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ANTIOXIDANT AND RAT BOWEL CYTOPROTECTIVE EFFECTS OF AN HERBAL DERIVED PRODUCT BASED ON POLYSACCHARIDES AND FLAVONOLS COMPOUNDS

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Abstract

The aim of studies was to demonstrate potential benefits of an herbal derived product combining polysaccharides compounds from leaves of marshmallow and quercetin derivates from external brown peels (scales) of yellow onion on castor oil induced colitis model on rats. Histological evaluation indicated castor oil damaging potency at the level of intestinal epithelial cells, as well as the capacity of the plant derived product (500mg/kg body) to counteract oleum ricini histological modifications. MTS test made on 3T3 and HUVEC cells has proved the lack of toxicity, as well as antioxidant activity of the test product, together suggesting cytoprotective properties of this herbal derived product upon inflammatory bowel lesions.

Keywords: *Althaea officinalis* L., *Allium cepa* L., cytoprotective, *oleum ricini* rat bowel injuries.

Introduction

Even though the mechanism of initiation and development of inflammatory bowel diseases (IBD) is only partly acknowledged, it is well known that the emergence of muscular contraction, tissue inflammation and chemokinesis of the affected intestines in the main are the consequences of the increased level of pro-inflammatory cytokines [1-10], high levels of reactive

oxygen/nitrogen species [11, 12] and corresponding modifications at the level and rate of antioxidant enzymes [13-16].

On the other hand, good potency and efficacy of some plant derived products on each, bowel spasm, bowel inflammation and consequent diarrhoeal process through non-clinical and clinical studies have been reported [17]. For example, studies on the alcoholic extracts of leaves of *Psidium guajava* (known to contain six major quercetin derivates) indicated that quercetin aglycone released from its glycosides by the intestinal microflora enzymes inhibit, in a dose-dependant manner, the spontaneous contractions of the guinea pig ileum [18] and guinea-pig isolated smooth muscle [19] thus confirming strong anti-diarrhoeal properties of this Mexican, folk medicine remedy. Other studies [20] on the isolated organ (guinea pig ileum sensitized at egg albumin in order to obtain anaphylactic smooth muscle contraction) indicated that quercetin aglycone can inhibit both, phasic and tonic components of the anaphylactic contraction, also in a concentration-dependant manner (IC_{50}=10 μM), concluding that quercetin and its derivate 3,3'-di-O-methylquercetin from *Psidium guajava* plant derived products either *inhibits* Ca^{2+} *influx, or* Ca^{2+} *release from intracellular stores but, more likely,* Ca^{2+} *binding to intracellular receptor proteins* [21] thus manifesting its strong anti-diarrhoeal properties.

Regarding the antioxidant - anti-inflammatory potency of quercetin and its glycosides, several studies focused on flavonoids' subclasses have shown that while 2',3',4'-OH substitution on the flavonoid B ring play a crucial role in their scavenging activity, antioxidant activity being thus very pronounced in (poly)hydroxylated species [22, 23], *the factual anti-inflammatory activity is due mainly to their similitude either with the enzymes substrate or receptors ligands* [24], polymethoxylated species being the most effective anti-inflammatory species. Moreover, studies [25, 26] have revealed the capacity of some flavonoids compounds such as genistein, biochanin A, daidzein, or kaempferol to modulate the gene expression of specific antioxidant enzymes (e.g., metalothionein/MT, catalase/CAT and superoxide-dismutase/SOD), in a structure-dependant manner.

As well, studies on dextran sulfate sodium/DSS colitis in mice in order to compare anticolitis effects of quercetin and quercetin-3-O-rutinoside/rutin indicated that a diet containing 0.1% rutin, but not quercetin, significantly improved colitis histological aspects and also attenuated the expressions of IL-1 beta and IL-6 mRNA pro-inflammatory markers in colonic mucosa, suggesting that rutin could be a preventive, but also an effective treatment of IBD and colorectal carcinogenesis [27]. Furthermore, studies on quercetin and its glycosides concluded that *rutin acts as a quercetin deliverer to the large intestine, its anti-inflammatory action being through quercetin-mediated inhibition of TNF-alpha-induced NF-kappaB activation* [28]. Similarly, it was demonstrated that

quercetin aglicone is responsible for the down-regulation of mmatory response by inhibition of NF-kappaB pathway [29], the microbial transformation of quercetin aglycone completely abolishing its anti-inflammatory effect [30].

Besides, studies on quercetin and its derivates at phenyl group indicated that spiraeosides markedly suppressed lipid peroxidation when tested on rat gastrointestinal mucosa homogenates incubated with $Fe(NO_3)_3$ and ascorbic acid, its effectiveness being greater than that of isoquercitrin or quercetin. Spiraeosides also yielded higher amounts of quercetin aglycone than isoquercitrin on incubation with homogenates suggesting that, by reason of its *efficient conversion to antioxidative aglycone on exposure to the mucosa*, spiraeosides can act as a powerful antioxidant on iron ion driven lipid peroxidation in the intestinal mucosa [31].

Concerning the effective destruction at the level of inflamed mucosa tissue, clinical studies have demonstrated that specific, water-soluble, plant polysaccharides are the inflavery effective cytoprotective agents. Proving these, studies carried out on an *ex vivo* system based on porcine buccal membranes indicated that polysaccharides compounds isolated from *Althaea officinalis*, *Plantago lanceolata*, *Malva moschata*, *Tilia cordata*, *Calendula officinalis* and *Fucus vesiculosus* have the capacity to efectively bind to the epithelial tissue (polysaccharides compounds from *Fucus vesiculosus* and *Calendula officinalis* have shown the strongest adhesive properties), practically confirming high effectiveness of mucilage-containing plant products known as mucosa healing folk remedies [32]. Subsequent studies [33] regarding the mechanism of polysaccharides' bioadhesivity have to reveal that if rhamnogalacturonans compounds with a low degree of esterification and linear oligogalacturonids derived from pectin provide significant bioadhesion against colonic mucous membranes, highly esterified pectins and neutral polysaccharides are ineffective. Also, it was demonstrated that strongly acidic homogalacturonides are the most adhesive agents, esterification process and branching as non-linear backbone structures being associated with the reduction of the adhesive properties. By using the fluorescent microscopy method, it has also been proved that the adhesion of the exogenous galacturonides on the tissue surface is mediated by the interaction with the endogenous mucin, the release of the endogenous mucins with a mucolytic agent resulting in the decrease of the bioadhesion of exogenous galacturonides. Furthermore, rheological studies indicated that the artificial mucin layers provide protective effects on colonic mucous membranes against toxic agents, as shown by incubation of the tissue with TritonX-100, all these suggesting effective therapeutical potential of high bioadhesive polysaccharides in the treatment of intestinal injuries in IBD [33]. Finally, a study [34] designed to assess potential beneficial effects of the water-soluble dietary fibber produced by

controlled partial enzymatic hydrolysis of guar gum beans (PHGG) on colonic mucosal damage and inflammatory response in DSS colitis model in mice have demonstrated that, after two weeks of pre-feeding of PHGG, the shortening of the colon was significantly reversed and the infiltration of the inflammatory cells (especially neutrophils), as well as the mucosal cell disruption was reduced compared to controls. Also, the pre-treatment with PHGG significantly inhibited the increase of both myeloperoxidase/MPO activity and thiobarbituric acid-reactive substances, but also of the intestinal TNF-alpha protein and its mRNA expression, thus demonstrating mucosal anti-inflammatory potential of the water-soluble dietary fibbers on mice.

After all, polysaccharides compounds engage real benefits against the enhanced chemokinesis and bowel microbial content too, both explained by their capacity to effectively retain high quantities of water, minerals but also microbial thus reducing the immunogenicity and toxicity of the luminal content.

Given these, we have considered that combining polysaccharides compounds from *Althaea officinalis* L. plant species described with bioadhesive properties at the level of the epithelial tissue with flavonoid compounds (quercetin and spiraeosides) from *Allium cepa* L. known with high bioavailability and strong antispastic, antioxidant, anti-inflammatory properties, one can be obtained a plant derived product with cytoprotective effects on rat bowel injuries; the plant derived product has been designed so as to achieve the exactly content of 4% (w/w) total flavones expressed as quercetin equivalents. As about the pro-inflammatory agent used in experiments, on basis of its compliant effects it was selected the oil from *Ricinus comunis* L., also known as *oleum ricini* or castor oil [35, 36, 37]. The present work continues our former studies [38] dedicated to this important and actual issue, IBD causes [39] and possible natural, vegetal remedies [17].

Materials and methods

Plant material description

Althaea officinalis L. plant product (*folium*), commonly marshmallow, has been purchased from a specialised Romanian Plant Product Company (it presents as packages of 50 grams of medium size plant powder); taxonomic apartness is certified by the Trade Company.

The outer paper layers (external brown peels or scales) of yellow onion (*Allii cepae bulbus*) have been purchased from a green market in Bucharest and verified by the botanists' team at the *National Institute for Chemical - Pharmaceutical Research and Development* (ICCF), Bucharest, Romania.

The two voucher specimens (Aoff-ac-06 and Ace-ac-06) are deposited in ICCF *Plant Material Storing Room*.

Extracts' preparation

Technological studies have as the final purpose the obtainment of the two selective fractions; polysaccharides fraction from the aqueous extract of leaves of marshmallow and flavonols fraction from 70% (v/v) ethanolic extract of external brown peels (scales) of yellow onion. This way, marshmallow leaves were extracted with distilled water at boiling temperature and continuously agitation state (1:20, w/v) for 1 hour. The aqueous extract was concentrated at exactly 1:2, w/v ratio, then the concentrate was treated with ethanol solvent (98%, v/v) in order to precipitate polysaccharides compounds (1:4, v/v). The precipitate has dried (30-35°C) and grinded (fine powder) thus resulting *Althaeae folium* total polysaccharides fraction (codified P). It must be noted that the total polysaccharides fraction also contains proteins, minerals and traces of polyphenols compounds, as the former studies have described [38].

Flavonols fraction was prepared by treating the scales of yellow onion with (70%) ethanol; the extraction process has been done at the reflux temperature and continuously agitation state. The ethanolic extract was cooled at low temperature (4°C) and the resulted precipitate product was filtered (at vacuum system) and dried (30-35°C) finally resulting *Alii cepae bulbus* flavonoids fraction (codified QT) [38]. The final test vegetal product (codified QTP) has been obtained by adding QT fraction to P fraction so as to achieve the exactly content 4% total flavones compounds expressed as quercetin equivalents [38].

Chemicals, reagents and references

Chemicals (sodium carbonate, sodium acetate and aluminium chloride), reagents (Folin-Ciocalteau and NP/PEG) and solvents (ethanol, ethyl acetate, formic acid and glacial acetic acid) similar to *reference products* as rutin (min. 95%), quercetin (95%), apigenin (>97%), kaempferol (95%), cosmosiin (97%), vitexin (>96%), apiin (>97%), chlorogenic acid (>95%), caffeic acid (99%), gentisic (95%) and gallic acid (95%) were purchased of *Fluka and Sigma-Aldrich* Co (Bucharest, Romania); the reference products, polyphenols, were further prepared as 10^{-3}M in (70%, v/v) ethanol solution.

Qualitative analytical determination

Studies have as the main purpose the assessment of the polyphenols profile in the two active fractions (QT and P) and they were performed according to *Plant Drug Analysis* [40] and *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plant* [41], as described in our previous work [38].

Total flavones content estimation

Total flavones content of the two active fractions (QT and P) and of the final test product (QTP) has been done by using *Romanian Pharmacopoeia* (FR X) standard method [42], as described in our previous work [38].

Pharmacological studies *in vitro*

Cell viability evaluation assays – MTS test

The cell viability was determined according to Technical Bulletin of *Promega Corporation*, *CellTiter 96 AQueous One solution Cell Proliferation Assay* [43]. As general principle, the MTS tetrazolium is bioreduced by cells into a coloured formazan product soluble in culture medium, as it has been described in our previous work [44]. The Cell Viability Kit, *CellTiter 96 Aqueous One solution Cell Proliferation Assay* (MTS) was purchased from *Promega Corporation.* Mouse fibroblasts 3T3-L1(ATCC-CL-173) and human umbilical vein endothelial cells - HUVECs (ATCC-PCS-100-010) were obtained from *ATCC* (LGC Standards, Germany) while Dulbecco's Modified Essential Media (DMEM), DMEM-F12, Foetal Bovine Serum (FBS) and antibiotics were purchased from *Sigma-Aldrich* Romania. This way, the fibroblasts were cultured in DMEM supplemented with 10% FBS and 1% antibiotics (10,000 units / mL penicillin and 10,000 μg / mL streptomycin in 0.85% saline), while human entothelial cells were grown in DMEM-F12, also supplemented with 10% FBS and 1% antibiotics, incubated at 37ºC in 5% CO_2 humidified atmosphere. After reaching confluence, the cells were detached with Trypsin-EDTA and the cell suspension was centrifuged at 2000 rpm for 5 min then resuspended in the growth medium. Cells were seeded in 96-well plates at a density of 10^5 cells / mL, and 200 μL of culture medium was administered to each well. Cells were allowed to attach and achieve approximately 80% confluence prior to starting test the experiments. This way, different concentrations of the *test vegetal product* (QTP) are prepared in complete culture media consisting of DMEM/DMEM-F12 supplemented with 10% FBS and 1% antibiotic solution. Subsequently, the effects of the QTP product on the viability of 3T3 fibroblasts and HUVEC are evaluated using CellTiter 96 AQueous One Solution Cell Proliferation Assay(a), MTS test respectively. Accordingly, the culture medium from the wells was removed and replaced with 200 μL new culture media containing the QTP product at increasing doses (dilution series 1, 5, 10, 50 and 100 μg QTP / mL respectively). After 12 hours of exposure, the culture medium was removed. After other two hours of incubation with MTS solution, viability of the adherent cells was determined using MTS reduction; the MTS-formazan absorbance was detected by using *Chameleon V Plate Reader, LKB Instruments* and the recorded values, sample absorbance at 490 nm, used for cell viability estimation (see formula bellow).

$$\%\ cell\ viability = \frac{A490\ of\ treated\ cells}{A490\ of\ control\ cells} \times 100$$

Cytoprotective effect of QTP product against H_2O_2-induced oxidative stress

Cytoprotective effects were appraised by MTS test to determine the survival rate of the HUVECs. Briefly, after pretreatment of the HUVECs with the test vegetal product (100, 50, 10, 5 and 1 μg QTP / mL) for 24 h at 37º C, the cells were washed out with PBS and then, new medium with 1 mM hydrogen peroxide and 20 μM $FeSO_4$ was added to the wells and incubated for 30 minutes. Further experiment was performed as described above. The wells consisting of cells that haven't been exposed to the extract or H_2O_2 were considered as negative control.

Measurement of (anti)oxidative activity-lipid peroxidation assay

The malondialdehyde/MDA concentrations were determined by measuring thiobarbituric acid-reactive substances using dedicated kits (Lipid peroxidation assay kit) according to the manufacturer's protocols (Sigma-Aldrich) [45].

Pharmacological studies *in vivo*

In vivo studies, castor oil induced colitis model on rats respectively, have fulfilled on three groups of *Wistar* rats, male, as described in our previous work [38]; briefly, the rats received 16ml *oleum ricini per* kg body, *p.o*. in the first day and 8ml *oleum ricini per* kg body, *p.o.* in the second day, thus obtaining moderate diarrhoeal process on rats. The experiments respected FELASA (Federation of European Laboratory Animal Science Associations) and ARSAL (Romanian Association for Laboratory Animal Science) regulations: animals were housed under standard conditions meaning temperature at 20±2°C, relative humidity 50-60%, 12 h dark-light cycle, standard pellet diet and water *ad libitum.*

The three rat groups were as follows: **Group 1 - Control group** received standard food and water *ad libitum* all period of the studies (7 days); **Group 2 - Exposed untreated group** received 16 ml *oleum ricini per* kg body, *p.o*. in the first day, 8 ml *oleum ricini per* kg body, *p.o*. in the second day, the next five days the animals were observed as concerning diarrhoeal intensity and euthanized in the seventh day of experiment; **Group 3 - Exposed treated group** received first two days the dose of 500 mg QTP *per* kg body *p.o*, next two days the same dose of QTP but concomitantly with the *oleum ricini* dose (16 ml *oleum ricini per* kg body, *p.o*. in the third day and 8 ml *oleum ricini per* kg body, *p.o*. in the fourth day) after that three days they received the same dose of the test vegetal product (500 mg QTP *per* kg body *p.o*) and euthanized in the seventh day of experiment; similar to control group (group 1), the two exposed groups (group 2 and 3) received standard food and water *ad libitum* all period of the studies.

At the end of the experiment (the 7th day), the animals were euthanized using diethyl ether overdose, the abdomen was opened and 5 cm of the small intestine (situated 5 cm after duodenum) was excised, incised longitudinally, then washed with saline solution. The samples excised were evaluated as concerning macroscopic aspects then they were subjected to biochemical and histological studies.

Biochemical studies

Samples for biochemical studies had as the main purpose the estimation of the antioxidant - anti-inflammatory activity of the QTP treatment by measuring the intestinal level of malondialdehyde/MDH and reduced glutathione/GSH as well as superoxide dismutase/SOD and catalase/CAT) activities; the obtained results have been presented in our previous work [38].

Histological studies

Samples for histological study were fixed in 10% formalin then the tissues were embedded in paraffin, processed and sectioned into thick slices and the resulted pieces were stained with hematoxylin and eosin followed by viewing them as concerning the intensity of coloration and the degree of vacuolation of cells that line the intestinal microvillus, two general qualitative characteristics that give indications on the extent of damage of the intestinal mucosa; microscopic studies were done by a microscope equipped with a color video camera for digital imaging. The obtained results are presented in the following.

Statistical analysis

Results were calculated as mean ± SD, n=5.

Results and discussion

Analytical studies results

Figure 1 shows qualitative (HP)TLC aspects referring to polyphenols content of the two active fractions [38], *Alii cepae bulbus* flavonols fraction (T6-T8) and *Althaeae folium* polysaccharides fraction (T9-T11), face to several polyphenols compounds, reference products mixtures (T1-T5) respectively.

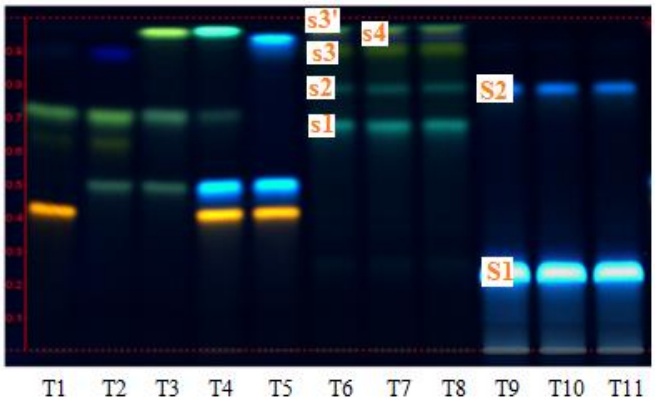

Figure 1. Polyphenols profile of the two active fractions, flavonols fraction from scales of *Alii cepae bulbus* and polysaccharides fraction from *Althaeae folium* raw material face to several reference products, polyphenols mixtures (ref.).

T1 track – quercetin-3-O-rutinoside/rutin, apigenin-8-C-glucoside/vitexin, apigenin-7-O-glucoside/cosmosiin and gentisic acid (ref.); T2 track - apigenin-7-(2-O-apiosylglucoside)/apiin, apigenin-8-C-glucoside/vitexin, cosmosiin and gallic acid (ref.); T3 track - apiin, cosmosiin, quercetin and apigenin (ref.); T4 track - rutin, chlorogenic acid, cosmosiin and kaempferol (ref.); T5 track - rutin, chlorogenic acid and caffeic acid (ref.); T6 - T8 tracks – *Allii cepae bulbus* flavonols fraction (QT) – triplicate;

T9 - T11 tracks – *Althaeae folium* polysaccharides fraction (P) – triplicate.

As shows Figure 1, flavonols fraction prepared from external brown peels (scales) of yellow onion (QT product, T6-T8 tracks) reveals two blue-green fluorescent (fl.) spots (s1/*Rf*~67 and s2/ *Rf*~78) attributed to spiraeosides (quercetin-3/4'-glycosides) compounds, two yellow-orange fl. spots attributed to quercetin aglycone (s3 and s3') and one small, blue fl. spot at the FRONT of chromatogram attributed to caffeic acid (s4). Polysaccharides fraction prepared from marshmallow leaves (T product, T9-T11 tracks) revealed two blue fl. spots (S1/*Rf*~0.23 and S2/*Rf*~78) that were attributed to kaempferol glycosides based on UV-Vis studies on the respective spots (the two spots were extracted with 80% (v/v) methanol after that studied as concerning maximum absorption wavelength).

Such as, analytical studies confirmed polysaccharides [38], quercetin and spiraeosides presence in the two active fractions of QTP, so that a chemical composition attributed with potential cytoprotective and antioxidant effects on the intestinal irritated mucosa.

Pharmacological studies results

QTP cytotoxicity – MTS test on 3T3 and HUVEC cells

The main purpose of the *in vitro* studies was the evaluation of QTP potential toxicity on 3T3 (fibroblasts) and HUVEC (human umbilical vein endothelial cells) cells. As it has previously been presented (Section *Cell viability evaluation assays – MTS test*), the effects of QTP on 3T3 and HUVEC cells viability were evaluated using a colorimetric test based on the selective ability of viable cells to reduce the tetrazolium component of MTS into purple coloured formazan crystals. The quantity of formazan product, as measured by absorbance at 490nm, is directly proportional to the number of living cells in culture, so that the effect of the test-product on the proliferation of 3T3/HUVEC cells can be calculated as cell viability percentage (%).

This way, MTS assays (Figure 2 and Figure 3) non-significant inhibitory effects of QTP alone (1 - 100 µg / mL, 12 hours time of exposure) to HUVECs and 3T3 cells proliferation have revealed. Also, cells viability dynamic evaluation only modest modifications to endothelial cells and fibroblast after QTP treatment at higher concentrations (100 µg / mL) have been revealed, suggesting the lack of toxicity of the test vegetal product QTP.

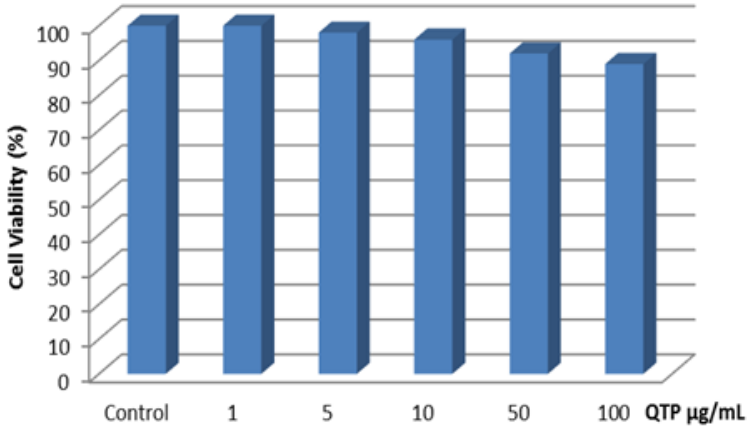

Figure 2. MTS assay, percentage of cell viability on 3T3 cells (fibroblasts) exposed to different concentrations of vegetal extract (QTP)

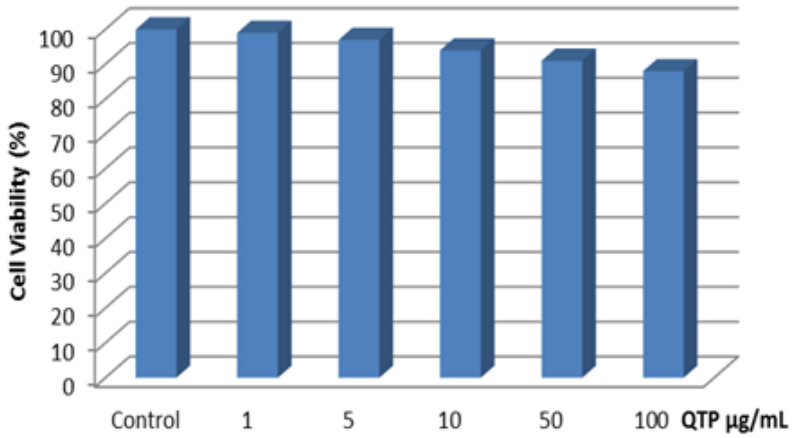

Figure 3. MTS assay, percentage of cell viability on HUVECs exposed to different concentrations of vegetal extract

Cytoprotective effect of QTP against H_2O_2-induced oxidative stress

Figure 4, dose response of H_2O_2 treatments on HUVECs cell viability respectively, also suggest the cytoprotective effect of the QTP product against oxidative stress-induced cell injury on HUVECs, also using MTS assay.

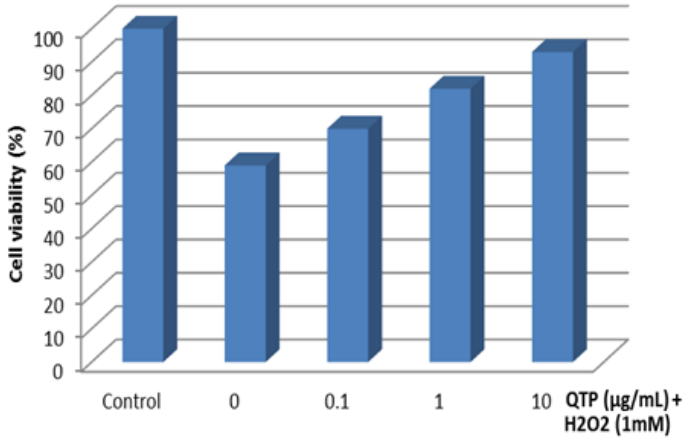

Figure 4. Cytoprotective effect of QTP on H_2O_2-induced oxidative stress in HUVECs; cells were incubated with H_2O_2 (1mM, 30 min) after pretreatment with different concentrations of QTP for 24 hours.

As shown, the exposure of HUVECs to 1 mM H_2O_2 and 20 µM $FeSO_4$ for 30 minutes resulted in a reduction of cell viability. Pretreatment of HUVECs (for 24 hours) with QTP decreased the cell death resulted from the exposure to hydrogen peroxide in a concentration-dependant manner. The protective effect was

significant at concentration of 10 µg QTP / mL of the product suggesting that QTP plant product has a protective effect against H_2O_2-induced toxicity in HUVECs at the concentration range of 0.1 - 10 µg / mL, a result which also suggests a very strong antioxidant activity for this vegetal product. Further lipid peroxidation assay and malondialdehyde (MDA) appraisal has been made too.

Lipid peroxidation assay - Effects of QTP on MDA formation

MDA is a product of lipid peroxidation and the amounts of MDA formed during cells' incubation can be determined by measuring the sample absorbance at 532 nm. Therefore, a high absorbance is an indication of a high concentration of formed peroxides (MDA) and the inhibition of lipid peroxidation (%) can be appraised by using the following equation:

$$(\ \%\ )\quad cytoprotection = \left(1 - \frac{T - M}{I - M}\right) x100$$

Where: T - Absorbance value in the presence of the sample, M - Absorbance value of the negative control reaction and I - Absorbance value of the positive control reaction.

The data resulted from our studies (the levels of cellular MDA, Lipid Peroxidation Index respectively) demonstrate (Figure 5**)** that, compared to the control, the incubation of HUVECs with QTP product significantly decreased the MDA levels, in a concentration-dependant manner respectively, thus confirming high antioxidant potency of the test vegetal product QTP.

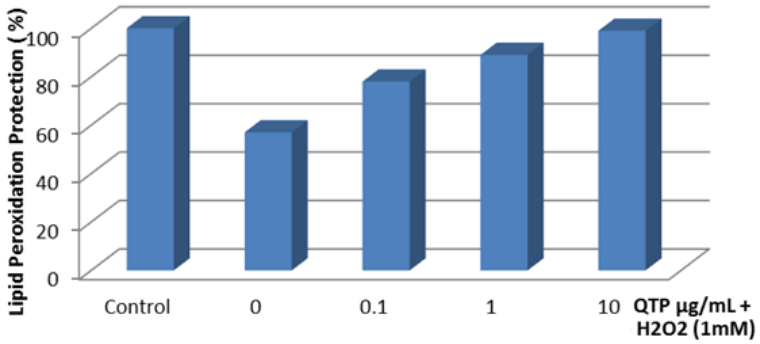

Figure 5. Effects of QTP on inhibition of lipid peroxidation in HUVECs; cells were incubated with H_2O_2 (1mM, 30 min) after pretreatment with different concentrations of QTP for 24 hours.

Histological aspects

Histological studies aimed at assessing the degree of damage to the cell edge of the intestinal mucosa of the three rat groups. Based on the fact that intense haematoxylin coloration and a reduced number of vacuoles are associated with a healthy tissue, the control group sample (group 1, Figure 6) suggests a normal bowel tissue and no intestinal injuries. Presence of several vacuoles in the cells lining of the marginal intestine represents the normal characteristic of an intestinal mucosa, as a result of its rapid and permanent regeneration of cells; it must be noted that, in humans, the regeneration of the superficial cells of the intestinal mucosa occurs every 2 - 3 days.

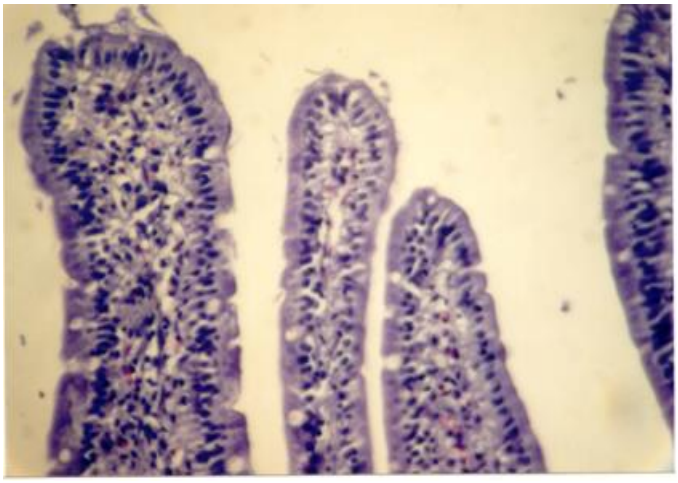

Figure 6. Histological aspects of the intestinal tissue of control group (group 1); haematoxylin coloration (x200).

Group 2 (Figure 7a and Figure 7b), respectively the group of the stressed and untreated animals showed a low intensity staining peripheral cells, a large number of vacuolated cells and mucosal lesion regions strongly detached from the body of the intestinal microvillus. Therefore, getting a weak staining along with the presence of a large number of vacuoles are clear indications of damage the integrity of the intestinal cells and the presence of damaged mucosal regions confirm strong irritative effect of the castor oil/*oleum ricini* product.

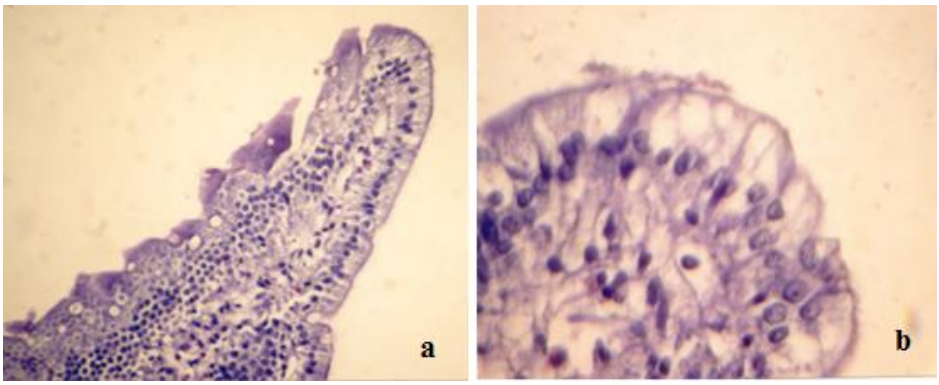

Figure. 7. Histological aspects of the intestinal tissue of group stressed and untreated (group 2); haematoxylin coloration (x200/**a**, x400/**b**).

Group 3 (Figure 8), respectively the group stressed and treated with the vegetal product QTP indicated an intense haematoxylin coloration and a reduced number of vacuoles, two histological aspects associated with a healthy tissue and a normal intestinal mucosa, un-affected by the presence of *oleum ricini* product.

Therefore, our results suggest antioxidant and rat bowel cytoprotective effects of the plant derived product made, QTP, a plant product designed on basis of literature data consistent with the fact that polysaccharides compounds with bioadhesive properties (such as pectins and mucilages from specific plant products) and flavonols (such as quercetin and spiraeosides) with effective antispastic and antioxidant properties could be one of the numerous compatible combination for new natural products able to protect irritated intestinal tissue.

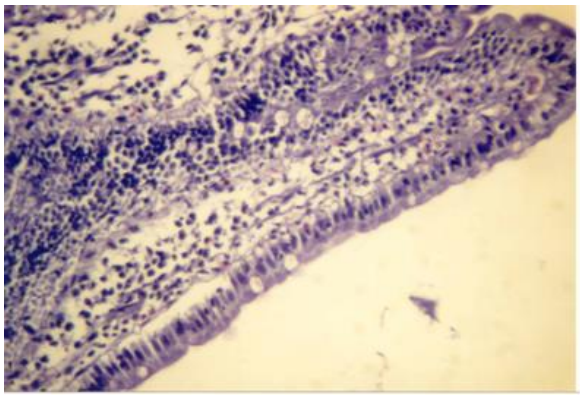

Figure 8. Histological aspects of the intestinal tissue of group stressed and treated with test vegetal product (group 3); haematoxylin coloration (x200).

In support, studies [46] aiming to asses whether *Althaea officinalis* roots aqueous extract (AE) and separate polysaccharides (RPS) may provide an active influence on mucosal or connective tissue cells (an effect useful for better tissue regeneration) indicated that, if the whole water extract (1, 10 μg / mL) had a stimulating effect on cell viability and proliferation of epithelial KB cells, the separate polysaccharides (1, 10 μg / mL) significantly stimulated cell vitality of epithelial cells, without triggering them into higher proliferation status. Also, it was revealed [46] that the two extracts did not exert any effect on fibroblasts while microarray analysis indicated an up-regulation of genes related to cell adhesion proteins, growth regulators, extracellular matrix, cytokine release and apoptosis, finally concluding that both, whole aqueous extracts and separate polysaccharides fraction from roots of *Althaea officinalis* are effective in the treatment of irritated mucous membranes and tissue regeneration as well.

Conclusions

Based on the numerous literature data reporting good potency and efficacy of the plant derived products and particular plant molecules on each, bowel spasm, bowel inflammation and corresponding intestinal tissue damages and enhanced chemochinesis, related with inflammatory bowel disease status, we have considered that combining vegetal polysaccharides with bioadhesive properties able to protect but also repair mucosal tissue, with flavonoids with high bioavailability able to reduce spastic activity of the smooth muscle, but also oxidative stress at the level of the affected tissue, one can obtain a natural product able to offer protection against intestinal external pro-inflammatory agents.

Based on these reasoning, the manufactured plant derived product (codified QTP) included polysaccharides fraction from leaves of marshmallow and quercetin and spiraeosides from outer paper layers (external brown peels also known as scales) of yellow onion at precise concentration of 4% (w/w) total flavones content expressed as quercetin equivalents. It must be also noted that the selection of marshmallow leaves and not roots pieces has been sustained by their polysaccharides similitude but lack of starch compounds.

Our previous studies on castor oil model colitis on rats indicated that the treatment with doses of 500 mg QTP / kg body, *p.o*, seven days consecutively, completely counteracted *oleum ricini* negative effects on the intestinal tissue; these protective effects were related with the inhibition of production of pro-inflammatory malondialdehyde (MDH) and the enhancement of activity of antioxidant superoxide dismutase (SOD) enzyme, most probably through polyphenols and polysaccharides synergistic activity.

The current study regarding cytotoxic and antioxidant potency of QTP product, MTS test on 3T3 and HUVEC cells respectively, indicated its lack of

toxicity at doses of 1 - 100 μg / mL. Also, our findings shown cytoprotective effect of QTP against oxidative stress induced by H_2O_2 in HUVECs at the concentration range of 0.1 - 10 μg / mL. Furthermore, histological evaluation on *Wistar* rats indicated intense haematoxylin coloration and a reduced number of vacuoles at the level of the intestinal pieces excised from the group stressed with *oleum ricini* but treated with the test vegetal product, QTP, so that an intestinal tissue un-affected by the presence of *oleum ricini* irritative and pro-inflammatory product. Differently, the group stressed with *oleum ricini* but untreated with QTP product has demonstrated a low intensity staining peripheral cells and a large number of vacuolated cells and mucosal lesion regions strongly detached from the body of intestinal microvillus clearly suggesting the damage of the integrity of intestinal cells and rat bowel irritative effects of the castor oil treatment.

Together, our results confirm antioxidant and rat bowel cytoprotective activity of a plant derived product combining polysaccharides compounds with bioadhesive properties and flavonols compounds (quercetin and spiraosides) with high bioavailability and effective antispastic, antioxidant, anti-inflammatory properties, as the literature data has been proposed [17].

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