Gastroprotective effects of the fatty acid esters and ethanol-amides synthesised from extra-virgin *Oleum Olivae* - on pharmacological induced ulcers and gastric secretion on rat

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Abstract

Bioactive foods are gaining increasing attention due to the scientific evidences related to the correlations between diet and health status. The mediterranean diet is associated with reduced risk of developing chronic degenerative disorders such as cardiovascular diseases, type 2 diabetes and cancer, it can improve diseases symptoms in inflammatory degenerative diseases, acting both at local and systemic levels. The role of the extra virgin olive oil (EVOO) is essential, many studies showed the importance of a rich in olive oil diet. The role of polyfenols and free fatty acids in the EVOO is extensive investigated, corelating the action of the fatty acids and the endogenous endocannabinoid system. In this purpose, we measured the protective effect of the esthers and ethanolamides of the fatty acids from extra-virgin Oleum Olivae on the experimental gastric ulcers and on the gastric secretion on rat. The therapy with the ethanolamides of the fatty acids in the presence of indometacin reduced the number of ulcers with 89.96% compared with indometacin and methylic esthers, reduced with 53.61%. The gastric acidity of the animals treated with indometacin and ethanolamides of the fatty acids decreased with 60.66% compared with the animals treated with indometacin only, and with 38.11% for the ethylic esters and indometacin; the ethanolamides of the fatty acids from the extra-virgin Olive oil presented a protective effect in the ulcers induced by indometacin.

Keywords: faty acids methylic esters, oleamides, anandamide, cannanbinoid, fatty acids ethanol amides, extra-virgin olive oil, gastric ulcers

Introduction

The extra-virgin olive oil (EVOO) is a bioactive food that contains polyphenols and mainly fatty acids, glicerids of the linoleic acid and arahidonic acid, trioleine, tripalmitine, sterols (beta-sitosterol) delta 7-stigmasterols, delta 5-avanasterol, insaponificable substances represented by iridoids, triterpens and lignanic compounds [**1**]. Many studies showed the importance of a rich in olive oil diet **[2, 3, 4]** the extra virgin olive oil contains polyphenols having a biological action in the treatment of the arterial hypertension and the prevention of the cardio-vascular diseases, improving the endothelial markers involved in blood pressure control in hypertensive women. [**5,6,7**]. According to the current research data, olive oil polyphenols revert endothelial dysfunction induced by high glucose/free fatty acids. The regular consumption of extra virgin olive oil (EVOO) inputs high content of polyphenols with a recognised role in modulating several molecular pathways and protects the plasmatic lipids from the oxidative stress; to the phenolic compounds but also due to the monounsaturated fats content, that have antioxidant, anti-inflammatory and immunomodulatory properties [**8**]. Polyphenols comprise thousands of compounds of plant secondary metabolites including flavonoids, isoflavonoids, phenolic acids, proantho cyanidins and other tannins, and lignans

with different biological activities. The major polyphenols in olive oil are phenolic acids (hydroxytyrosol, tyrosol), secoiridoids (e.g. oleuropein) and lignans (e.g. pinoresinol)[9]. Polyphenols have been found to decrease heart disease risk factors by lowering blood pressure and cholesterol, reducing blood clotting and improving the health of artery linings. In the EU EFSA Regulation 432/2012 [**9**] the health claim regarding "the protection of blood lipids from oxidative stress" that can be attributed to the EVOO relates to the level of olive phenolic compounds, respectively "may be used only for olive oil which contains at least 5 mg of hydroxyl-tyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 mg of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20mg of olive oil" [**10].**

The olive oil comprises mainly of the monounsaturated fatty acid oleic acid (C18:1); a EFSA approved health claim on the unsaturated fatty acids (Commission Regulation (EU) 432/2012) reccomends that replacing saturated fats in the diet with unsaturated fats contributes to the maintenance of normal blood cholesterol levels.

Considering the fact that in the olive oil are present mono- and poly-unsatturated fatty acids, principaly in the form of triglycerides [**Table 1**] we elaborated a method of transformation of the extra virgin olive oil **fatty acids** in the corresponding **methyl esters** and **ethanol amides** at the National Institute of Chemical and Pharmaceutical Research and Development.

Researches demonstrated that the ethanol amides of the fatty acids represent a new class of biologically active compounds "endocannabinoidome" [**11**] with potential anti-inflammatory and immune modulatory action **[12]** CNS function regulation of food intake and sleep **[13]**, cicatrizing action **[14]** According to the protective effects of the endocannabinoid system against the gastric lesions and its regulatory role in feeding behavior and inflammatory bowel disease [**15, 16**, **17]**, this ubiquitous signalling system is considered as an emerging target for the therapeutic interventions in the gastrointestinal (GI) disorders [**18**].

We approached two synthesis strategies based on previous experience in oleamide synthesis **[19]** to obtain the fatty acid ethanol amides starting form the *Olivae oleum virginale:* a) the aminolysis of the glycerides b) the transformation of the glycerides in the corresponding methyl esters.

Synthesis of amides like anandamide (N-arachidonoyl-ethanolamine – AEA - lipid mediator that acts as an endogenous ligand of CB1 receptors, exerts an overall modulatory effect on the brain reward circuitry **[20])**, prostamides, fatty acids amides, macamides, could be realized by several methods: direct estherification of acids with amines or with carbonyl-di-imidazole, acid chloride and amines**,** esters with amines catalyzed by bases like MeONa, we synthesized the tested products according with **[11, 21,19].**

Fig. 1 The structure of oleamide – *(Z)-Octadec-9-enamide* ($C_{18}H_{35}NO$) – amide of oleic acid

The final product is a mixture of the corresponding ethanol amides in which predominates according to the procentual composition the ethanolamide of the oleic acid, the ethanolamide of the linoleic acid and a small quantity of the ethanolamide of the stearic acid [**19**].

The structure of the methylic esters and of the 1-ethanolamides obtained was confirmed by the 1H-RMN spectres and IR. The present synthesis is subject of a patent.

In the present paper we tested the cicatrising action of the fatty acids esters and ethanol amides from the extra-virgin olive oil on the experimental ulcers provoked by indometacin and on the gastric secretion on rat.

Materials and methods

The *Olivae oleum virginale* (olive oil) used in the study was manufactured in Greece, obtained by cold pressing and centrifugation of the mature fruits of the species *Olea europea L.* with quality specifications according to the European Pharmacopoeia [**22**], the monography *Olivae oleum virginale.* We transformed the olive oil fatty acids in the corresponding methylic esters and ethanol amides. We took into study pharmaceutical grade raw materials processed and controlled in our laboratory.

We took into study batches of white, male Wistar rats, weighing 200±10 g, kept into mobile cages, in special shelters, in standard laboratory conditions. The animals were purchased from the Animal Biobase of the University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania.

With 24 hours before the study the animals were restricted from food during 24 hours and water "*ad libitum*". All experiments were performed in compliance with European Communities Council Directive 1986 (86/609/EEC) and Ordinance No. 37 of the Romanian Government from 2nd February 2002. The animals were distributed in six batches of eight animals each.

The first batch was the control, with 24 ours food restriction before being sacrificed. The second batch was orally treated with indometacin 10 mg/body weigh (b.w), orally, during 4 days, the third batch received indometacin 10 mg/b.w. orally and methylic esters of the fatty acids in dose of 250 mg/b.w. orally during a 4 days period; the fourth batch received indometacin 10 mg/b.w. orally and the ethanol amides of the fatty acids from the olive oil in dose of 250 mg/b.w. orally during a 4 days period and the batch 5 was treated with indometacin 10 mg/b.w. orally and 5 ml/b.w. orally extra-virgin *Oleum olivae*. The batch 6 received ranitidine 10 mg/b.w. orally as a reference substance because inhibits the basal secretion and stimulated and it has a long acting activity.

The measurement of the gastric secretion was done by the Shay method of ligature of the pillory. After two hours the animals were sacrificed under chloroform anesthesia. The stomach was excised and dissected on the large curve margin, the gastric secretion was collected and the stomachs fixed on the contention plates, the mucous was examined with the magnifying lens, marking the ulcers appeared and their dimension.

After the measurement of the gastric secretion volume, the gastric content was centrifuged and the gastric acidity was measured by the Topfer Linossier method. The percentage index of protection (IPP) is calculated with the formula:

$$IPP = M/Tx100 \quad [8]$$

M=gastric acidity for the control animals; T=gastric acidity for the treated animals

The statistical evaluation of the results was made by the "t" student test**.** Data were analyzed using t tests (for single between-group comparisons). Data were expressed as mean ± standard error. A p value of 0.05 ± SD was considered statistically significant.

Results and discussions

The macroscopic analysis

After the sacrification of the animals the stomach was excised, cut on the large curve margin, washed for eliminating the eventual impurities and fixed on contention plates. The anatomopathological preparates are examined with the magnifying lens and measured the lenght of the lesions for each stomach.The indometacine treated batches presented multiple ulcers of variate dimensions (**Fig. 1-5**).

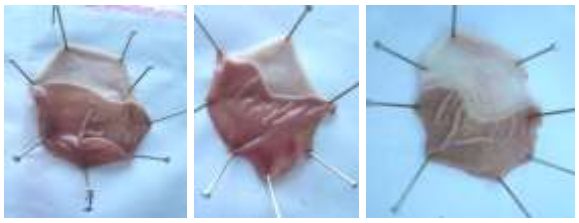

Fig. 2 Anatomo-pathological aspect of the gastric mucosa in the control batch

Fig. 3 Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine

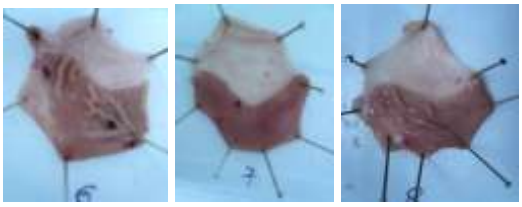

Fig. 3 Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine and olive oil

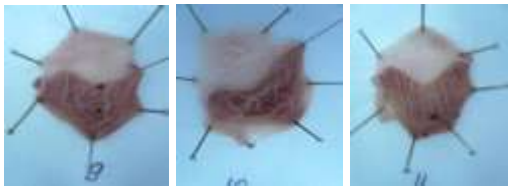

Fig. 4 Anatomo-pathological aspect of the gastric mucosa after administration of indomethacine and amides

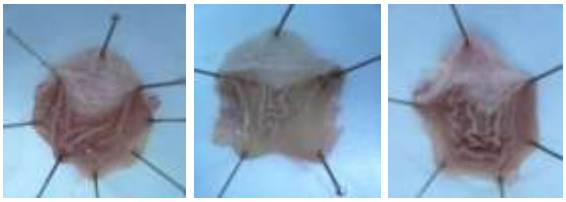

Fig. 5 Anatomo-pathological aspect of the gastric mucosa after administration of indometacin and famotidine

Fig. 6 Anatomo-pathological aspect of the gastric mucosa after administration of indometacin and esters

The association of the indometacin with the ethanolamides of the fatty acids from the olive oil had a protection of 89,96% compared with the treated batch only with indometacine and a protection of 53,61% for the batch treated with the mehylic esthers. Also the extra-virgin *Olivae oleum* presented a protection of 66,26% compared with the indometacine (**Table 1-2**).

Table 1 The influence of the ethanolamides and of the esters of the fatty acids from extra-virgin *Oleum Olivae* on the experimental ulcers produced by indometacin

Tested product	**Ulcers no. x±e.s.**				**media**	**Effect%**
	< 0,5 mm	**2 mm**	**4 mm**	**6 mm**		
Untreated controls	2,3±0,40	1,6±0,50	1±0,6	0	1,22	-
Indometacine	5,2±0,70	6±0,80	3,2±0,4	2,2±0,3	4,15	-
Indmethacine + methilic esthers	4,4±0,30	3,3±0,4	0	0	1,92	-53,61
Indometacine +ethanolamides	1,75±0,20	0	0	0	0,43	-89,96
Indometacine + *Oleum Olivae*	2,6±0,20	3±0,25	0	0	1,40	-66,26
Ranitidin	1±0,09	0	0	0	0,25	-93,97

x±e.s. = media ± standard error, p<0,05

Determination of gastric secretion acidity

The animals treated with the methylic esters of the fatty acids from *Oleum olivae* presented a decrease of the gastric acidity with 38,11% compared with indometacine, and those treated with ethanolamides presented a decrease of 60,66%. Also the extra-virgin *Olivae oleum* reduced the gastric acidity with 32,84% compared with the animals treated with indometacine.

Table 2 The influence of the ethanolamides and of the esters of the fatty acids from extra-virgin *Oleum Olivae* on the gastric secretion produced by indometacin

Batch	**Gastric volume content** (mL)		**Total acidity** (mEq/L)		
	x±e.s.	*Efect%*	*x±e.s.*	*Effect %*	*IPP*
Untreated controls	2,75±0,3	-	14,67±1,3	-	-
Indometacine	4,10±0,45	49,09	27,10±2,90	84,73	-
Indmethacine + methilic esthers	3,28±0,40	19,27	16,77±1,40	-38,11	87,47
Indometacine + ethanolamides	2,36±0,32	-14,18	10,66±0,95	-60,00	137,61
Indometacine + *Oleum Olivae*	3,10±0,35	12,72	18,2±1,60	-32,84	80,60
Ranitidin	1,3±0,22	-52,72	6,85±0,70	-74,72	214,16

x±e.s.=media ± standard error, p<0,05, IPP = percentage protection index

Conclusions

The ethanolamides of the fatty acids from extra-virgin *Olivae oleum* presented the most pronounced effect in the ulcers provoked by indometacine and of decrease of the gastric acidity on rat.

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