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PATHOLOGICAL CHANGES IN THE DIGESTIVE SYSTEM OF RATS RECEIVED ORAL APPLICATIONS OF THERMOPEROXIDE SUNFLOWEROIL

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Abstract

Inadequate fat diet is one of the most important reasons for the development of the most widespread non-communicable diseases, such as atherosclerosis, coronary heart disease, type 2 diabetes mellitus, obesity, and metabolic syndrome. One of the most common forms of such nutrition is the consumption of thermally processed fats and oils, which have general toxicity, embryotoxicity, and carcinogenic effects due to the accumulation of peroxides, epoxides, aldehydes, trans compounds, and other toxic compounds.

Aim: To determine the condition of the organs and tissues of the digestive system when consuming heat-treated sunflower oil.

Methods: Thermal peroxidation of sunflower oil was carried out in the presence of H_2O_2 at temperatures of 125, 150 and 180 °C. The degree of peroxidation was assessed by the level of diene conjugates and the content of malondialdehyde (MDA). The effect of oral applications of thermal peroxide sunflower oil (TPSO) on the condition of the digestive system was assessed in rats by the degree of increase in the level of biochemical markers of inflammation: elastase activity, urease activity and MDA content.The level of these parameters was determined in the mucous membranes of the cheeks (MMOC), stomach, small and large intestines, as well as in the liver.

Results: The maximum increase in the content of diene conjugates and MDA is observed when the oil is heated for 60 minutes at +180 °C. After 5 days of TPSO applications at a dose of 2.25 g/kg, a significant increase in elastase activity by 36-156% (most in the stomach), urease activity by 30-278% (most in the liver) and MDA content by 26-210% (most in the liver). When summing up the degree of increase in the level of biochemical parameters in percent, it turned out that the highest degree of damage when consuming TPSO is observed in the liver (524%), in second place (244-270%) were the mucous membranes of the stomach and colon, and the lowest sensitivity to TPSO was MMOC.

Conclusion: Even short-term consumption of thermally processed sunflower oil causes serious pathological changes in the digestive system, especially the liver.

Keywords: *thermal peroxidation of fats, inadequate fat nutrition, pathology of the digestive system*

Introduction

Inadequate fatty nutrition (IAFN) is one of the most important causes of the development of the most widespread non-infectious diseases, such as atherosclerosis, coronary heart disease, type 2 diabetes mellitus, obesity, and metabolic syndrome [1].

One of the most common forms of IAFN is the consumption of thermally processed fats and oils both in home cooking in everyday meals and in the food industry [2-6].

The disadvantage of thermal processing of fats and fat-containing products (meat, fish, milk), is the formation of toxic products from unsaturated fatty acids, such as peroxides, epoxides, aldehydes, trans-compounds during such processing [7-10].

Established not only their general toxicity, but also embryotoxicity and carcinogenic effect [11].A number of works [6, 10, 12, 15] have shown the pro-inflammatory and pro-dysbiotic effects of thermoperoxide oils on the tissues of the oral cavity, on the colon, liver and kidneys.

Analysis of the above results of experimental studies showed that the pathogenic effect of thermoperoxide fats was assessed during their long-term consumption by animals (several weeks or even months).Taking into account the high toxicity of the compounds formed during the heat treatment of fats, it is possible to assume the appearance of pathological disorders in the body even with a shorter period of exposure to such factors.

In recent years, the structure of fats and fat-containing components used in human nutrition has changed significantly, in particular, due to a number of reasons, the consumption of sunflower oil, containing a large amount of linoleic acid ($C_{18:2}$), which is very easily amenable to thermoperoxidation, has increased significantly [16].

The aim of this study was to determine the pathogenic effect of thermo- peroxide sunflower oil (TPSO) on tissues and organs of the digestive system of rats under short-term exposure (3-5 days) by oral application of such oil.

To achieve this goal, the following tasks were set:

–To determine the effect of thermal peroxidation on the biological value of sunflower oil;

–To determine pathological changes in the organism of laboratory animals based on the determination of biochemical markers of inflammation and dystrophy.

Materials and methods

The work used unrefined sunflower oil, the fatty acid composition of which is presented in table.1 and fig. one.

The pathogenic effect of thermoperoxide sunflower oil is proposed to be evaluated by the nature of changes in the level of biochemical markers of inflammation and dystrophy, namely, by the degree of increase in the activity of the proteolytic enzyme elastase and the bacterial enzyme urease, as well as by the increase in the content of the end product of oxidation of unsaturated fatty acids malondialdehyde (MDA).

Leukocytes are the main source of elastase in tissues [17]. Urease is produced exclusively by bacteria, and it is itself a toxin [18], and, on the other hand, a marker of bacterial contamination [19]. As for MDA, its level reflects the degree of lipid peroxidation, which always accompanies the development of inflammatory-dystrophic processes [20].

Thermal peroxidation of the oil was carried out in the presence of 1.5% H_2O_2 (30%) by heating in a glycerol bath at 125 °C, 150 °C and 180 °C for 60 minutes.

The degree of peroxidation was assessed by two indicators: the content of diene conjugates [21] and MDA [22]. The peroxide number was not determined, since it does not reflect the process of thermoperoxidation due to the rapid destruction of peroxides at high temperatures [4].

Biological experiments were carried out on 37 white Wistar rats (females, 4-5 months old, 212-230 g) in two series of experiments. In the 1st series, TPSO obtained by heating to + 150 °C was tested. Oral applications were made at a dose of 0.5 ml of oil per rat (2.25 g/kg). There were 5 groups in total: 1st – control, 2nd – received oral applications of cold sunflower oil for 3 days, 3rd – received TPSOfor 3 days, 4th – received oral applications of cold sunflower oils within 5 days and the 5th – received TPSO also within 5 days. After the euthanasia of the animals (on the 4th and 6th days), respectively, in the 2nd, 3rd and 4th, 5th groups, the buccal mucosa (BM)

was excised, in the homogenate of which the activity of elastase [17], urease [23] and the content of MDA [22].

In the second series of experiments, TPSO was used, obtained by heating+180 °C. The rats were divided into 2 groups: 1st – control, 2nd – received TPSO applications at a dose of 2.25 g/kg for 5 days. After the euthanasia of the animals, on the 6th day, the mucous membranes of the cheeks, stomach, small and large intestines, and also the liver were excised. Before scraping the mucous membranes, they were washed with cold 0.9% NaCl from the contents and stored until the study at minus 30 °C.

In homogenates of isolated tissues, the activity of elastase, urease, and MDA content were determined.

The total pro-inflammatory effect (TPIE) of TPSO on an organ (or tissue) was estimated by the formula:

$TPIE = I_E + I_{MDA} + Iu$, where

I_E - increase in elastase activity,%;

I_{MDA} - increase in the content of MDA,%;

Iu - increased urease activity,%.

The results of experimental studies were subjected to standard statistical processing [24].

Experimental studies were conducted in accordance with the rules established by the Directive of the European Parliament and the Council (2010/63 / EU), by the order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 "On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions " and methodical recommendations.

Results

At the first stage of research in laboratory conditions, samples of thermo-peroxide sunflower oil were prepared and controlled without treatment, and the fatty acid composition was determined (Table 1, Fig. 1.).

In fig. 2 shows the content in TPSO of diene conjugates and MDA formed in sunflower oil at temperatures of +125 °C, +150 °C and +180 °C.

TPSO, obtained at +150 °C, was used in the 1st series of experiments, which showed that pathological changes in the BM are observed only after TPSO applications and more significantly after 5 days of applications (Table 2).

Therefore, in the second series of experiments, TPSO obtained by heating at +180 °C was used, and oral applications were made for 5 days.

Table 3 shows the results of determining the activity of elastase in different parts of the rat digestive tract.

It can be seen that the highest activity of elastase is observed in the mucous membrane of the small intestine, and the lowest in the mucous membrane of the stomach. A fairly high elastase activity is observed in the liver (possibly due to macrophages –Kupffer cells [25]).

In all rats that received oral TPSO applications, the activity of elastase, the main biochemical marker of inflammation, was significantly increased.

Table 4 shows the results of determining the content of the second biochemical marker of inflammation, MDA, in the tissues of the digestive system of rats.

The highest content of MDA was found in the liver, and the lowest in the mucous membrane of the small intestine. In rats that received oral TPSOapplications, the MDA content significantly increased in all tissues, especially strongly (by 3.1 times) in the liver.

Table 5 shows the results of determining the activity of urease, from which it can be seen that the highest (as expected) indicators were found in the intestinal mucous membranes, and the lowest in the liver.

The degree of increase (in %) in the level of all three markers of inflammation in the tissues of the digestive system of rats receiving oral TPSO applications indicates that the activity of elastase increases most of all in the gastric mucosa, while the content of MDA and the activity of urease – in the liver.

The results obtained agree with the numerous publications in the scientific literature on the toxic effect of thermoperoxidation products of edible fats and oils [6, 10, 11, 12, 15, 27, 28].

In fig. 3 presents a generalized assessment of pathological changes in the digestive system of laboratory animals after oral applications of TPSO.

Taking into account the values of all the studied markers of inflammation and dystrophy, the smallest changes in BM (115.6%) were found, and the liver was the most susceptible to the toxic effect of peroxidation products (524.1%) (Fig. 3).

As can be seen from Fig. 3, the sensitivity to the pathogenic effect of sunflower oil peroxidation products of the gastric and colon mucous membranes is quite significant and ranges from 244-270%, in the small intestine the same indicator is slightly lower – 190%, but also indicates the appearance of lesions.

Conclusions

1. It was found that oral applications of unoxidized sunflower oil did not significantly affect the level of biochemical markers of inflammation in the BM (elastase 69.7±7.9 μcat/kg, MDA 12.34±1.02 mmol/kg, urease 0.89±0.18 μcat/kg) and pathological processes that develop in the tissues of the digestive system of rats are caused by the action of peroxidation products.

2. A comprehensive assessment of the pathogenic effect when summing up the indicators of changes in the level of markers showed that the highest pro-inflammatory activity of sunflower oil peroxidation products is observed in relation to the liver (524.1%), and the lowest in relation to BM (115.6%), despite the fact that TPSO was introduced into the body by oral applications. In second place in sensitivity to the pathogenic effect of sunflower oil peroxidation products (244-270%) were the mucous membranes of the stomach and colon.

3. It has been established that even short-term exposure to TPSO peroxidation products (5 days of oral application at a dose of 2.25 g/kg) lead to pathological changes in the digestive system, which can subsequently cause deeper and systemic disorders (disturbance of the digestive, metabolic, antitoxic function, dysbiotic syndrome, etc.).

4. The established toxic effect of thermoperoxidation products of edible fats and oils on the body and the increasing consumption of thermally processed fat-containing products (due to their organoleptic attractiveness) exacerbate the need for further research, finding ways to solve the problem of preventing pathological complications of such inadequate fatty nutrition.

Acknowledgments

The authors declare that there are no conflicts of interest.

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Table 1. Fatty acid composition of non-oxidized and thermoperoxide sunflower oil

Number	Acid	sunflower oil sample	
		unoxidized	thermoperoxide
1	Myristic	0,23	0,18
2	Palmitic	7,85	8,14
3	Palmitooleic	0,19	0,18
4	Stearic	3,74	3,43
5	Oleic	29,77	30,00
6	Vaccenic	0,61	0,55
7	Linoleic	56,69	55,86
8	Linolenic	0,09	0,00
9	Arachinic	0,35	0,28
10	Eicosenic	0,24	0,17
11	Behenic	0,93	0,90
12	Lignoceric	0,33	0,20

Table 2. The level of biochemical markers of inflammation in the BM of rats receiving oral applications of sunflower oil (SO) and thermal peroxide sunflower oil (TPSO)

№	Groups	Elastase, mk-cat/kg	MDA, mmol/kg	Ureaza, mk-cat/kg
1	Control	73,0±6,2	13,20±0,92	0,74±0,06
2	SO, 3 days	66,9±6,9 p>0,3	13,34±1,27 p>0,5	0,76±0,07 p>0,5
3	TPSO, 3 days	88,8±5,3 p<0,05; p_1<0,05	14,87±1,41 p>0,05; p_1>0,05	0,81±0,02 p>0,05; p_1>0,05
4	SO, 5 days	69,7±7,9 p>0,3	12,34±1,02 p>0,3	0,89±0,18 p>0,3
5	TPSO, 5 days	91,1±2,7 p<0,05; p_1<0,05	15,63±1,35 p>0,05; p_1<0,05	1,02±0,11 p<0,05; p_1>0,05

p– in comparison with gr. one; p1 - in comparison with gr. 2 and 4.

Table 3. Activity of elastase in the digestive system of rats receiving oral applications of thermoperoxide sunflower oil (TPSO)

Organsandtissues	Elastase, mk-cat/kg	
	Control	TPSO
Buccal mucosa	70,3±5,2	112,2±5,0 p<0,05
Gastricmucosa	47,6±2,4	122,1±10,3 p<0,01
Smallintestinemucosa	1859±374	2681±220 p<0,05
Colonmucosa	117,8±5,0	230,7±8,2 p<0,001
Liver	332,7±13,4	451,9±17,1 p<0,001

Table 4. The content of malondialdehyde (MDA) in the digestive system of rats receiving oral applications of thermal peroxide sunflower oil (TPSO)

Organsandtissues	MDA, mmol/kg	
	Control	TPSO
Buccal mucosa	15,71±0,42	19,87±0,73 p<0,01
Gastricmucosa	7,00±0,69	9,83±0,62 p<0,05
Smallintestinemucosa	2,90±0,40	5,45±0,41 p<0,01
Colon mucosa MDA, mmol / kg	3,63±0,31	8,21±0,24 p<0,001
Liver	23,42±1,96	72,72±5,36 p<0,001

Table 5. Activity of urease in the digestive system of rats receiving oral applications of thermo- peroxide sunflower oil (TPSO)

Organs and tissues	Urease, mk-cat / kg	
	Control	TPSO
Buccal mucosa	0,44±0,02	0,57±0,05 p<0,05
Gastric mucosa	0,75±0,11	1,30±0,12 p<0,05
Smallintestinemucosa	2,54±0,42	3,94±0,34 p<0,05
Colon mucosa	2,37±0,34	2,89±0,80 p>0,3
Liver	0,09±0,01	0,34±0,05 p<0,01

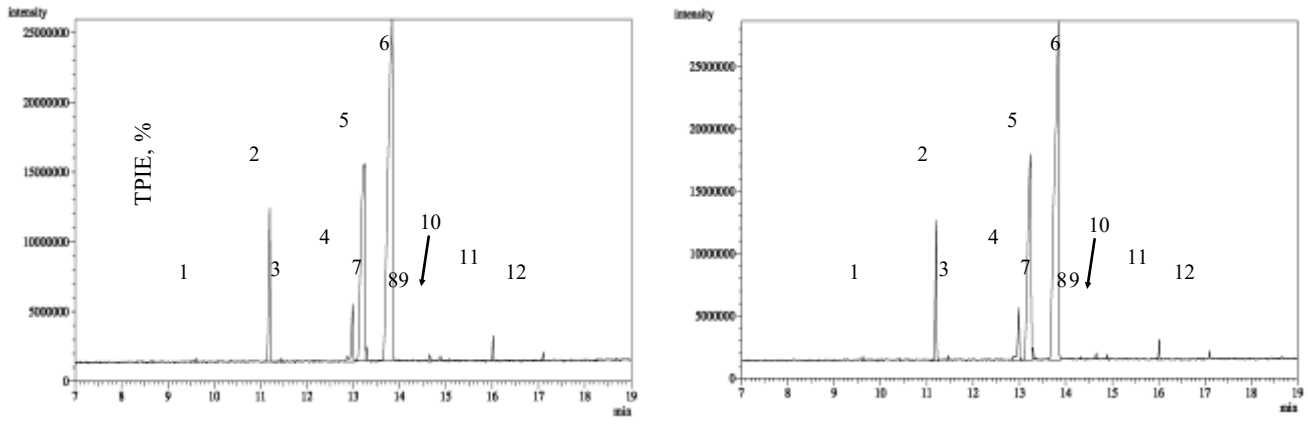

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Fig. 1. Chromatograms of unoxidized (A) and thermal peroxide (B) sunflower oil (TPPM). Thermoperoxidation conditions: temperature +150° C, 1.5% H_2O_2 (30%), 60 minutes.

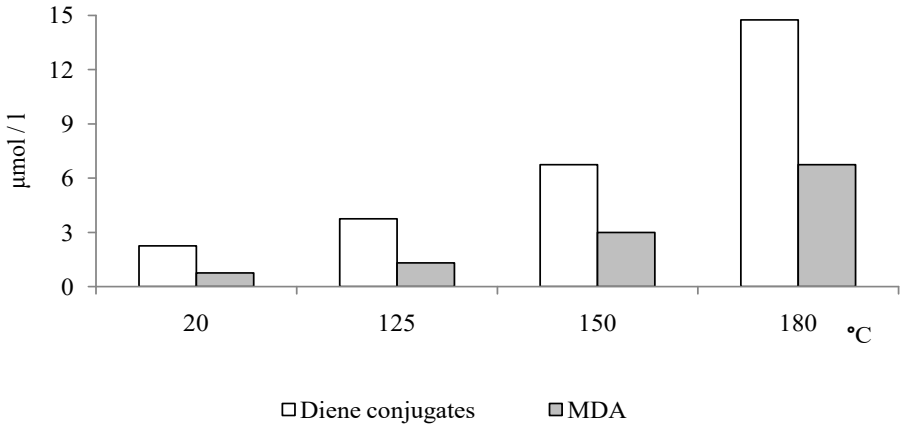

Fig. 2. Content of diene conjugates and MDA in TPSO after heating in the presence of 1.5% H_2O_2 (30%) for 60 minutes.

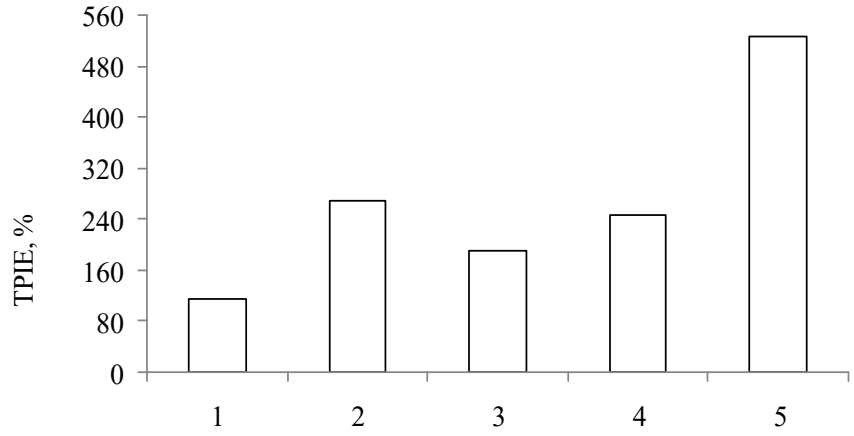

Fig. 3. The total pro-inflammatory effect (TPIE) of TPSO on the digestive system of rats (1 – BM, 2 – stomach, 3 – small intestine, 4 – large intestine, 5 – liver)