

EXPERIMENTAL EVALUATION OF SOME BIOCHEMICAL PARAMETERS AFTER ADMINISTRATION OF FATTY ACIDS ETHANOLAMIDES FROM EXTRA VIRGIN OLIVE OIL IN RATS

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Abstract

Extra virgin olive oil is rich in fatty acids, especially in mono unsaturated glycerides. The National Institute for Chemical and Pharmaceutical Research and Development (NICPRD) Bucharest has developed a transformation process of the monoglycerides in the appropriate ethanolamides, through an aminolysis reaction with ethanolamine.

On the fatty acids ethanolamides obtained from extra virgin olive oil there were performed pharmaco-toxicological studies. It was assessed their influence on biochemical parameters: glicemy, total plasmatic lipids, cholesterolemia, HDL-cholesterol, LDL-cholesterol, non-protein nitrogen compounds and the plasmatic transaminases activity compared with olive and sunflower oil.

Extra virgin olive oil does not present toxicity and fatty acids ethanolamides have a reduced toxicity.

The value of total lipids decreases after the treatment with olive oil fatty acids ethanolamides with 19.43% and with 14.02% for the olive oil.

Serum triglycerides level decreases by 11.33% for ethanolamides and 12.88% for olive oil. Cholesterolemia diminishes 19.84% for ethanolamides and 13.08% for olive oil.

LDL-c value diminishes 17.62% for ethanolamides and 11.86% for extra virgin olive oil.

The non-protein nitrogenous compounds, urea, creatinine and uric acid show a moderate decrease under the influence of ethanolamides and extra virgin olive oil studied. The activity of plasmatic transaminases and serum glicemy have not changed significantly under the influence of ethanolamides and olive oil.

Rezumat

Uleiul de măsline extra virgin este bogat în acizi grași, în special mononesaturați sub formă de gliceride. La Institutul Național de Cercetări Chimico-Farmaceutice (ICCF) București s-a elaborat un procedeu de transformare a acestor gliceride în etanolamidele corespunzătoare, printr-o reacție de aminoliză cu etanolamina.

Asupra amestecului de etanolamide ale acizilor grași din *Oleum olivae* extra virgin, obținute, s-au efectuat studii farmaco-toxicologice și s-a urmărit influența acestora

asupra parametrilor biochimici serici: glicemie, lipide totale, colesterolemie, HDL-colesterol, LDL-colesterol, compuși azotați neproteici și activitatea transaminazelor serice, comparativ cu uleiul de măsline și uleiul de floarea soarelui.

Uleiul de măsline extra virgin nu prezintă toxicitate, iar etanolamidele acizilor grași au o toxicitate redusă.

Lipidele totale scad în urma tratamentului cu etanolamidele acizilor grași din uleiul de măsline cu 19,43%, iar pentru uleiul de măsline cu 14,02%. Trigliceridele serice scad cu 11,33% pentru etanolamide și cu 12,88% pentru uleiul de măsline. Colesterolemia scade cu 19,84% pentru etanolamide și cu 13,08% pentru uleiul de măsline. LDL scade cu 17,62% pentru etanolamide și cu 11,86% pentru uleiul de măsline extra virgin.

Compușii azotați neproteici, ureea, creatinina și acidul uric prezintă scăderi moderate sub influența etanolamidelor luate în studiu și a uleiului de măsline extra virgin.

Activitatea transaminazelor serice și glicemia nu se modifică semnificativ sub influența etanolamidelor și a uleiului de măsline.

Keywords: fatty acids ethanolamides from olive oil, biochemical parameters

Introduction

Recent research studies worldwide have shown that the fatty acids ethanolamides are converted under the action of the appropriate cyclooxygenases in the cells of the body in prostamides, which is a new class of local hormones with a wide range of therapeutic actions. Thus, biological tests showed that fatty acids ethanolamides can inhibit the aggregation of blood platelets induced by different substances, the activity of lipooxygenase preventing the formation of free radicals, inhibit the proliferation of lymphocytes.[1,6,7,8].

The ethanolamide of arachidonic acid, anandamide (arachidonil ethanolamide AEA) has been identified as an endogenous ligand for the cannabinoid receptors in the brain. Although structurally different from cannabinoids, AEA shows pharmacological properties similar to cannabinoids. By the interaction with periferic CB₁ receptors and / or CB₂, AEA has a strong anti-inflammatory and immunomodulatory activity. [4,5,8].

Under the action of cyclooxygenase 2 (COX₂) but not of cyclooxygenase 1 (COX₁), AEA is oxidized through a similar mechanism with arachidonic acid cascade (AA) and it is transforming into the corresponding prostamides similar to those formed from the arachidonic acid.

In the present paper there were performed pharmaco-toxicological studies of fatty acids ethanol amides from extra virgin olive oil and it was observed their influence on some biochemical parameters.

Materials and methods

Determination of the acute toxicity

We took into study a group of 20 white mice of both sexes, weighing 22 ± 3 g, that were kept in standard laboratory conditions which were treated with increasing doses of ethanolamides. Each group was observed daily for 14 days, counting the number of dead animals, the clinical status of the animals, the dynamic increase in weight and the appearance. DL50 calculation was performed using graphics [2].

All procedures were carried out in accordance with the Directive 86/609/EEC of 24th November 1986, regarding the protection of animals used for experimental and other scientific purposes.

Evaluation of the biochemical parameters

We used groups of 8-10 white male Wistar rats, each, weighing 220 ± 10 g. The treatment was carried out with fatty acids ethanolamides in DL50/50 dose orally, daily for 7 days. The control group received a solution of NaCl 0.9% in a quantity of 0.5 mL/100g body weight.

The determinations were compared with the extra virgin olive oil and sunflower oil treatment in quantities of 0.5 mL/100g body weight.

Two hours after the last administration, animals were killed under anesthesia with chloroform, blood was collected and serum separated and used for the biochemical parameters assesment.

The determination of blood glucose was performed using the Cormay method [6].

The determination of total lipids, total cholesterol and HDL-cholesterol was performed using also the Cormay method [6]. LDL-cholesterol was performed using an enzymatic-colorimetric method and the plasmatic triglycerides using an enzymatic method [6].

Among non-protein nitrogenous compounds, serum urea was determined by the enzymatic method with urease, uric acid by the enzymatic method with uricase and serum creatinine by the colorimetric method. Plasmatic transaminases activity was determined by an enzymatic method [6].

Statistical evaluation of results was performed using student "t" test and ANOVA.

Results and discussion

Determination of the acute toxicity

Based on the obtained results the fatty acids ethanolamides from extra virgin olive oil present a low toxicity at a dose of 3326 mg/kg body weight orally.

The body weight of animals treated with ethanolamides decreased by 9.09% compared to the initial weight, while the control animals weight increased by 37.50%.

Evaluation of the biochemical parameters

Glicemy was not significantly altered after the treatment with ethanolamides taken into study (Table I), which shows that they do not affect the pancreatic function and do not inhibit the enzymes involved into the hydrolysis of polysaccharides.

Table I
The action of fatty acids ethanolamides
from extra virgin *Olivae oleum* on blood glucose, in rats

Tested product	Dose	Glicemy mg/dL		
		X $\pm$ SD	Effect %	p/control
Controls	NaCl 0.9% 0.5mL/100g bw	117.04 $\pm$ 5.84	-	-
Fatty acids ethanol amides from olive oil	66.52mg/kg bw orally	114.58 $\pm$ 4.37	-2.10	0.07549
Extra virgin <i>Oleum olivae</i>	0.5mL/100g bw	115.61 $\pm$ 3.98	-1.22	NS
<i>Oleum helianthi</i>	0.5mL/100g bw	116.5 $\pm$ 6.2	-0.046	NS
F (ANOVA)	23.87654			
P (ANOVA)	0.35965			

X $\pm$ SD = mean $\pm$ standard deviation

NS = not significant

Total lipids decreased by 19.43% for ethanolamides and by 14.02% for the extra virgin olive oil, and serum triglycerides decreased by 11.33% and 12.88% respectively (Table II, fig. 1).

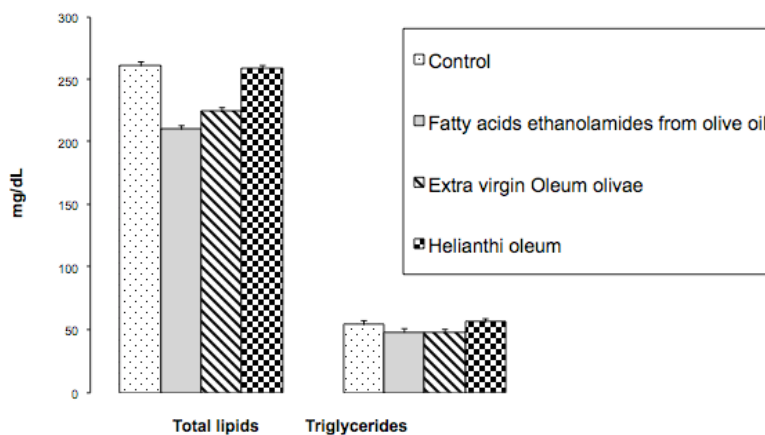
Table II

The fatty acids ethanolamides from extra virgin *Olivae oleum* on total lipids and plasmatic triglycerides, in rats

Tested product	Dose	Total plasmatic lipids mg/dL			Plasmatic triglycerides mg/dL		
		X±SD	Effect %	p/control	X±SD	Effect %	p/control
Control	NaCl 0.9% 0.5mL/ 100 g bw	260.90 ± 16.21	-	-	54.18 ± 3.48	-	-
Fatty acids ethanolamides from olive oil	66.52 mg/kg bw orally	210.19 ± 15.84	- 19.43	0.02049	48.05 ± 3.22	- 11.33	0.01438
Extra virgin <i>Oleum olivae</i>	0.5 mL/100 g bw	224.30 ± 19.51	- 14.02	0.01953	47.2 ± 3.50	- 12.88	0.01683
<i>Helianthi oleum</i>	0.5 mL/100 g bw	258.6 ± 17.7	-0.88	NS	56.3 ± 2.88	+3.91	NS
F (ANOVA)	53.61136				51.30613		
P (ANOVA)	0.17421				0.12096		

X ± SD = mean ± standard deviation

NS = not significant

**Figure 1**

The fatty acids ethanolamides from extra virgin *Olivae oleum* on total lipids and plasmatic triglycerides, in rats

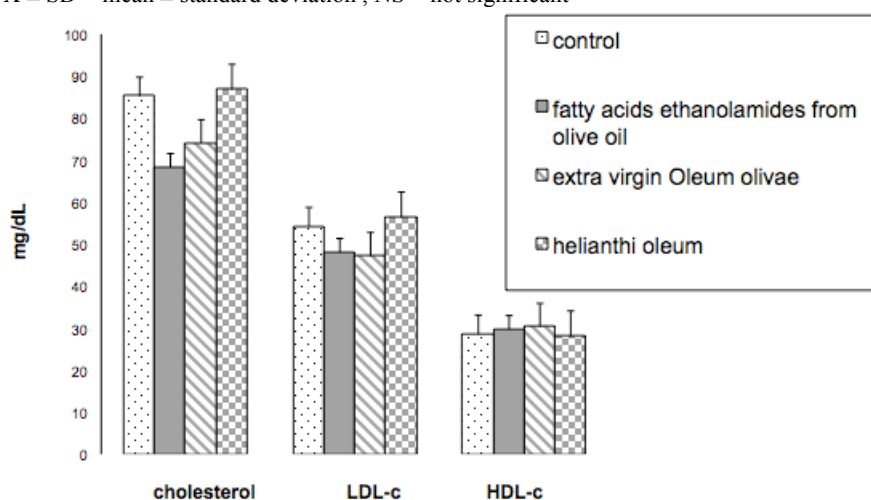
Cholesterolemia decreased by 19.84% with ethanolamides, LDL-cholesterol with 17.62%, while HDL-cholesterol did not change significantly (Table III, fig. 2).

Table III

The activity of fatty acids ethanolamides from extra virgin *Olivae oleum* on plasmatic cholesterol, in rats

Tested product	Dose	Total cholesterol mg/dL			LDL-c mg/dL			HDL-c mg/dl		
		X±SD	Effect %	p/control	X±SD	Effect %	p/control	X±SD	Effect %	p/control
Control	NaCl 0.9% 0.5mL/ 100 g bw	85.16 ± 4.58	-	-	54.18 ± 3.48	-	-	28.54 ± 2.47	-	-
Fatty acids ethanolamides from olive oil	66.52 mg/kg bw orally	68.26 ± 3.29	-19.84	0.0494	48.05 ± 3.22	-11.33	0.01438	29.72 ± 2.13	+4.13	0.28651
Extra virgin <i>Oleum olivae</i>	0.5 mL/100g bw	74.02 ± 5.6	-13.08	0.0325	47.2 ± 3.50	-12.88	0.01683	30.31 ± 2.60	+6.2	NS
<i>Helianthi oleum</i>	0.5 mL/100g bw	86.7 ± 6.1	+180	NS	56.3 ± 2.88	+3.91	NS	28.01 ± 1.6	-	NS
F ANOVA		81.08631			51.30613			35.17831		
P ANOVA		0.23309			0.12096			0.17532		

X ± SD = mean ± standard deviation, NS = not significant

**Figure 2**

The activity of fatty acids ethanolamides from extra virgin *Olivae oleum* on plasmatic cholesterol, in rats

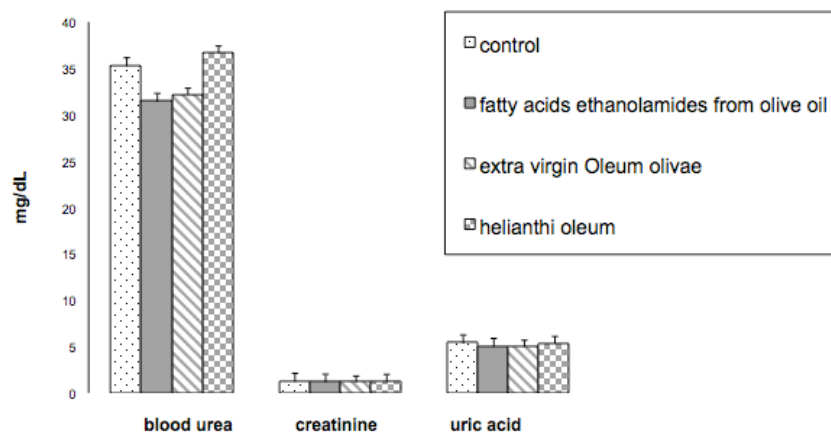
Among non-protein nitrogenous substances, serum urea decreases significantly, with 10.85%, after the treatment with ethanolamides taken into study. Uric acid and serum creatinine do not change the values significantly from a statistical viewpoint, which shows that these compounds did not affect the renal and hepatic function (Table IV, fig.3).

Table IV

The action of fatty acids ethanolamides from extra virgin *Olivae oleum* on non-protein nitrogenous substances, in rats

Tested product	Dose	Blood urea mg/dL			Creatinine mg/dL		Uric acid mg/dL	
			X±SD	Effect %	X±SD	Effect %	X±SD	Effect %
Control	NaCl 0.9% 0.5mL/ 100 g bw		35.20 ± 4.03	-	1.18 ± 0.94	-	5.31 ± 3.92	-
Fatty acids ethanolamides from olive oil	66.52mg/kg bw orally		31.38 ± 4.15	- 10.85	1.1 ± 0.92	-6.77	4.96 ± 2.73	-6.59
		p/control	0.054331		NS		NS	
Extra virgin <i>Oleum olivae</i>	0.5 mL/100g bw		32.15 ± 4.60	-8.66	1.12±0.7	-5.08	4.98 ± 3.56	-7.72
		p/control	0.02351		NS		0.02751	
<i>Helianthi oleum</i>	0.5 mL/100g bw		36.60 ± 4.8	+3.29	1.20 ± 0.8	+1.69	5.29 ± 3.28	- 0.376
		p/control	NS	-	NS		NS	
F(ANOVA)	43.07862				11.96433		52.10862	
P(ANOVA)	0.37541				0.46419		0.64311	

X ± SD = mean ± standard deviation, NS = not significant

**Figure 3**

The action of fatty acids ethanolamides from extra virgin *Olivae oleum* on non-protein nitrogenous substances, in rats

Plasmatic transaminases activity was not significantly altered by fatty acids ethanolamides and neither by olive oil, which shows that these compounds do not harm the hepatocyte. (Table V, fig. 4).

Table V
The action of fatty acids ethanolamides from *Oleum olivae* on the activity of plasmatic transaminases, in rats

Tested product	Dose	AST U/L			ALT U/L		
		X±SD	Effect %	p/control	X±SD	Effect %	p/control
Control	NaCl 0.9% 0.5mL/100 g bw	140.67 ± 7.8	-	-	31.72 ± 1.88	-	-
Fatty acids ethanolamides from olive oil	66.52mg/kg bw orally	136.18 ± 8.3	-2.75	NS	29.80 ± 1.36	-6.05	NS
Extra virgin <i>Oleum olivae</i>	0.5 mL/100g bw	137.84 ± 9.2	-2.01	NS	30.2 ± 1.88	-4.79	NS
<i>Helianthi oleum</i>	0.5 mL/100g bw	139.02 ± 8.8	-1.17	NS	32.3 ± 1.64	+1.82	NS
F(ANOVA)		52.08912			47.90321		
P(ANOVA)		0.21765			0.37378		

X ± SD = mean ± standard deviation

NS = not significant

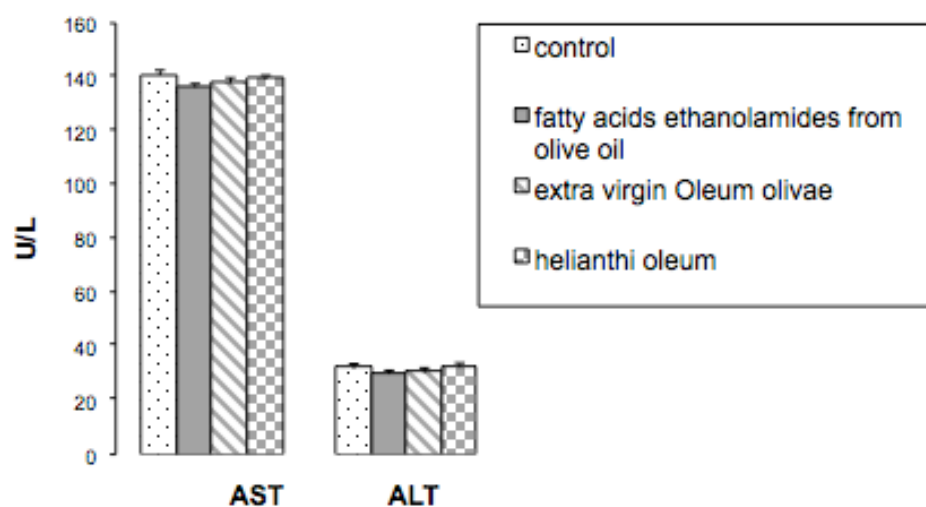


Figure 4

The action of fatty acids ethanolamides from *Olivae oleum* on the activity of plasmatic transaminases, in rats

Conclusions

From the extra virgin olive oil there were obtained fatty acids ethanolamides through an aminolysis reaction with ethanolamine.

Fatty acids ethanolamides from olive oil present a very low toxicity.

The tested ethanolamides decrease moderately the lipemy, cholesterolemia, plasmatic triglycerides and the LDL-cholesterol.

Non-protein nitrogenous compounds, urea, creatinine and uric acid register moderate decreases under the influence of the ethanolamides and of the extra virgin olive oil taken into study.

Transaminases activity and serum glycemie not changed significantly under the influence of ethanolamides and olive oil.

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