



Effects of high-dose alpha tocopherol supplementation combined with a vitamin E-rich diet on body weight and in liver and kidney tissue in adult mice

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ABSTRACT

Background. Numerous reviews report the beneficial effects of alpha tocopherol in preventive supplementation and also as an adjuvant in the treatment of some pathologies (cardiovascular, cancers). In this work, we analyzed the effects of vitamin E at high doses on some biochemical parameters.

Methods. Thirty-two adult male and female mice (CD1 albino mice) were randomly selected for a 4-week experiment. The mice were supplemented with alpha tocopherol at doses of 150, 400 and 750mg/day. With a high dietary intake of vitamin E.

Results. According to our analyses, we can note Excess weight predominated in groups 4 to 7.

All the blood lipid parameters showed an abnormal concentration, as of the 400 mg dose of α -T-acetate.

Hyperglycemia and hyperlipidemia were observed. These variations were more pronounced for total cholesterol and triglycerides than for HDL and LDL fractions.

Conclusion. This study revealed that the consumption of high doses of vitamin E conferred on the animals a pro-oxidant status which induced a disturbance in lipid metabolism. This dysfunction resulted in obesity, kidney and liver abnormalities.

Key words: Alpha-tocopherol, vitamin E-rich diet, body weight, kidney tissue, liver tissue

1. Introduction

Vitamin E is an essential micronutrient for humans. It is a fat-soluble molecule grouped into two major families (tocopherols and tocotrienols) with four iso forms each of the alpha, beta, gamma, and delta families (Jihyeung J et al. 2009)

Human diets including vegetable oils and cereals are generally fortified with α -tocopherol to meet basic nutritional requirements (Moukobolo F et al. 2021). Although there are other circumstances that call for supplementation.

Vitamin E plays a vital role in physiological processes, such as fertility, oxidative homeostasis, signal transduction and gene regulation, and in diseases, such as obesity and cancers. (Rimbach G et al.,2010;Ulatowski L et al. 2015; Marlene M, et al. 2017;).

α -Tocopherol is best known for its antioxidant property which gives it the potential to reduce oxidative damage in cancer cells to DNA, genome instability, lipids and proteins caused by chemotherapy and radiotherapy. It increases apoptosis, inhibits cell proliferation and decreases the activities of reactive oxygen species (ROS) or nitrogen species (RNS) in the body (Shen, X.-H et al.2010)

And, it is established today a relationship between oxidative stress and the appearance of type 2 diabetes (T2D) which makes vitamin E (α -tocopherol) an ally in the fight against hyperglycaemia and thus to insulin resistance in obesity (Renfan X et al 2014; Monique E et al. 2014).

This is why it is often used in preventive supplementation and also as an adjuvant in the treatment of some pathologies (cardiovascular, cancers); (Brigelius-Flohé R 2002; Landrier J.F2011). However, at high doses, vitamin E can be responsible for deleterious effects, which can promote the appearance or even recurrence of certain cancers (Monnier L.2017).

Hence the interest of this work which aims to analyse in mice the effects of vitamin E on some biochemical parameters.

2. Material and methods

The experiment was carried out at the Institut National de Recherche en Sciences de la Santé du Congo (IRSSA). The protocol was approved by the ethics commission of Médical Congo, under the study number 048/MRSIT/IRSSA/CERSSA.

2.1. Materials

The materials used for the experiment on mice were

Mouse subjects

A total of 32 male and female CD1 albino mice were selected, all 9 weeks old. They were randomly divided into seven groups (n = 4 in each group). They were obtained from the IRSSA where they lived in good conditions.

Drug

We used synthetic vitamin E: alpha tocopherol acetate (α -T-acetate), viscous liquid capsules, 500 mg, Laboratoire Pharma GDD, France.

Dietary intake rich in vitamin E

The diet (Table 1) was based on patties (about 35g per day) consisting mainly of a mixture of leafy vegetables, oil and eggs. All these foods were purchased in the public markets of Brazzaville.

Table 1. **Vitamin E intake of rich foods by mice.**

Catégories	Food	Quantities (g)
Oils	Unrefined palm oil	30
Vegetables	Spinach	50
	Squash	50
Cereals	Soya	450
	Wheat	450
Protein	Eggs	1(of 50)
Fruit	Avocado	50

2.2 Methods

Alpha tocopherol supplementation

The α -T-acetate was administered in each group at different doses. It was dissolved in mineral water (450 mg/ml) and administered by gavage, 4 days a week for 3 months. The dose of α -T-acetate was increased according to the group. A maximum dose of 750 mg was administered in group 6. After 3 months, we evaluated the morphological and tissue changes in the body (kidney and liver).

The experiment was repeated twice under the same conditions to re-evaluate the results.

Anthropometric parameters (weight)

In view of the drug intake, body mass was measured daily.

Histopathological analysis.

After three (3) months the animals were sacrificed and necropsied in order to collect kidney and liver tissue from each mouse. The tissues were fixed in 10% formaldehyde for three days and then stored in paraffin-embedded blocks. For histopathological analysis, the tissues were stained with haematoxylin-eosin (HE) according to the usual protocol. Tissues in paraffin-embedded, HE-stained sections from kidneys of α -T-acetate and control mice were visualised using the Optika microscope, and images were acquired using the Micro Cam Labo II software camera

Statistical analysis

The data were organised and analysed using the following software: Microsoft Excel version 2016 for the creation of the database, Epi-Info version 7.2. The Anova test allowed us to conclude that there was a significant difference between the batches when the p-value was less than or equal to 0.5. The data were expressed as mean \pm standard deviation. The F-test statistics and the degree of freedom (DDR) associated with each comparison were expressed.

3. RESULTS

3.1. Anthropometric parameters

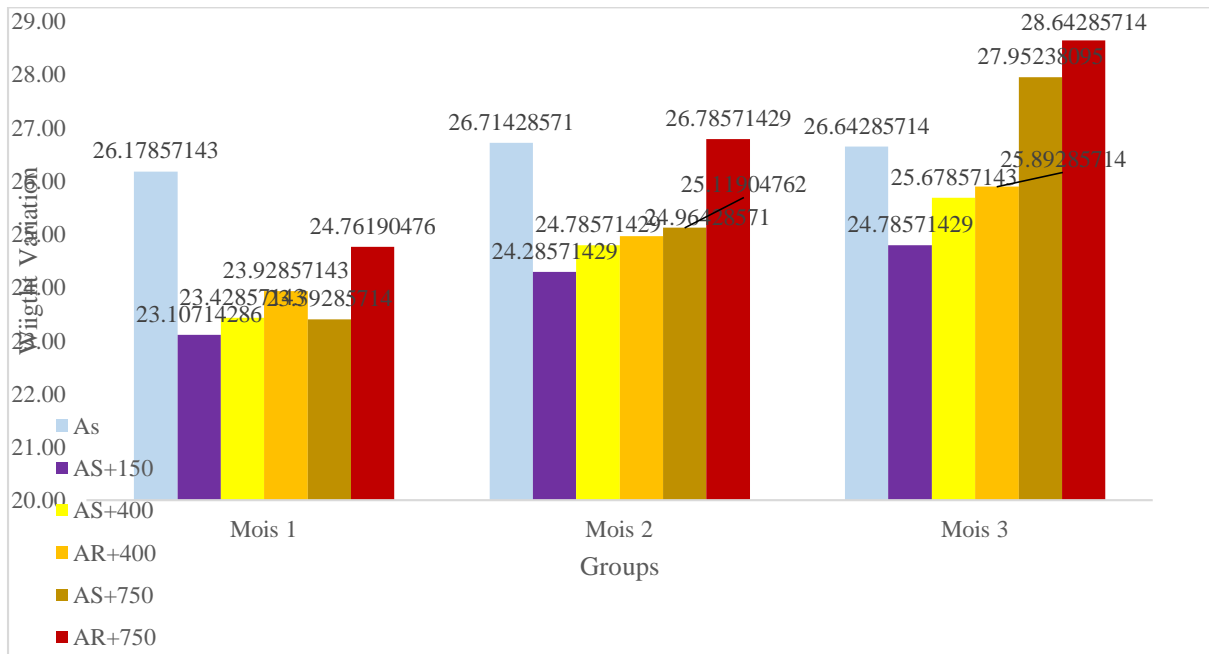


Figure 1: **Weight variation**

Results are expressed as mean average. The F-test statistics and the degree of freedom (DOF) associated with each comparison.

AS= control ;

AS+150= batch consuming standard feed + vit E at a dose of 150mg;

AR +400 = batch consuming rich food + vit E at 400mg;

AS +400 = batch consuming standard food + vit E at 400mg;

AS + 750 = batch consuming standard feed + vit E at 750;

AR + 750 = batch consuming rich feed + vit E at 750mg.

According to figure 1, the evolution of body weight increases as the months go by.

In fact, the difference in weight was very visible from the third month of the experiment with a very significant P value ($P=0.19$). The results obtained at the end of the experiment clearly show an increase in the weight of the mice predominating in the batches (5 to 8) that consumed high doses of alpha tocopherol.

3.2. Histological analyses

The histological sections of the kidneys and liver taken during the biopsies show multiple morphological anomalies (Figure 2 and 3), particularly in the batches (5 to 8) that consumed high doses of α -T.

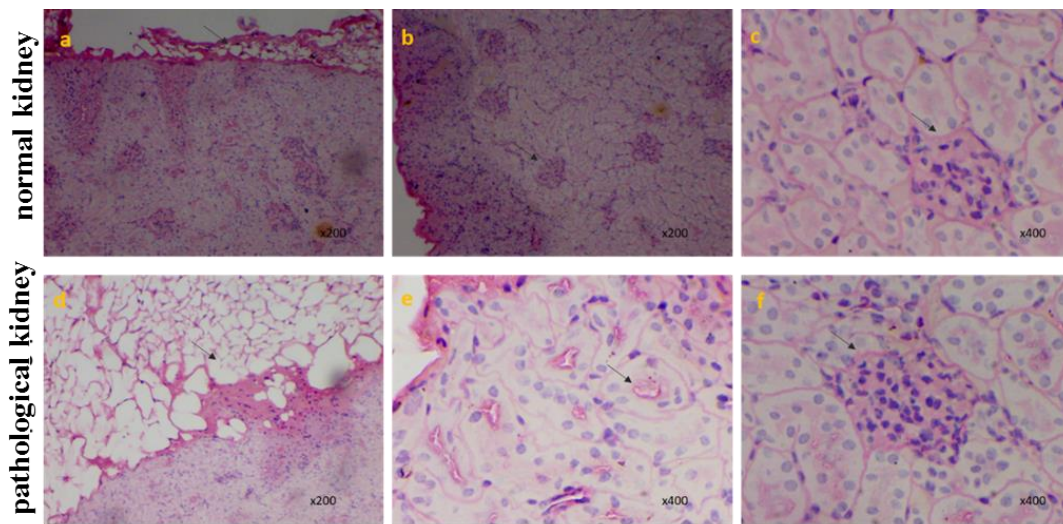


Figure 2. **Histology of the kidney of mice**

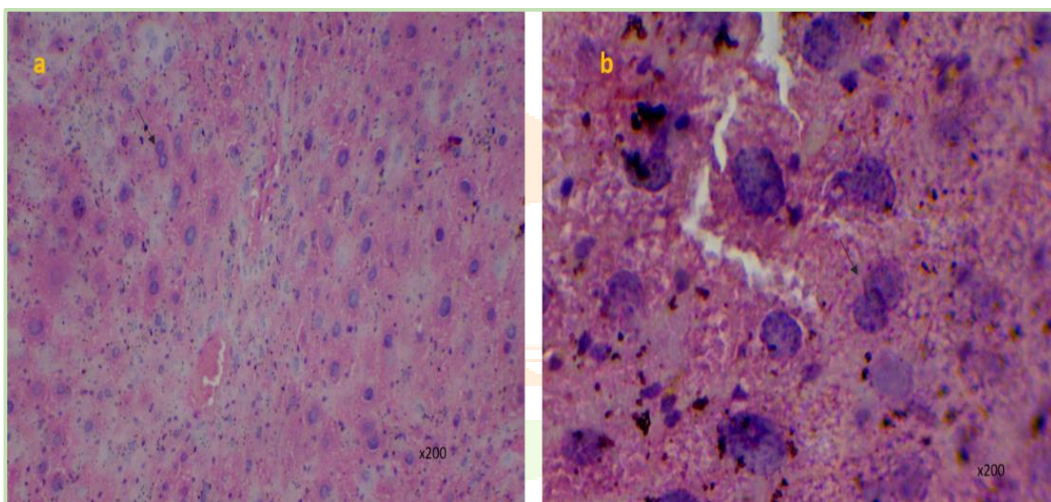


Figure 3. **Histology of the liver**

4. Discussion

4.1 Obesity

The study showed that high-dose α -T supplementation resulted in excess body mass in mice with a significant thickening of adipose tissue, especially in batches of mice 4 to 8, indicating true obesity (Figure 8c). Unfortunately, obesity is known to be a risk factor for diabetes and several molecules in synergy with α -T could be the cause of this obesity (Lu Zhao et al. 2016).

And yet, vitamin E thanks to its antioxidant power could regulate body weight and effectively prevent oxidative stress-related obesity especially in its tocotrienol form (Lu Zhao et al. 2016; Marlene M, et al 2017). This fat regulating role of vitamin E does not only depend on its antioxidant property but also to its ability to regulate certain signalling pathways according to Landrier J.F, 2011, vitamin E was able to regulate body weight by inducing adiponectin expression in adipose tissue in an animal model via a peroxisome proliferator-activated receptor γ (PPAR γ) dependent mechanism. Other molecules involved in the regulation of fat accumulation are: CD36, SR-B1, lipoproteins [Galmés et al., 2018].

CD36 interacts with LDL, its excess can induce obesity. α -T reduces lipoproteins and inhibits CD36 expression. SR-B1 encoded by the SCARB1 gene, interacts with HDL and its deficiency improves obesity (Galmés et al., 2018). ABCA1 acts in the circulation of lipophilic compounds like cholesterol, vitamin E. ABCG1 interacts with HDL and its inactivation may cause accumulation of vitamin E in target tissues such as adipose tissue composed of adipocytes (Galmés et al., 2018).

Under the control of the adipogenesis marker genes CEBP α , and PPAR γ , adipocytes secrete leptin, adiponectin which are adipokines involved in glucose and lipid regulation (Landrier, 2011).

In conclusion, a disorder in the vitamin E transporter molecules can lead to pathological variations in several biochemical parameters such as lipids, lipoproteins, glucose and lead to obesity, which is the main risk factor for type 2 diabetes (Galmés et al., 2018).

4.2. Tissue damage

High doses of vitamin E induce kidney damage by reducing proximal tubules (Farid M. et al.2009)

This study shows the significant imbalance in the oxidant/antioxidant balance when a diet containing a high vitamin E diet and supplemented with α -T is administered to high in adult mice.

Kidney damage

Microscopic analysis of the kidney shows that at high doses, α -T induces adipocyte hyperplasia and hypertrophy (Figure 1 c, d). This damage is related to lipid dysfunction caused by fat accumulation. Other renal lesions are: decrease in proximal tubular and glomerular surface area. All these lesions have been reported in previous studies (Jansen et al., 2016). Vitamin E, which should protect the kidney, has been shown to be harmful at high doses and to impair renal function especially when supplemented in combination with a co-antioxidant such as vitamin E (Saadia R. 2014).

Hepatic lesions

Histological sections of the liver tissue of the control lot 1 show normal liver cell architecture (Figure 2a), whereas histological sections of the lot 8 with high doses of α -T reveal foci of cellular abnormalities and liver cell proliferation (Figure 2b).

These tissue alterations indicate an imbalance in the antioxidant system caused by obesity. At high concentrations, α -T (with a high fat content) acts as a pro-oxidant and not as an antioxidant. At reasonable doses, In addition to adipose tissue, the liver is an important organ that regulates obesity by controlling lipid metabolism in the body [Lu Zhao et al., 2016].

Indeed, when the antioxidant system is balanced, the pro-oxidant action of α -tocopheroxyl is inhibited by co-antioxidants like ascorbic acid. Conversely, high concentrations of α -T overproduce α -tocopheroxyl radicals, which can no longer be effectively detoxified by co-antioxidants. The concentration of ascorbic acid is important because it is this co-antioxidant that will regenerate α -T. When the concentration of ascorbic acid is low, the pro-oxidant effect of α -T occurs, as alpha-tocopheroxyl, will extract an electron from the fatty acids of the phospholipids of the cell membranes and thus propagate the chain reaction of lipoperoxidation at the origin of ROS AND NRS deleterious to the cells and systems of the body (Rouaki et al., 2019).

Limitations of the study

Our study although preliminary had some limitations including:

The absence of blood levels of alpha tocopherol before and after experimentation to relate blood levels to the occurrence of disturbances that appeared during and after experimentation.

5. Conclusion

The animal experiments revealed that the consumption of high doses of vitamin E conferred on the latter a pro-oxidant status which induced a disturbance in lipid metabolism. This dysfunction resulted in obesity, kidney and liver abnormalities. The latter is a condition strongly associated with oxidative stress which leads to excessive production of ROS and RNS.

However, the consumption of low doses of vitamin E leads to a decrease in body mass and could be exploited in the management of cardiovascular diseases.

