% carbohydrate, 14% protein, 19.5% other components without caloric value; 3.20 kcal/g).
35 Housing conditions and experimentation procedures were strictly in accordance with the European
36 Union ethical regulations on the care of animals for scientific research. According to the recognized
37 ethical principles of “Replacement, Refinement and Reduction of Animals in Research”, colon
38 specimens were obtained as residual material from vehicle-treated rats randomized in our previous
39 experiments approved by Local Ethical Committee (G. d’Annunzio University) and Italian Health
40 Ministry.
41

42 Rats were sacrificed by CO₂ inhalation (100 % CO₂ at a flow rate of 20 % of the chamber volume
43 per minute) and colon specimens were immediately collected and maintained in humidified
44 incubator with 5% CO₂ at 37 °C for 4 h, in DMEM buffer with added bacterial LPS (10 µg/ml)
45 (incubation period). During the incubation period, tissues were treated with scalar sub-toxic
46 concentrations of chamomile extract (100-1000 µg/ml). The efficacy of chamomile extract was
47 evaluated in comparison with sulfasalazine (5 mM). Tissue perfusates were collected and PGE₂ and
48 8-iso-PGF_{2α} levels (ng/mg wet tissue) were measured by radioimmunoassay (RIA), as previously
49 reported (Chiavaroli et al., 2010; Menghini et al., 2010). On the other hand, individual colon
50 specimens were dissected and subjected to extractive procedures to evaluate MPO activity (mU/mg
51 wet tissue), 5-HT steady state level (ng/mg wet tissue), IL-6 and TNFα gene expression, as
52 previously reported (Krawisz et al., 1984; Brunetti et al., 2013).
53
54
55
56
57
58
59
60

STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA). Means \pm S.E.M. were determined for each experimental group and analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keuls comparison multiple test. Statistical significance was set at $P < 0.05$. As regards to gene expression analysis, the comparative $2^{-\Delta\Delta Ct}$ method was used to quantify the relative abundance of mRNA and then determine the relative changes in individual gene expression (relative quantification) (Livak et al., 2001). Finally, as regards to the animals randomized for each experimental group, the number was calculated on the basis of the "Resource Equation" $N = (E+T)/T$ ($10 \leq E \leq 20$) elaborated by the "National Centre for the Replacement, Refinement and Reduction of Animals in Research" (NC3RS) and reported on the following web site: <https://www.nc3rs.org.uk/experimental-designstatistics>.

RESULTS

Our *in vitro* study showed that chamomile extract was well tolerated by HT29 cell line in the range (10-1000 $\mu\text{g/ml}$) (Fig.1). Moreover, we observed a dose-dependent protective effect exerted by chamomile extract (10-1000 $\mu\text{g/ml}$) as revealed by the significant reduction of H_2O_2 -induced (1 mM) ROS production (Fig.1).

(Please insert Figs.1-2)

The preliminary *in vitro* test revealed a valuable index of non-toxic and effective doses to define the concentration for colon tissue treatment. In the *ex vivo* experiments, in colon tissues exposed to LPS-induced inflammatory stimulus, we found that chamomile extract (100-1000 $\mu\text{g/ml}$) was effective in reducing the oxidative stress, inflammation and immune response biomarkers, such as MPO, 5-HT, IL-6, $\text{TNF}\alpha$, PGE_2 and 8-iso- $\text{PGF}_{2\alpha}$ (Figs. 3-8). The efficacy was comparable to sulfasalazine (5 mM).

(Please insert Figs. 3-8)

DISCUSSION

Oxidative stress is an imbalance in the pro-oxidant/antioxidant homeostasis, characterized by overproduction of reactive oxygen/nitrogen species (ROS/RNS) that could drive to disruptive peroxidation reactions on cellular substrates such as proteins, lipids, and nucleic acids (Uttara et al., 2009). In particular, lipid peroxidation has been recognized as a crucial step in the pathogenesis of several disease states, including IBDs (Achitei et al., 2013). ROS and RNS are mainly produced by macrophages and neutrophils, and the effects of these reactive species include neutrophil recruitment at the inflamed epithelial colon tissue. 8-iso- $\text{PGF}_{2\alpha}$, deriving from ROS/RNS peroxidation of membrane arachidonic acid, represents a stable marker of oxidative stress, *in vivo* (Praticò et al., 2002), and our experiments demonstrate that 8-iso- $\text{PGF}_{2\alpha}$ production is reduced in inflamed rat colon (Fig.4), after administration of chamomile extracts. This could partially derive by the radical scavenging activity of chamomile (Lee et al., 2002), that was confirmed by our evaluations on HT29 cell line exposed to H_2O_2 (1 mM)-induced oxidative stress (Fig.2), and could explain the observed inhibitory effect on MPO activity (Fig.3), a biomarker of neutrophil infiltration (Talero et al., 2007). We also found a significant reduction in PGE_2 levels in the

1
2
3 chamomile-treated colons (Fig.4). This reduction could be, albeit partially, related to a possible
4 inhibitory effect on cyclooxygenase 2 activity, as previously suggested by Srivastava and
5 colleagues (Srivastava et al., 2009). Finally, we tested the modulatory effects of chamomile on
6 colonic TNF α , IL-6 and 5-HT production. 5-HT pro-inflammatory role in IBDs has been previously
7 suggested (Regmi et al., 2014), possibly involving the activation of 5-HT₃ receptors
8 (Mousavizadeh et al., 2009). Our results showed that chamomile extracts are able to reduce colonic
9 5-HT levels (Fig.6). Multiple comparative studies confirmed that neurotransmitter steady state level
10 is a valuable index of neurotransmitter release in vivo (Brunetti et al., 2014). Actually, our findings
11 of reduced colon 5-HT levels induced by chamomile extract is consistent with the apigenin-induced
12 inhibition of gut 5-HT release, in vitro (Zhao et al., 2010). In ulcerative colitis, the infiltration of
13 intestinal mucosa by macrophages and neutrophils also enhances the local levels of pro-
14 inflammatory cytokines, such as TNF α and IL-6, which are known to play a key role in mediating
15 tissue damage (Bounguen et al., 2011). In this context, we have investigated the possible immune-
16 modulatory effects of chamomile extracts, finding a significant inhibition of both basal and LPS-
17 induced TNF α and IL-6 activity in colon specimens, as revealed by the reduction of their mRNA
18 levels (Fig.7-8). These data corroborate the previous reported inhibitory effects induced by both
19 apigenin and chamomile extracts on cytokine production, in vitro (McKay et al., 2006; Drummond
20 et al., 2013).

21
22 The inhibitory effects exerted by chamomile extract on the tested biomarkers are consistent with the
23 observed positive clinical effects induced by chamomile herbal formulations on IBDs symptoms
24 (Langhorst et al., 2013; Albrecht et al., 2014).

25
26 In conclusion, in the present work we have investigated the possible efficacy of a commercial
27 chamomile extract in modulating the inflammatory and immune response, in an ex vivo
28 experimental model of IBD (Bahar et al., 2012). The observed modulatory effects support a
29 rationale use of chamomile supplementation as a promising pharmacological tool for the prevention
30 and management of ulcerative colitis in humans. Since each technique measures something
31 different and has its own inherent limitations, further investigations, comparing different analytical
32 methods and experimental paradigms for detection and quantification of oxidative stress,
33 inflammation and immune response biomarkers are required for an accurate evaluation of
34 chamomile efficacy.

35 36 37 38 39 40 41 42 ACKNOWLEDGEMENTS

43
44 This work was supported by grants from the Italian Ministry of University.

45
46 *This work is dedicated to the loving memory of Professor Giovanni Ciabattini (1951-2014), man of*
47 *science, culture and humanity, to whom the authors would once again express their gratitude for*
48 *his precious and priceless teachings.*

49 50 51 52 53 CONFLICT OF INTEREST

54
55 Authors declare no financial/commercial conflicts of interest.

56 57 58 59 REFERENCES

1
2
3 Achitei D, Ciobica A, Balan G, Gologan E, Stanciu C, Stefanescu G. 2013. Different profile of
4 peripheral antioxidant enzymes and lipid peroxidation in active and non-active inflammatory bowel
5 disease patients. *Dig Dis Sci* 58(5): 1244-1249.
6

7
8 Albrecht U, Müller V, Schneider B, Stange R. 2014. Efficacy and safety of a herbal medicinal
9 product containing myrrh, chamomile and coffee charcoal for the treatment of gastrointestinal
10 disorders: a non-interventional study. *BMJ Open Gastroenterol* 1(1): e000015.
11

12
13 Bahar B, O'Doherty JV, Hayes M, Sweeney T. 2012. Extracts of brown seaweeds can attenuate the
14 bacterial lipopolysaccharide-induced pro-inflammatory response in the porcine colon ex vivo. *J*
15 *Anim Sci* 90(Suppl 4): 46-48.
16

17
18 Bouguen G, Chevaux JB, Peyrin-Biroulet L. 2011. Recent advances in cytokines: therapeutic
19 implications for inflammatory bowel diseases. *World J Gastroenterol* 17(5): 547-556.
20

21
22 Brunetti L, Orlando G, Ferrante C, Recinella L, Leone S, Chiavaroli A, Di Nisio C, Shohreh R,
23 Manippa F, Ricciuti A, Vacca M. 2013. Orexigenic effects of omentin-1 related to decreased CART
24 and CRH gene expression and increased norepinephrine synthesis and release in the hypothalamus.
25 *Peptides* 44: 66-74.

26
27 Brunetti L, Orlando G, Ferrante C, Recinella L, Leone S, et al. 2014. Peripheral chemerin
28 administration modulates hypothalamic control of feeding. *Peptides* 51: 115-121.
29

30
31 Chiavaroli A, Brunetti L, Orlando G, Recinella L, Ferrante C, Leone S, Di Michele P, Di Nisio C,
32 Vacca M. 2010. Resveratrol inhibits isoprostane production in young and aged rat brain. *J Biol*
33 *Regul Homeost Agents* 24(4): 441-446.

34
35 Chung HL, Yue GG, To KF, Su YL, Huang Y, Ko WH. 2007. Effect of Scutellariae Radix extract
36 on experimental dextran-sulfate sodium-induced colitis in rats. *World J Gastroenterol* 13(42): 5605-
37 5611.
38

39
40 Drummond EM, Harbourne N, Marete E, Martyn D, Jacquier J, O'Riordan D, Gibney ER. 2013.
41 Inhibition of proinflammatory biomarkers in THP1 macrophages by polyphenols derived from
42 chamomile, meadowsweet and willow bark. *Phytother Res* 27(4): 588-594.
43

44
45 Feghali CA, Wright TM. 1997. Cytokines in acute and chronic inflammation. *Front Biosci* 2: 12-26.
46

47
48 Koutroubakis IE, Malliaraki N, Dimoulios PD, Karmiris K, Castanas E, Kouroumalis EA. 2004.
49 Decreased total and corrected antioxidant capacity in patients with inflammatory bowel disease. *Dig*
50 *Dis Sci* 49(9): 1433-1437.
51

52
53 Krawisz JE, Sharon P, Stenson WF. 1984. Quantitative assay for acute intestinal inflammation
54 based on myeloperoxidase activity Assessment of inflammation in rat and hamster models.
55 *Gastroenterology* 87(6): 1344-1350.
56

57
58 Langhorst J, Varnhagen I, Schneider SB, Albrecht U, Rueffer A, Stange R, Michalsen A, Dobos GJ.
59 2013. Randomised clinical trial: a herbal preparation of myrrh, chamomile and coffee charcoal
60 compared with mesalazine in maintaining remission in ulcerative colitis--a double-blind, double-
dummy study. *Aliment Pharmacol Ther* 38(5): 490-500.

1
2
3 Lee JS, Park SY, Thapa D, Choi MK, Chung IM, Park YJ, Yong CS, Choi HG, Kim JA. 2010.
4 Grifola frondosa water extract alleviates intestinal inflammation by suppressing TNF-alpha
5 production and its signaling. *Exp Mol Med* 42(2): 143-154.
6

7
8 Lee KG, Shibamoto T. 2002. Determination of antioxidant potential of volatile extracts isolated
9 from various herbs and spices. *J Agric Food Chem* 50(17): 4947-4952.
10

11 Lenoir L, Joubert-Zakeyh J, Texier O, Lamaison JL, Vasson MP, Felgines C. 2012. Aloysia
12 triphylla infusion protects rats against dextran sulfate sodium-induced colonic damage. *J Sci Food*
13 *Agric.* 92(7): 1570-1572.
14

15 Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time
16 quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408.
17

18
19 McKay DL, Blumberg JB. 2006. A review of the bioactivity and potential health benefits of
20 chamomile tea (*Matricaria recutita* L). *Phytother Res* 20(7): 519-530.
21

22 Menghini L, Genovese S, Epifano F, Tirillini B, Ferrante C, Leporini L. 2010. Antiproliferative,
23 protective and antioxidant effects of artichoke, dandelion, turmeric and rosemary extracts and their
24 formulation. *Int J Immunopathol Pharmacol* 23(2): 601-610.
25

26
27 Menghini L, Leporini L, Scanu N, Pintore G, La Rovere R, Di Filippo ES, Pietrangelo T, Fulle S.
28 2011. Effect of phytochemical concentrations on biological activities of cranberry extracts. *J Biol*
29 *Regul Homeost Agents* 25: 27-35.
30

31 Mousavizadeh K, Rahimian R, Fakhfour G, Aslani FS, Ghafourifar P. 2009. Anti-inflammatory
32 effects of 5-HT receptor antagonist, tropisetron on experimental colitis in rats. *Eur J Clin Invest*
33 39(5): 375-383.
34

35
36 Nagib MM, Tadros MG, ElSayed MI, Khalifa AE. 2013. Anti-inflammatory and anti-oxidant
37 activities of olmesartan medoxomil ameliorate experimental colitis in rats. *Toxicol Appl Pharmacol*
38 271(1): 106-113.
39

40
41 Praticò D. 2002. Alzheimer's disease and oxygen radicals: new insights. *Biochem Pharmacol* 63(4):
42 563-567.
43

44 Regmi SC, Park SY, Ku SK, Kim JA. 2014. Serotonin regulates innate immune responses of colon
45 epithelial cells through Nox2-derived reactive oxygen species. *Free Radic Biol Med* 69: 377-389.
46

47 Rezaie A, Parker RD, Abdollahi M. 2007. Oxidative stress and pathogenesis of inflammatory bowel
48 disease: an epiphenomenon or the cause?. *Dig Dis Sci* 52(9): 2015-2021.
49

50
51 Sintiprungrat K, Singhto N, Sinchaikul S, Chen ST, Thongboonkerd V. 2010. Alterations in cellular
52 proteome and secretome upon differentiation from monocyte to macrophage by treatment with
53 phorbol myristate acetate: insights into biological processes. *J Proteomics* 73: 602-618.
54

55
56 Srivastava JK, Pandey M, Gupta S. 2009. Chamomile, a novel and selective COX-2 inhibitor with
57 anti-inflammatory activity. *Life Sci.* 85(19-20): 663-669.
58
59
60

1
2
3 Talero E, Sánchez-Fidalgo S, Calvo JR, Motilva V. 2007. Chronic administration of galanin
4 attenuates the TNBS-induced colitis in rats. *Regul Pept* 141(1-3): 96-104.
5

6 Uttara B, Singh AV, Zamboni P, Mahajan RT. 2009. Oxidative stress and neurodegenerative
7 diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr*
8 *Neuropharmacol* 7(1): 65-74.
9

10 Zhao G, Qin GW, Wang J, Chu WJ, Guo LH. 2010. Functional activation of monoamine
11 transporters by luteolin and apigenin isolated from the fruit of *Perilla frutescens* (L) Britt.
12 *Neurochem Int* 56(1): 168-176.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Figure 1

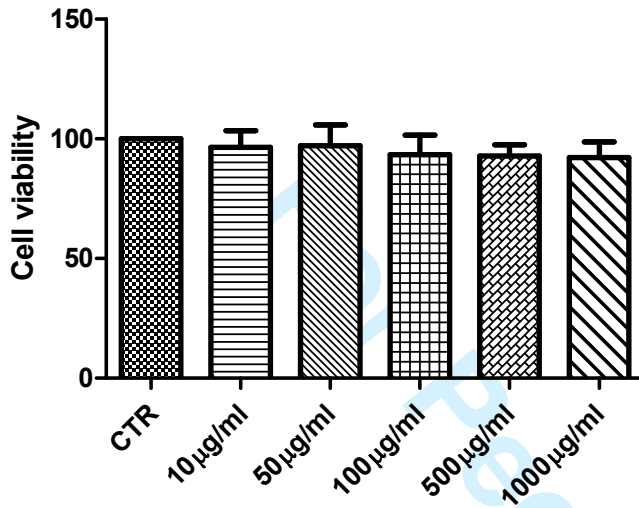


Figure 1: Effect of chamomile extract (10-1000 µg/ml) on HT29 cell line viability.

Figure 2

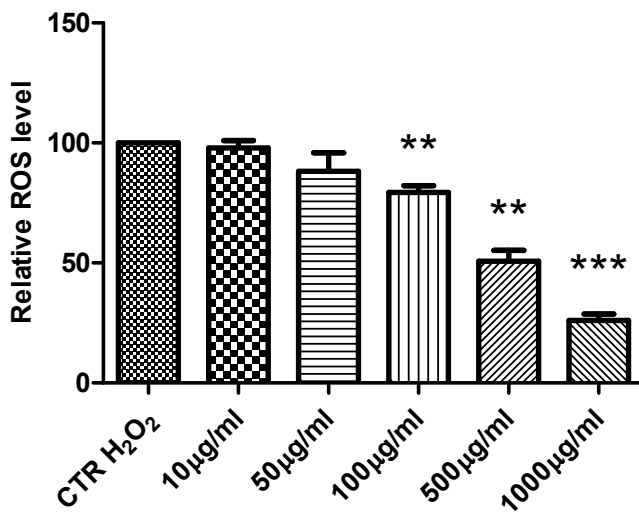


Figure 2: : Effect of chamomile extract (10-1000 $\mu\text{g/ml}$) on H_2O_2 ROS production in HT29 cell line. ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs H_2O_2 -treated group

Figure 3

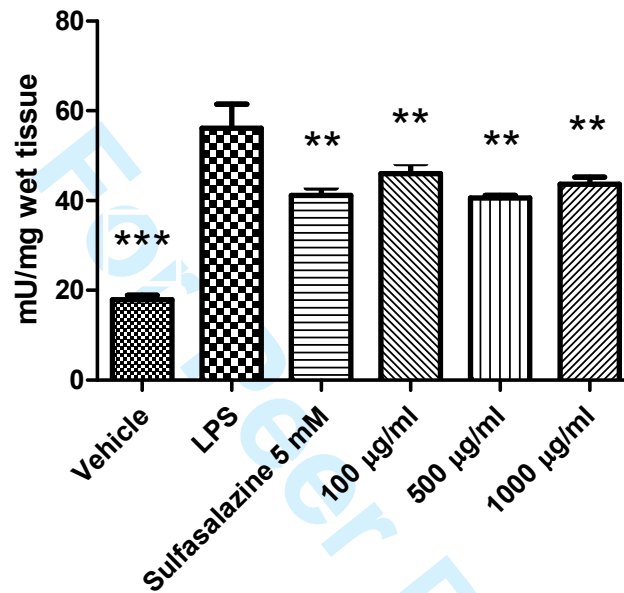


Figure 3: Effect of chamomile extract (100-1000 $\mu\text{g/ml}$) on mieloperoxidase (MPO) activity (mU/ng wet tissue). ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs LPS-treated group.

Figure 4

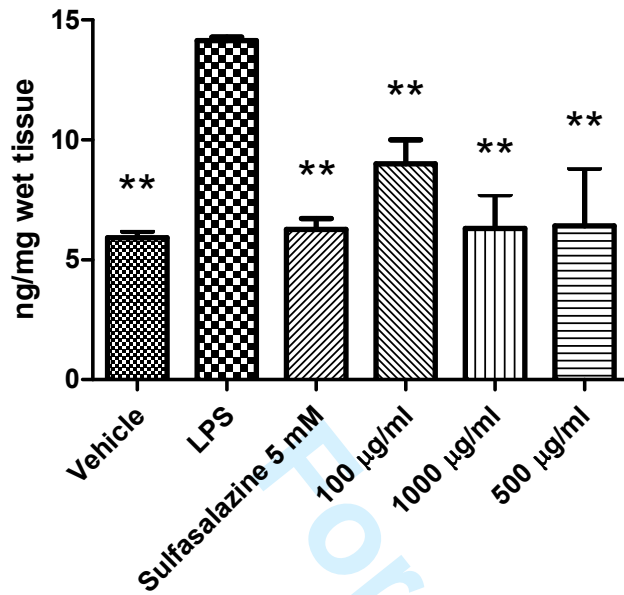


Figure 4: Effect of chamomile extract (100-1000 µg/ml) on prostaglandin E₂ (PGE₂) levels. ANOVA, $P < 0.01$, post hoc $**P < 0.01$ vs LPS-treated group.

Figure 5

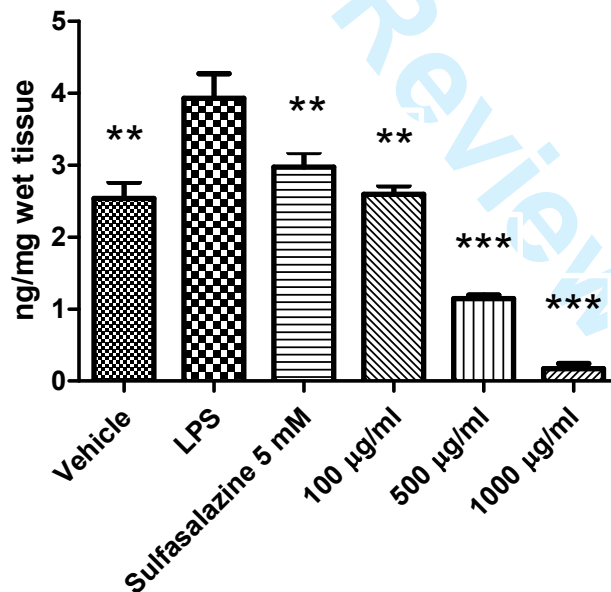


Figure 5: Effect of chamomile extract (100-1000 µg/ml) on 8-iso-prostaglandin F_{2α} (8-iso-PGF_{2α}) levels (ng/mg wet tissue). ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs LPS-treated group.

Figure 6

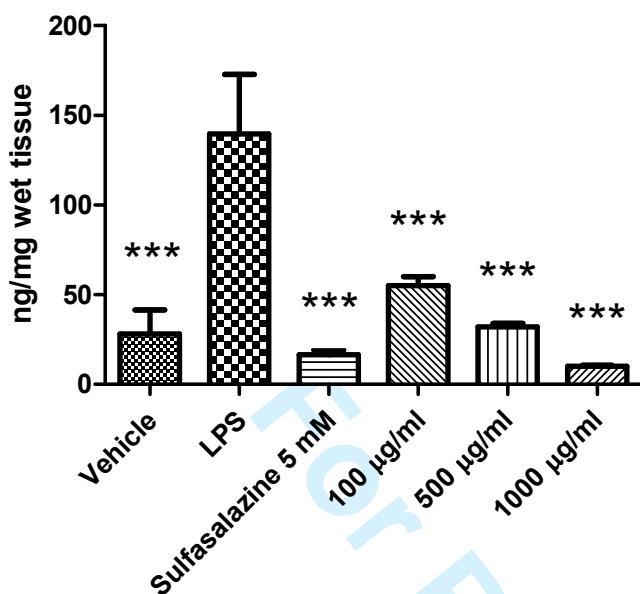


Figure 6: Effect of chamomile extract (100-1000 µg/ml) on serotonin (5-HT) levels (ng/mg wet tissue). ANOVA, $P < 0.01$, post hoc $***P < 0.001$ vs LPS-treated group.

Figure 7

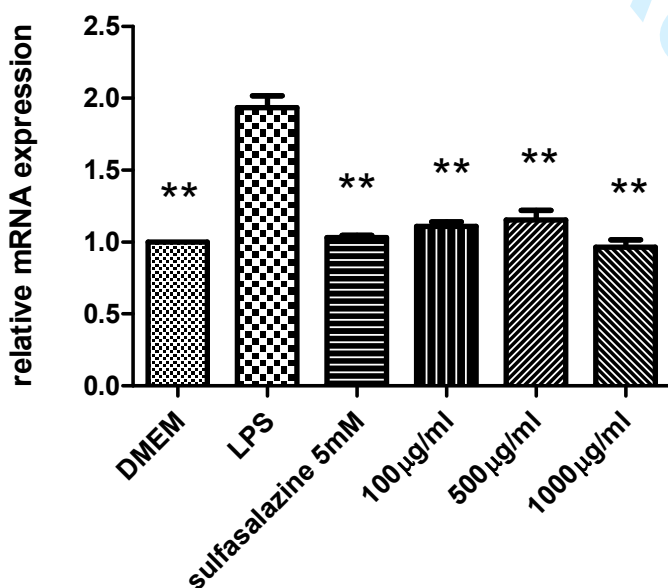


Figure 7: Effect of chamomile extract (100-1000 µg/ml) on tumor necrosis factor α (TNF α) and gene expression. ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs respective LPS-treated group.

Figure 8

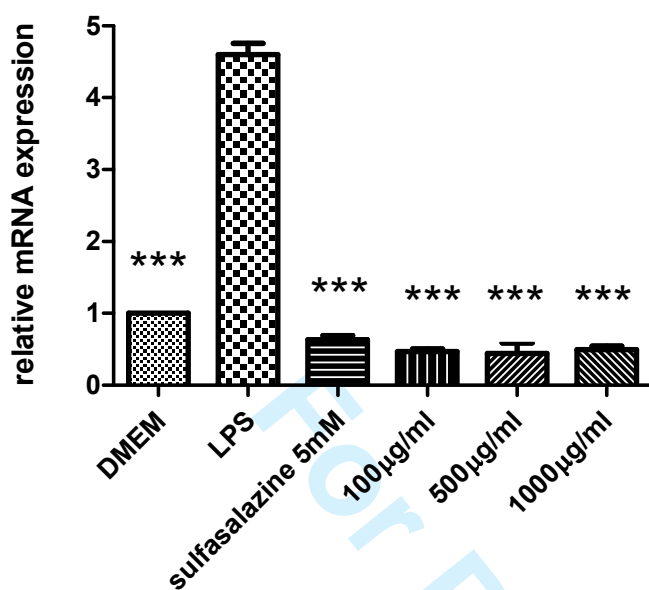


Figure 8: Effect of chamomile extract (100-1000 µg/ml) on interleukin-6 (IL-6) and gene expression. ANOVA, $P < 0.0001$, post hoc $***P < 0.001$ vs respective LPS-treated group.

Table I: Phytochemical composition of the tested chamomile extract.

Table I	
Total apigenin (as sum of apigenin, apigenin-7-glucoside and apigenin-7-(6-acetyl)glucoside)	4.52±0.28 %
Total phenols (as mg of gallic acid equivalents per g of extract)	56.62±1.31 mg/g d.e.
Total flavonoids (as mg of rutin equivalents per g of extract)	21.53±0.86 mg/g d.e.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

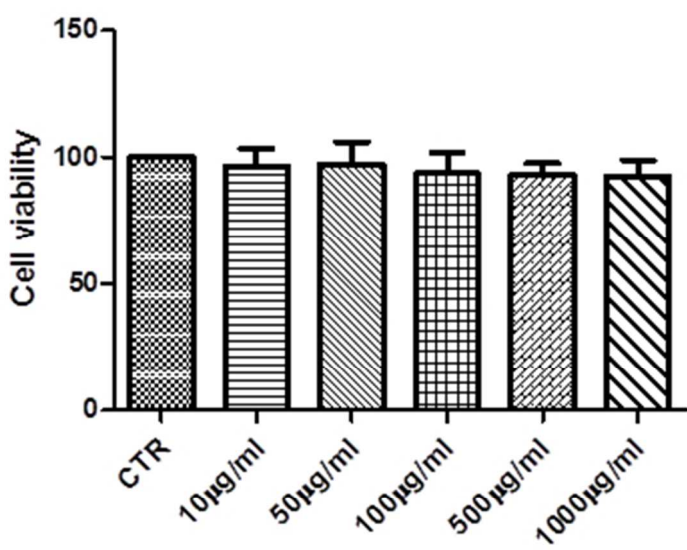


Figure 1: Effect of chamomile extract (10-1000 µg/ml) on HT29 cell line viability.
128x115mm (72 x 72 DPI)

Review

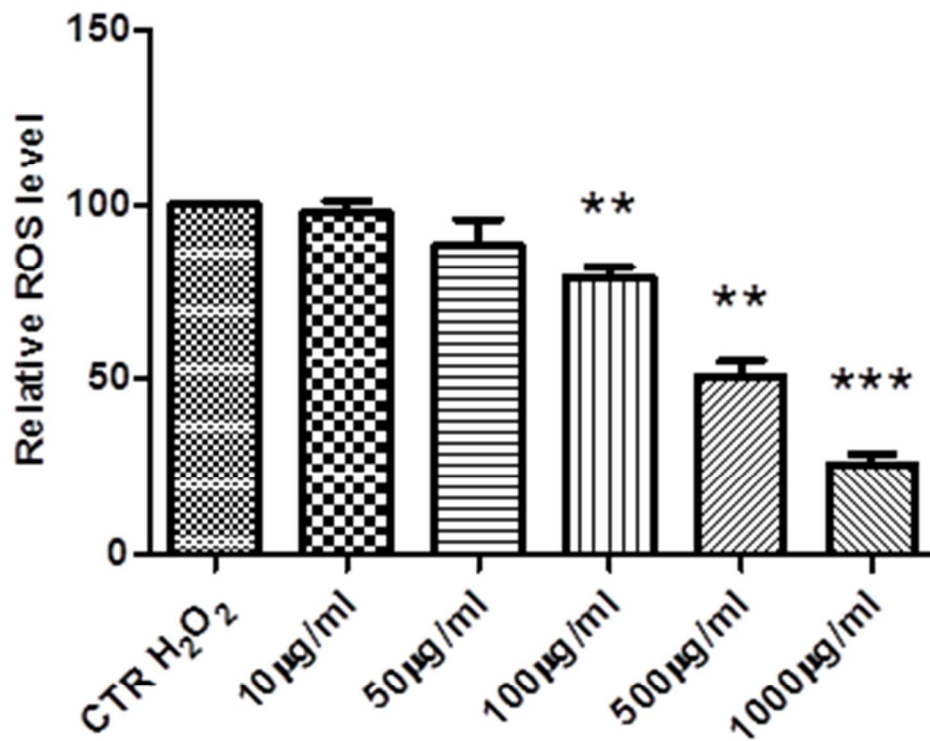


Figure 2: : Effect of chamomile extract (10-1000 µg/ml) on H₂O₂ ROS production in HT29 cell line. ANOVA, $P < 0.0001$, post hoc ** $P < 0.01$, *** $P < 0.001$ vs H₂O₂-treated group
128x106mm (300 x 300 DPI)

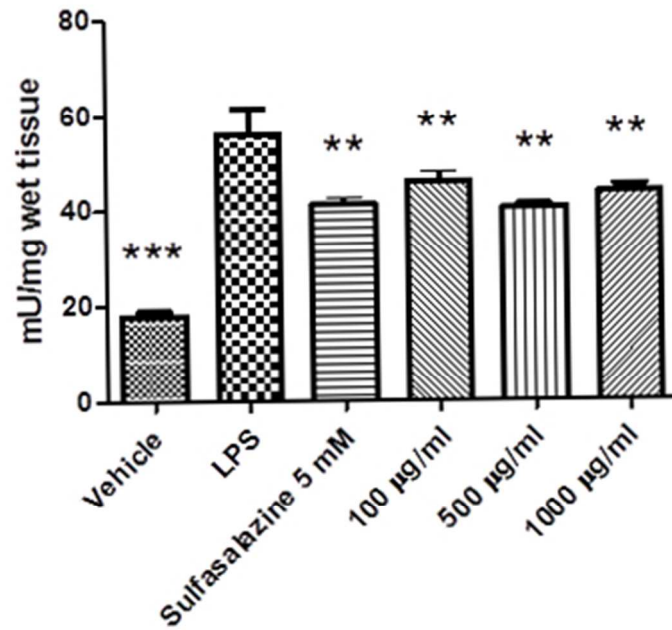


Figure 3: Effect of chamomile extract (100-1000 µg/ml) on mieloperoxidase (MPO) activity (mU/ng wet tissue). ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $**P < 0.001$ vs LPS-treated group.
31x27mm (300 x 300 DPI)

Review

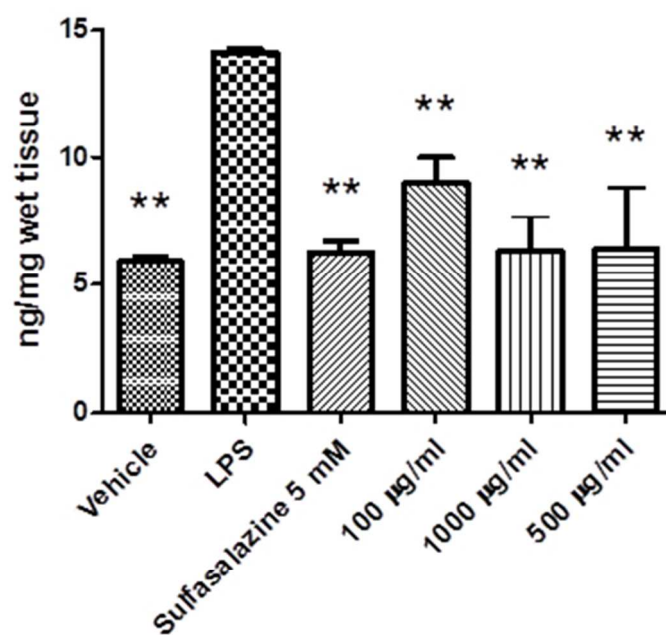


Figure 4. Effect of chamomile extract (100-1000 µg/ml) on prostaglandin E2 (PGE2) levels. ANOVA, $P < 0.01$, post hoc $**P < 0.01$ vs LPS-treated group.
30x31mm (300 x 300 DPI)

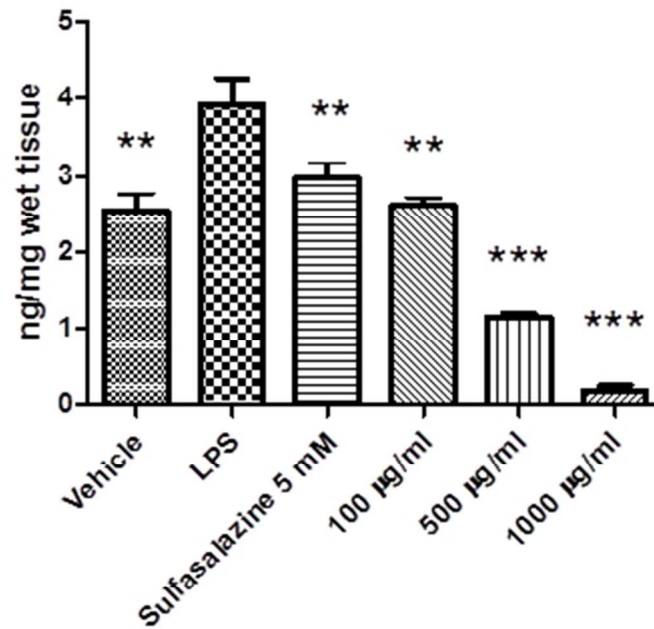


Figure 5: Effect of chamomile extract (100-1000 µg/ml) on 8-iso-prostaglandin F2α (8-iso-PGF2α) levels (ng/mg wet tissue). ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs LPS-treated group.
30x31mm (300 x 300 DPI)

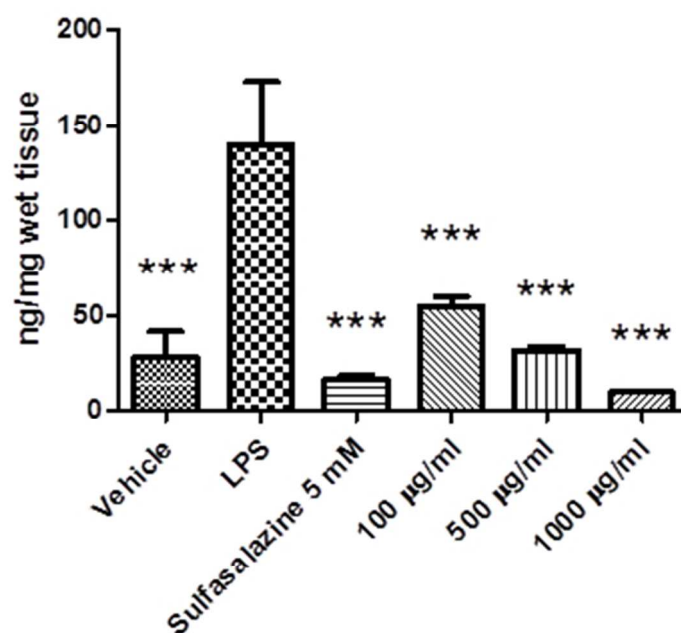


Figure 6: Effect of chamomile extract (100-1000 µg/ml) on serotonin (5-HT) levels (ng/mg wet tissue). ANOVA, $P < 0.01$, post hoc $***P < 0.001$ vs LPS-treated group. 30x31mm (300 x 300 DPI)

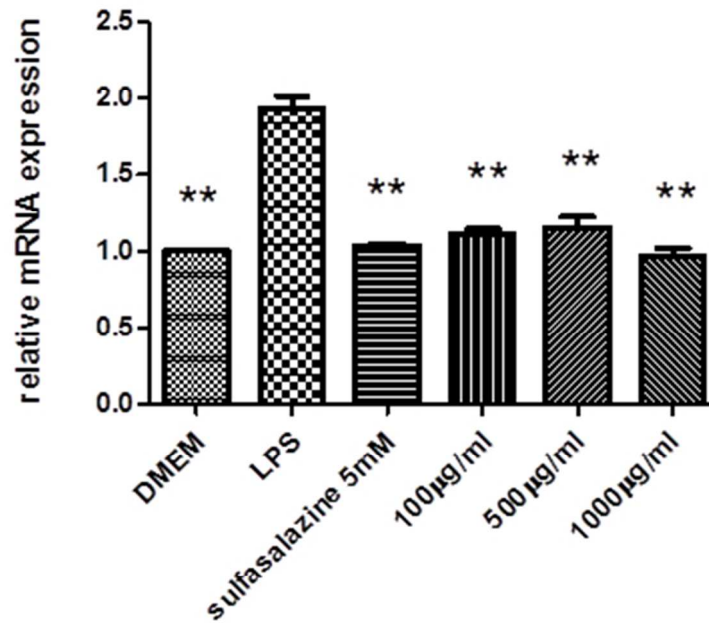


Figure 7: Effect of chamomile extract (100-1000 µg/ml) on tumor necrosis factor α (TNF α) and gene expression. ANOVA, $P < 0.0001$, post hoc $**P < 0.01$, $***P < 0.001$ vs respective LPS-treated group.
32x30mm (300 x 300 DPI)

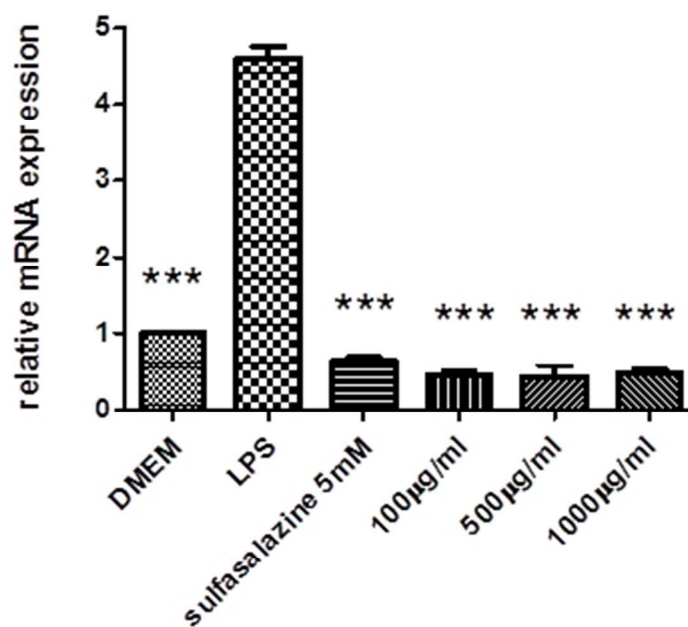


Figure 8: Effect of chamomile extract (100-1000 µg/ml) on interleukin-6 (IL-6) and gene expression. ANOVA, $P < 0.0001$, post hoc $***P < 0.001$ vs respective LPS-treated group. 31x30mm (300 x 300 DPI)