The anti-inflammatory action of the ethanol amides of the extra-virgin *Oleum olivae* fatty acids by experimental acute inflammation methods on rats

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Abstract

Unsaturated or polyunsaturated fatty acid ethanol amides represent a new class of active biological compounds. Recent researches at a global level has demonstrated that in the body, at the cellular level, under the action of the cyclooxigenasess, the ethanolamides of unsaturated fatty acids are transformed into the corresponding prostamides that represent a new class of local hormones with a wide therapeutic range.

Taking into account that the mono and poly-unsaturated fatty acids in the extra-virgin olive oil are mainly present in the form of triglycerides, we have elaborated a technique of their transformation into the corresponding ethanol amides through an ethanolamide reaction. Studies were carried out regarding the anti-inflammatory action of the 5% gel of the amides of the fatty acids from the olive oil (extra virgin Olivae oleum) by local application of the gel and by oral administration of 5% of the solution.

The anti-inflammatory action was tested on white male Wistar rats, using as edemogen agents the Dextran solution 6%. The determination was made by the plethysmometric measurement of the edema. The ethanolamides of the fatty acids from Olivae oleum presented an anti-inflammatory action in local application and in oral administration.

In the inflammatory model produced by the 6% Dextran solution, the most pronounced effect was presented by the 5% gel of the ethanolamides of the fatty acids, of 33.33% compared to the phenylbutazone gel, of 43.33% anti-inflammatory effect after 120 minutes from the induction of the inflammation. The orally administration of the 5% solution of the ethanolamides of the fatty acids decreased the inflammation after 120 minutes with 26.66%.

Keywords: extra-virgin *Olivae oleum,* ethanolamides

Introduction

The extra-virgin olive oil is obtained by cold pressing and centrifugation of the mature fruit of *Olea europea L.* The extra-virgin olive oil contains glycerides of the linoleic and arahidonic acids, trioleine, tripalmitine, tristearine, sterols (beta-sitosterol), delta 7–stigmasterol, delta 5 –avenasterol, unsaponifiable substances represented by iridoids, triterpens and lignanic compounds (Table 1) [1].

Many studies have shown the importance of a rich in olive oil diet [2,3,4,5] in the treatment of high blood pressure and the prevention of the cardio-vascular diseases.

Taking into account that in the composition of the olive oil, mono- and poly-unsaturated fatty acids are principally present as glycerids, at the National Institute of

Chemical and Pharmaceutical Research and Development, a transformation method of the fatty acids into the corresponding methylic esters and ethanol amides was developed.

Table 1. The extra-virgin olive oil characteristics [1]

Relative density	0.913.
Acid value	< 2.0, determined on 5.0 g.
Peroxide value	< 20.0.
Unsaponifiable matter	< 1.5 %
Absorbance	$T_{270\ nm} < 0.20$. $A_{232\ nm}/A_{270\ nm} > 8$
Composition of fatty acids	
- saturated fatty acids of chain length less than C16	< 0.1 %
- palmitic acid	7.5% - 20.0 %
- palmitoleic acid	< 3.5 %
- stearic acid	0.5 % - 5.0 %
- oleic acid	56.0 % - 85.0 %
- linoleic acid	3.5 % - 20.0 %
- linolenic acid	< 1.2 %
- arachidic acid	< 0.7 %
- eicosenoic acid	< 0.4 %
- behenic acid	< 0.2 %
- lignoceric acid	< 0.2 %
Sterols	
- sum of β-sitosterol, Δ5,23-stigmastadienol, clerosterol, sitostanol, Δ5-avenasterol and Δ5,24-stigmastadienol	> 93.0 %
- cholesterol	< 0.5 %
- Δ7-stigmasterol	< 0.5 %
- campesterol	< 4.0 %
- stigmasterol	< campesterol

To obtain the fatty acid ethanolamides starting form the *olivae oleum virginale* were approached two synthesis strategies: a) the aminolysis of the glicerides b) the transformation of the glicerides in the corresponding methylic esters. The final product is a mixture of the corresponding ethanolamides where predominates according to the percentual composition the ethanolamide of the oleic acid and of the linoleic acid respectively and also a small quantity of the ethanolamide of the stearic acid. The structure of the methylic esters of the 1-ethanolamides obtained was confirmed by the 1H-RMN specters and IR. The current synthesis is subject of a patent.

Research has demonstrated that the ethanol amides of the fatty acids represent a new class of biologically active compounds [6,7]. The anandamide (arahidonoil ethanol amide AEA) is the ethanolamide of the arahidonic acid and it was identified as an endogenous ligand for the canabinoid receptor in the brain. Even if from the structural point of view AEA is different from canabinoids, it presents pharmacological properties that are similar to those of the canabinoids. By the interaction with the periferic receptors CB1 or /and CB2, AEA produces a strong immuno-modulatory and anti-inflammatory activity. Under the action of the cyclooxigenase 2 (COX-2) but not of the COX 1, AEA is oxidized to similar products with the substances formed from the arahidonic acid. Palmitethanolamides possess anti-inflammatory properties [8] and the oleamide is a sleep induced agent [9,10].

In the present study we have tested the anti-inflammatory action of ethanol amides from extra-virgin olive oil at a concentration of 5% in **local** application formulated as hydrogel compared to the phaenylbutasone gel 4% and in **oral** administration of the 5% solution compared to the phaenylbutasone solution in concentration of 125 mg/kg body weight administered intraperitoneally (i.p.).

Materials and Methods

Materials. The olive oil used in the study was manufactured in Greece, with specifications according to the European Pharmacopoeia [11], monography *Olivae oleum virginale*.

All materials were pharmaceutical grade raw materials processed and controlled in our laboratory. For the pharmaco-toxicological studies, the ethanol amides and the olive oil were locally administered under the form of gels in concentration of 5% prepared from Carbopol 940, propylene glycol, triethanolamine (TEA) and purified water.

For the oral administration the water insoluble ethanolamides were administered as a 5% solution in sunflower oil. The oral route was used because of the impossibility to administer intraperitonealy the ethanolamides oil solution. The phenylbutazone prepared from pharmaceutical grade raw phenylbutazone and sterile saline solution 0.9% was used intraperitonealy because the orally administration is unsafe and is not able to achieve the maximum of biodisponibility.

The Dextran 70 sterile solution 6% in saline solution 9 g/L was produced by Sicomed, Romania.

Methods. Batches of 6 white, male Wistar rats were taken for study, for the local administration and batches of 8 rats for the oral and intraperitonealy administration route, weighing 190±10 g, kept into mobile cages, in special shelters, in standard laboratory conditions.

The Dextran solution 6% was used as edemogen agent to determine the anti-inflammatory action. A quantity of 0,1 mL of Dextran solution 6% was injected subcutaneously into the rear paw. Treatment was performed locally with the gel of the ethanolamides in the fatty acids, at a concentration of 5 g% mL and orally, by gavage with 0,5 mL of solution 5g % mL of ethanolamides/100 g b.w and 2 mL of the saline solution of 125 mg/b.w., intraperitoneally.

All the locally applied substances were administered in amount of 0,2 mg, on the skin surface of the inflamed paw, by massage until the complete penetration of the gel.

The research was performed between 9,00 AM and 16,00 PM, at 22°C. The research bioethics regulations for the laboratory animals were met [12].

Determinations. The measurements were made comparative to the phenylbutazone gel 4% and with the gel of extra-virgin olive oil in concentration of 5%. During the oral treatment, the determinations were made in comparison with phenylbutazone 125 mg/ kg body weigh (b.w.) i.p.

The evolution of the inflammatory process was quantified by plethysmometric measurement of the rat paw volume from 30 to 30 minutes for 120 minutes. The paw volume was measured with a 7140 – Plethysmometer. The anti-inflammatory effect was calculated comparing the inflamed treated paws with the inflamed untreated paws.

Data analysis. The statistical analysis was done by Student test, using XLSTAT software.

Results and Discussion

The inflammation produced by the 6% Dextran solution decreased with 33.33% for the gel of the ethanolamides fatty acids from the of extra-virgin olive oil (Fig. 1) and with 26.66% in orally administration of the fatty acids ethanolamides 5% (Table 2).

Table 2. The anti-inflammatory action of the ethanolamides of the fatty acids from extra-virgin *Oleum Olivae* administered locally on the edema produced by the 6% Dextran solution on rat

Tested product	Paw edema, mL					Inhibition of the oedema, %			
	Initial volume	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Untreated controls	0.20± 0.022	0.34± 0.04	0.360± 0.05	0.410± 0.06	0.380± 0.065	-	-	-	-
Gel base 0,1 mg	0.17± 0.02	0.30± 0.04	0.38± 0.05	0.44± 0.06	0.40± 0,04	11.76	5,55	7,31	5,26
Phaenyl-butasone gel 4%	0.19± 0.021	0.28± 0.03*	0.294± 0.03**	0.30± 0.05**	0.240± 0.03*	17.64	18.33	26.82	36.84
5% gel of ethanolamides of the fatty acids	0.20± 0.022	0.298± 0.04*	0.302± 0.03*	0.316± 0.04*	0.253± 0.03**	12.35	16.11	22.92	33.43
5% gel with extra-virgin *Oleum Olivae*	0.21± 0.023	0.324± 0.04*	0.335± 0.03*	0.369± 0.05*	0.315± 0.03*	4.70	6.94	10.00	17.10

x±e.s. = media ± standard error, *p<0.05, **p<0.01

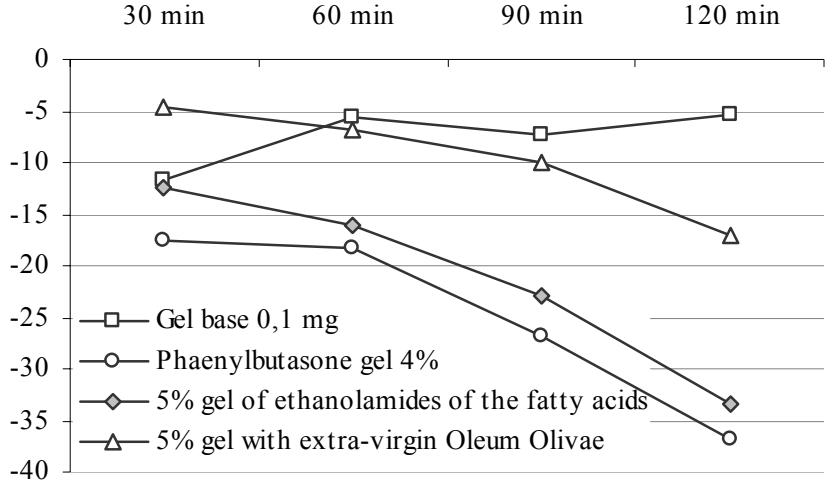

Figure 1. The anti-inflammatory action of the fatty acids ethanolamides from extra-virgin *Oleum Olivae* on the edema administered locally

The maximum effect of inflammation decrease was observed after 120 minutes. The olive oil gel presented a lower effect of 16.66%.

The inflammation produced by the 6% Dextran solution decreased for the fatty acid ethanolamides from the extra-virgin *Oleum Olivae* with 33.43% after 120 minutes in comparison with the decrease of 36.84% induced by the phenylbutazone at the same time

span. The 5% extra-virgin *Oleum Olivae* gel decreased the inflammation with 17.10% after 120 minutes from the induction of inflammation.

Table 3. The anti-inflammatory action of the fatty acids ethanolamides from extra-virgin *Oleum Olivae* administrated orally

Tested product	Paw edema, mL					Inhibition of the oedema, %			
	Initial volume	30 min	60 min	90 min	120 min	30 min	60 min	90 min	120 min
Untreated controls	0.20± 0.022*	0.34± 0.04*	0.360± 0.05*	0.410± 0.06	0.380± 0.065*	0	0	0	0
Sunflower oil 0.25 mL/100 g b.w.	0.20± 0.0192	0.33± 0.03*	0.354± 0.032*	0.40± 0.042*	0.38± 0.052*	2.94	1.66	2.43	0
Extra – virgin olive oil 0.25 mL /100 g b. w orally	0.211± 0.016*	0.285± 0.032*	0.315± 0.023	0.308± 0.023*	0.298± 0.027*	16.76	12.50	24.84	21.57
Ethanolamide solution 0.5 mL 5 mg/100 mL. g b.w orally	0.21± 0.020*	0.330± 0.04*	0.340± 0.05*	0.352± 0.04*	0.281± 0.03*	2.94	5.55	14.14	26.05
Phenyl butasone 125mg/100 g animal i.p.	0.18± 0.019*	0.270± 0.03*	0.280± 0.03**	0.285± 0.03*	0.210± 0.02*	20.58	22.22	30.48	44.73

x±e.s. = media ± standard error. *p<0.05, **p<0.01

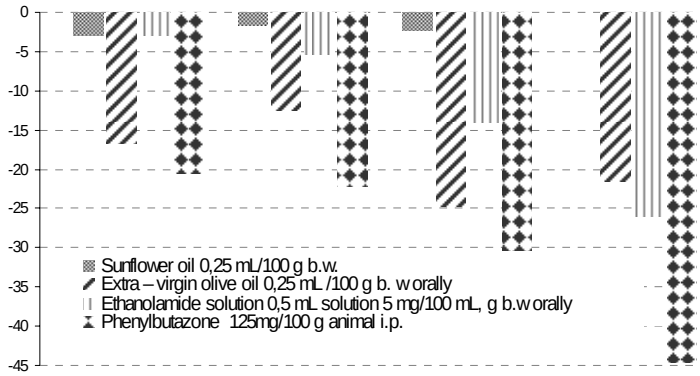

Figure 2. The anti-inflammatory action of the fatty acids ethanolamides from extra-virgin *Oleum Olivae* administrated orally

After the orally administration of 0.5 mL solution 5% ethanolamides in sunflower oil the maximum decrease effect (26.05%) was observed after 120 minutes from the induction of inflammation (Table 3).

The olive oil had an anti-inflammatory effect of 10% in the local administration after 120 minutes. After 150 minutes from the induction of inflammation in local and oral administration of the ethanolamides of the extra-virgin *Oleum Olivae*, the decrease of inflammation effect was reduced, almost 12.60 % for the locally administration and 5.12 % for the oral route (Fig. 3).

Conclusions

The substances containing fatty acids ethanolamides from the extra-virgin olive oil tested have an anti-inflammatory action on the acute inflammation produced experimentally by the 6% Dextran solution for locally and orally administration.

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