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Comparative studies of influence of palm oil on activity of xanthine oxidase and free radicals in liver and blood: Diabetes and metabolic syndrom

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ABSTRACT

Diabetes is a chronic metabolic disease, which has affected approximately 10.5% of the global population [1]. The most frequent types of diabetes are type-1 and type-2. The third frequent type is gestational diabetes. The pathogenicity of diabetes is multifactorial and involves various factors such as genetic, food, stress, environmental pollutants, and others, in most of the cases combination of two or more factors are noticed. Comparative studies of the influence of palm oil on the activity of xanthine oxidase (XOD) and free radicals in liver and blood. The research compared the effect of palm oil on carbohydrate metabolism in male rats: in young rats and adult rats. The research compared the effect of palm oil on free radicals in male rats: in adults and juvenile rats. Experimental animal body weight change research, 30 days after palm oil treatment. For this scientific research we utilized experimental animals of the male genus: mice of the Wistar type, small mice (45-60 g) and large mice (160-180 g). Experimental animals were divided into groups of six-eight experimental mice and details are shown in the corresponding part of the results. The results are shown in six figures and two tables. Results indicate an increase in the formation of free radicals in blood plasma, both in experimental juvenile mice and in experimental large mice under the influence of palm oil. Palm oil increases the activity of XOD in blood, both in small animals and in large experimental animals. Palm oil increases the formation of XOD in the black liver in both elderly groups. In all groups during the experiment there was an increase in body weight. Results from studies of the effect of palm oil on carbohydrate metabolism showed changes in glucose tolerance test. Current studies for the first time convincingly demonstrate the risk of using palm oil. Analysis of the results of two studies on XOD and carbohydrate metabolism showed a correlation between increased XOD levels and the development of prediabetes.

Keywords: diabetes, palm oil, xanthine oxidase, free radicals

INTRODUCTION

Diabetes is a chronic metabolic disease, which has affected approximately 10.5% of the global population [1]. The most frequent types of diabetes are type-1 and type-2. The third frequent type is gestational diabetes.

The pathogenicity of diabetes is multifactorial and involves various factors such as: genetic, food, stress, environmental pollutants, and others, in most of the cases combination of two or more factors are noticed. In type 2 diabetes leading factor is insulin resistance and or impairment of pancreatic beta-cell function, which produces and secretes the hormone insulin, important for carbohydrate metabolism, human metabolism. Diabetes is characterized by disturbance of carbohydrate, protein, and lipid metabolism and is associated with risk of multiple complications. Despite significant scientific and clinical progress, there are a large number of risk factors as obesity, increased formation of free radicals, certain cytokines, and others can be triggers for the development of diabetes. In addition, the accumulation of a significant amount of fatty tissue in the organism leads to the development of a series of complications during diabetes. Increased formation of free radicals is due to various causes, including the pharmacological substances and foods. Free radicals are particles, which have toxic effects on cells and as their concentration increases the balance between antioxidant protection of the cell and oxidative stress decays. During decay of the balance and predominance of oxidative stress, cell function is disrupted, including beta cells, reducing insulin production in the organism. The main studies in this paper deals on the effects of various substances on free radicals and carbohydrate metabolism. One of the modern problems in diabetes therapy is insulin resistance. The role of free radicals in the development of insulin deficiency is also discussed. In individual cases, when there is an increase in mass and an increase in free radicals, the risk of developing insulin resistance is greater.

Metabolic syndrome is a condition that is characterizes by a cluster of several physical diseases, with together increase the vulnerability of a person to developing cardiovascular disease, diabetes mellitus, and vascular and neurological complications such as cerebrovascular accidents. World Health Organization consultation proposal [2] was the first attempt to define metabolic syndrome, with the notion that modification is needed according to new research information and the predictive power of the proposed criteria.

Subsequently, the criteria of metabolic syndrome are modified according to the proposed formulated definitions of different committees, including the European Group for the study of Insulin Resistance [3], National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATP III) and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) [4], American Association of Clinical Endocrinology [5], International Diabetes Federation [6], and American Heart Association/National Heart, Lung, and Blood Institute [7]. Adipose tissue has an important role in controlling cardiovascular, endocrine, metabolic and other functions. The growth of adipose tissue is in the pathogenesis of said functions. It is accepted to discuss the state of obesity during adipose tissue growth. The triggers for weight gain can be grouped, as follows:

1. Endogenous: genetic, epigenetic, and associated endocrine disease.
2. Exogenous, which are associated with increased consumption of animal fats, sugar, salt, alcohol, decreased physical activity, stress, etc. [8].

Objectives

1. Compare studies of the influence of palm oil on the activity of xanthine oxidase (XOD) and free radicals in liver and blood.
2. Compare the effect of palm oil on carbohydrate metabolism in male rats: in young rats and adult rats.
3. Compare the effect of palm oil on free radicals in male rats: in adults and juvenile rats.
4. Make experimental animal body weight change research, 30 days after palm oil treatment.

MATERIALS & METHODS

We conducted the scientific experiments during the period September 2009-January 2014, while this scientific experiment for this paper was specifically carried out during the period September-November 2012, in the Pharmacology and Toxicology Laboratory at the State University of Sofia.

For this scientific research we utilized experimental animals of the male genus: mice of the Wistar type, small mice (45-60 g) and large mice (160-180 g). Experimental animals were divided into groups of six-eight experimental mice and details are shown in the corresponding part of the results**.** The growth of experimental animals was carried out under standard temperature conditions and on the basis of the respective requirements for the room, where the experiments were carried out. For experimental animal feed, we used pellets for experimental mouse (mainly for the control groups) as well as palm oil enriched diets (for the study groups). The approach for water of all experimental animals, was Ad Libitum.

Pharmacological Substances

Palm oil

Del Alma palm oil, made in Italy, is a vegetable fat (double fraction of palm oil), which does not oxidize, does not burn at high temperature and is ideal for frying.

Methodology for Determining Impact of Obesity on Activity of Enzyme Xanthine Oxidase

The influence of the fat-enriched diet on XOD activation was based on a comparison between the activity of the enzyme in tissues of experimental animals fed with a fat-enriched diet and those fed with standard food for rodents.

The group receiving standard food was designated as the control group, while the group receiving fat-rich food was designated as the experimental group.

Conducting experiment

The supernatant is prepared and the protein content in it is measured according to the procedures noted above. After that we made the measurements in blank samples on a plate containing the supernatant. In one ml of blank test sample there is 0.025 ml of solution of xanthine and phosphate buffer. In one ml of sample containing the supernatant there is 0.01 ml of supernatant, 0.025 ml of solution of xanthine and phosphate buffer. In our conditions we proved that, under the influence of the constituents in solution, the absorption maximum characteristic for uric acid is observed at 293 nm. First of all the signal absorption at 293 nm, A (293), was measured and 10 minutes after the addition of the xanthine solution to the blank sample. After that the same is done with the test, containing the supernatant. The instrumental error of our measurement was ±0.002. To account for errors in instrumental measurements, five parallel measurements were performed for each of the investigated samples.

Processing of Experimental Data

Initially from the database for absorption at 293 nm major experimental errors (Romanovski's method) are excluded. The data that remains after this procedure are used to determine A_{Expert}^{XO} $A_{Control}^{XO}$, resulting in two databases–one for control group, other for experimental group. From these two databases errors that may occur in the calculation are eliminated. The remaining values are used to calculate the effect values (*Effect*). The data set for the control group animals was used to determine the standard deviation around the baseline for comparison (deviations around *Effect*=100%).

The statistical significance of the observed effects was assessed using INSTAT statistical package, using all effect databases.

RESULTS

Scientific research was conducted in four groups of Wistar rats (from six-eight mice per group), distributed, as follows:

1. large mice (sexually mature)–controlling group,
2. large mice–which were treated daily with palm oil for 8 weeks (1 ml/100 kg),
3. small mice of male sex–controlling group, and
4. small mice–research group, treated with palm oil 1 ml/100 kg for eight weeks.

Four groups mentioned refer to two stages of research, namely the first stage for adult and sexually mature experimental animals aged three months and the second stage for juvenile experimental mice (28 days) with an initial weight of 45-55 g. During the time of the experiment the animals had free access to water and food.

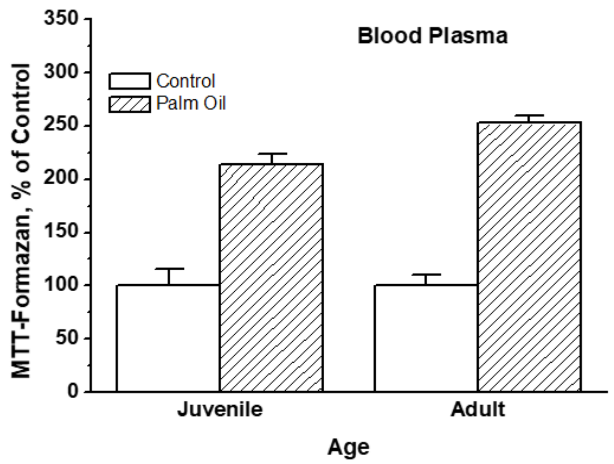

Figure 1. Effect of palm oil on MTT-Formazan: Blood plasma (Source: Author's own elaboration)

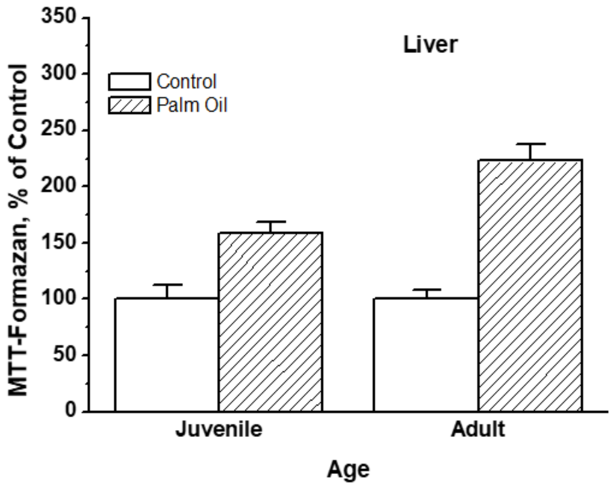

Figure 2. Influence of palm oil on MTT-Formazan: Liver (Source: Author's own elaboration)

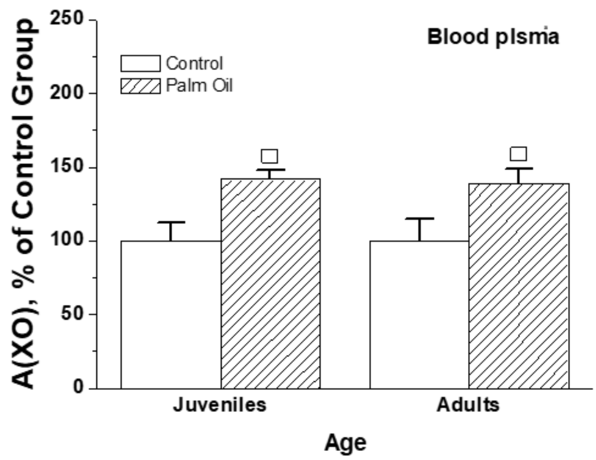

Figure 3. Influence of palm oil on XOD in blood plasma in small mice & large experimental mice (Source: Author's own elaboration)

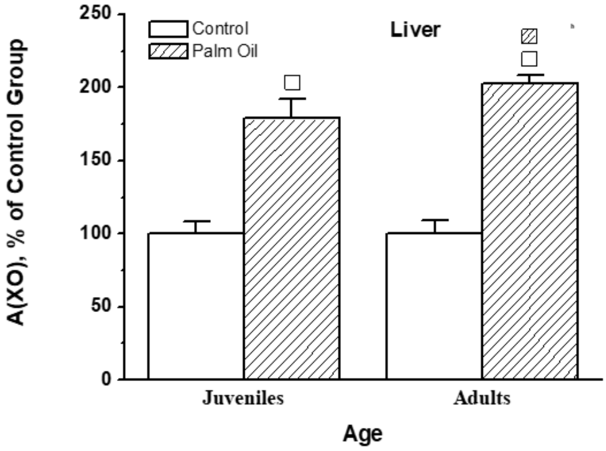

Figure 4. Influence of palm oil on XOD in black liver in adult & juvenile male mice (Source: Author's own elaboration)

Palm oil was administered once daily from 9-11 AM. The body weight of the experimental rats was determined weekly, while their behavior was observed daily. There are no mortality data. During experiments, indicators of carbohydrate metabolism (blood sugar and glucose load test)–glucose tolerance test (GTT)–were determined.

The results are shown in six figures and two tables.

In **Figure 1** and **Figure 2**, we present the results of the measurement of total radical formation, respectively in blood plasma and liver of four groups of experimental animals.

Results indicate an increase in the formation of free radicals in blood plasma, both in experimental juvenile mice and in experimental large mice under the influence of palm oil. There are no statistically significant differences between the two ages when determining free radicals in blood.

Insignificant is the formation of free radicals under the influence of palm oil in the liver of large mice as opposed to small mice (**Figure 2**).

Figure 3 and **Figure 4** present results from research of XOD activity, respectively in the blood plasma and the liver of adult (adults), and juvenile (juveniles) rats.

Palm oil increases activity of XOD in blood, both in small and large experimental animals. In this indicator, there are no significant statistical data for both age groups (**Figure 3**).

Palm oil increases the formation of XOD in the black liver in both elderly groups. The increase in XOD formation in experimental animals was statistically significant compared to control group animals $p<0.001$.

Also, significant statistical differences were found between large and small palm oil treated animals during $p<0.050$. Between the two groups there are no statistically significant differences of XOD. In these comparative all-study, it was found that palm oil had a more significant effect on XOD in black liver mice compared to the effect on XOD in blood plasma. This difference was more pronounced in small mice compared to large mice, where the same trend was noted. Palm oil has 25.0-40.0% more effect on XOD in the black liver compared to the effect on XOD in plasma.

Changes in the total weight of experimental animals will be presented in **Figure 5**.

In all groups during the experiment there was an increase in body weight. This increase was gradual and the difference between the weights in two successive periods was insignificant **Figure 5**. Palm oil had a weak but statistically significant effect on body weight after day eight. The most important is the increase in body weight of experimental mice in both age groups on the 30th day.

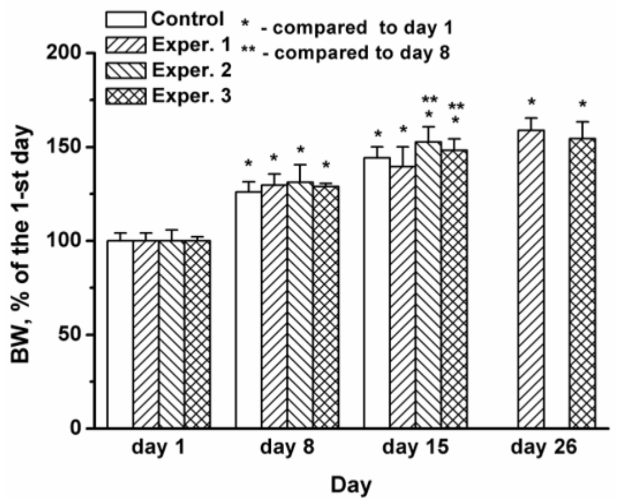

Figure 5. Total weight in research dynamics, which is compared to baseline on first day of experiment (control–controlling group & experiments 1, 2, & 3 were three parallel experimental groups that received palm oil) (Source: Author's own elaboration)

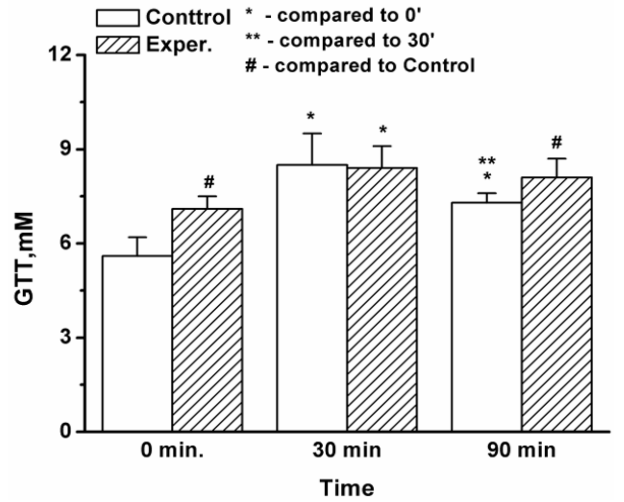

Figure 6. Effect of palm oil loading on tolerant glucose in small mice (30 g) from Wistar line (Source: Author's own elaboration)

Table 1. Blood sugar during GTT realism in adult male mice on **day 15**

Group	0 minute	30 minutes	90 minutes
Controller	5.5±0.2	7.3±0.3	6.2±0.4
Palm oil	5.8±0.2	7.7±0.3	6.5±0.2

Table 2. Blood sugar during GTT realism in adult male mice on **day 30**

Group	0 minute	30 minutes	90 minutes
Controller	5.6±0.12	8.3±0.6	7.0±0.20
Palm oil	6.3±0.20*	9.2±0.2	8.0±0.30*

Comparative studies of the influence of palm oil on carbohydrate metabolism in juvenile and adult rats. The experiments were carried out in four groups of male small and adult animal (details are shown before). The experimental animals from the research group were treated daily with palm oil at a dose of 1 ml/100 g, by oral application for eight weeks. The analysis showed significant statistical differences between the blood sugar of the research group and the control group on the 30th day after application of palm oil, respectively, at zero, 30th minute, and 90th minute of GTT.

Results from experiments conducted in young experimental animals indicate that, after 15 days of palm oil treatment, there are no statistically significant differences between the control and the study group, at zero, 30, and 90 minutes indicating the level of blood sugar (**Table 1**).

During continuous palm oil treatment of experimental juvenile rats, statistically significant increases in blood sugar was found, both in the 30th and 90th minute, between the control and experimental groups (**Table 2**).

Figure 6 shows the effect of palm oil loading on tolerant glucose in small mice (30 g) from the Wistar line.

DISCUSSION

Results from studies of the effect of palm oil on carbohydrate metabolism showed changes in GTT. Analysis of changes under glucose load showed **the development of pre-diabetes in both age groups of experimental animals.** No early changes were reported, as shown by the results of the 15th day of the study. Pre-diabetes development was detected on the 30th day of palm oil treatment.

The results shown also correlate with the increase in body weight of experimental animals in both age groups. The data from the insulin studies support our view (opinion) **that it is possible to develop prediabetes after prolonged palm oil intake**. There has been no data on this issue in the literature so far. Current studies for the first time convincingly demonstrate the risk of using palm oil, which is for different age periods of development. Analysis of the results of two studies on XOD and carbohydrate metabolism showed a correlation between **increased XOD levels and the development of pre-diabetes**. It is known that, after long-term consumption of high-fat foods, enzymes such as NOX XOD are activated [9, 10]. There is evidence that some fats damage liver cells and the resulting XOD falls into the blood plasma. Current research was also demonstrated in the literature for the first time [11]. **The new facts point to the risk of continuous intake of food containing palm oil**, this applies to every age.

The results provide reason to allow increasement **of oxidative stress and the deleterious action of experimental animal free radicals under the influence of prolonged palm oil uptake**. In support of this are also the results of other research [12] , which revealed increased oxidative stress, obesity, and alterations that demonstrate the development of metabolic syndrome. Palmitic acid, a saturated fatty acid, is the principal constituent of refined palm oil. In the last few decades, controversial studies have reported potential unhealthz effects of palm oil due to the high palmitic acid content. In this review we provide a concise and comprehensive update on the functional role of palm oil and palmitic acid in the development of obesity, type 2 diabetes mellitus, cardiovascular diseases, and cancer [13].

CONCLUSIONS

The results mentioned above in the study give reason to assume that the activation of oxidative stress under **the**

influence of continuous intake of refined palm oil in male sex mice adds risk to the occurrence of **pre-diabetes.**

If we compared with people, we find that: the use food product in countries, where palm oil is mainly used can cause pre-diabetes and if you continue to use food products with palm oil, those people will be predisposed to be affected by diabetes.

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Ethical statement: The author stated that scientific experiments within Sofia Medical University, Pharmacology Laboratory, have lasted from 2009-2014. The author further stated that, for all scientific research, permission from Ethical Committee & the Institute responsible for the use of experimental animals, throughout period of scientific research, was granted with number N=P-1383 in the meeting dated 1 September 2009. Experiments presented in this study were conducted during the period September-November 2012.

Declaration of interest: No conflict of interest is declared by the author.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the author.

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