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RESEARCH ARTICLE

ANTIOXIDANT EFFECTS OF THE ACTIVE INGREDIENT OF PREVENOX® WITH ITS FATTY ACIDS FREE AND IN THE FORM OF POTASSIUM SALTS

EFECTOS ANTIOXIDANTES DEL INGREDIENTE ACTIVO DEL PREVENOX® CON SUS ÁCIDOS GRASOS LIBRES Y EN FORMA DE SALES DE POTASIO

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ABSTRACT

The active ingredient (AI) of Prevenox® contains a reproducible mixture of saturated fatty acids (FA), extracted and purified from sugar cane wax (*Saccharum officinarum*). This product is registered in Cuba as an antioxidant nutritional supplement in tablet form, which has a high demand in the population due to its pharmacological effects together with its high safety profile. Therefore, in order to achieve greater productive efficiency that allows this demand to be met, technological modifications were implemented to obtain its AI through a simpler and more economical process, through which FA are obtained as potassium salts. The objective of this work was to compare the antioxidant effects of AI with free FA with those of AI with FA in the potassium salts form. For this, oxidative damage was induced by intraperitoneal administration of carbon tetrachloride to rats and similar doses of both AI (25, 100 and 200 mg/kg) were compared. Both AI, at the doses assayed, significantly reduced the values of malondialdehyde and oxidized thiol groups, while restoring the enzymatic activity of catalase both in plasma and in liver homogenate, reaching in all the cases percentages of inhibition greater than 75 %. The comparison between similar doses of both AI did not show significant differences for any of the variables analyzed. In conclusion, the AI in the form of potassium salts exerts similar pharmacological efficacy as an antioxidant to that of AI with its free FA.

Keywords: Prevenox®; free fatty acids; potassium salts of fatty acids; antioxidant.

RESUMEN

El ingrediente activo (IA) del Prevenox® contiene una mezcla reproducible de ácidos grasos (AG) saturados, extraídos y purificados de la cera de la caña de azúcar (*Sacharum officinarum*). Este producto está registrado en Cuba como suplemento nutricional antioxidante en forma de tabletas, el cual tiene una alta demanda en la población debido a sus efectos farmacológicos unidos a su alto perfil de seguridad. Por tanto, con el fin de lograr una mayor eficiencia productiva, que permita satisfacer esta demanda, se implementaron modificaciones tecnológicas para obtener su IA mediante un proceso más simple y económico, mediante el cual se obtienen los AG en forma de sales de potasio. El objetivo de este trabajo fue comparar los efectos antioxidantes del IA con AG libres con los del IA con AG en forma de sales de potasio. Para ello, se indujo un daño oxidativo mediante la administración intraperitoneal de tetracloruro de carbono a ratas y se compararon dosis similares de ambos IA (25, 100 y 200 mg/kg). Ambos IA, a las dosis ensayadas, redujeron significativamente los valores de malondialdehido y de grupos tioles oxidados, a la vez que restablecieron la actividad enzimática de la catalasa tanto en plasma como en homogenato de hígado, alcanzando en todos los casos porcentajes de inhibición superiores al 75 %. La comparación entre dosis similares de ambos IA no arrojo diferencias significativas para ninguna de las variables analizadas. En conclusión, el IA en forma de sales potásicas ejerce similar eficacia farmacológica como antioxidante que el IA con sus AG libres.

Palabras clave: Prevenox®; ácidos grasos libres; sales potásicas de ácidos grasos; antioxidante.

INTRODUCTION

Oxidative stress is defined as an imbalance between pro-oxidant substances or factors and antioxidant defense mechanisms due to excessive production of reactive oxygen species and/or a deficiency in antioxidant mechanisms. This imbalance produces alterations in the structure-function relationship of organs and systems or specialized cell groups, which leads to the deterioration of the vital functions of the organism. Thus, various preclinical and clinical studies demonstrate that oxidative stress is involved in the etiopathogenesis of multiple chronic-degenerative diseases with a high epidemiological incidence, such as osteoporosis, osteoarthritis, Alzheimer's, Parkinson's, diabetes mellitus, cancer, among others (Sánchez & Méndez, *2013;* Chatterjee, *2016;* Sies, *2020;* Mukherjee & Das, *2024;* Plotkin *et al. 2024;* Tonin *et al. 2024;* Abdelazim & Abomughaid, *2024).* In this sense, some authors suggest that the consumption of supplements or additives with antioxidant action in the diet prevents the development and progression of these diseases (Esposito *et al. 2002;* Guan *et al. 2019;* Mishra *et al. 2023*; Su *et al. 2024*).

Prevenox® is the commercial name of tablets that contain as active ingredient (AI) a reproducible mixture of high molecular weight saturated free fatty acids (FFA) extracted and purified from sugar cane wax (*Saccharum officinarum* L.). This mixture is composed of octacosanoic acid (C28:0), as the main component, followed by acids between C24:0 and C36:0 (González *et al. 2002;* Marrero *et al. 2013).*

Several studies have shown that this mixture of FFA inhibit lipid peroxidation ex vivo and in vivo, since it inhibits the generation of total peroxides and decreases malondialdehyde levels. Additionally, the FFA mixture reduces total thiol groups (a marker of protein oxidation) and increases the activity of the antioxidant enzymes catalase, glutathione peroxidase and glutathione reductase (Menéndez *et al. 2002;* Pérez *et al. 2007;* Pérez *et al. 2008* (a y b)). In correspondence with these preclinical results, studies in healthy volunteers have shown that FFA mixture inhibits the susceptibility of plasmatic lipoproteins to lipid peroxidation and increases the body's antioxidant response (Castaño *et al. 2002;* Castaño *et al. 2003*).

These pharmacological results, together with the demonstration of non-toxicity (preclinical toxicological studies) as well as of the safety and well tolerability (clinical trials) associated with the treatment, allowed the registration and marketing, in Cuba, of Prevenox® as an antioxidant nutritional supplement (Gámez *et al. 2002*; Castaño *et al. 2002;* Castaño *et al. 2003;* Gámez *et al. 2007* (a y b); Pérez *et al. 2010;* Ceballos *et al. 2011;* Ceballos *et al. 2015;* Quesada *et al. 2019*); being also the currently support of the high demand from the population that consumes it.

In order to achieve greater productive efficiency in obtaining this FFA mixture as well as satisfy the demand for this product (Prevenox®), technological modifications were implemented in obtaining it through a simple, more economical and efficient process, obtaining the same fatty acids and in the same proportion but in the form of potassium salts (PSFA).

Therefore, the objective of this work was to compare the antioxidant effects of AI in the form of FFA with those of AI in PSFA form.

MATERIALS AND METHODS

Animals and experimental design

Animals and standard food for rodents were supplied from CENPALAB- (National Center for the Production of Laboratory Animals, Havana, Cuba). The handling of the animals was carried out according to the norms established in the "Ethical Guide for the Handling of Laboratory Animals" (Havana, Cuba, 1992) and the ethical principles for the use of laboratory animals recommended in the international guidelines and in the Republic of Cuba. In addition, it was complied with chapter VIII of Decree-Law 31/2021 "On Animal Welfare" (Havana, Cuba, 2021), the regulations established by the Center for State Control of Medicines, Equipment and Medical Devices (CECMED) (Cuba): Regulation No.64/2012 "Guidelines for the Constitution and Operation of the Institutional Committees for the Care and Use of Laboratory Animals (CICUAL)", Regulation No.39/04 "Principles of Good Non-Clinical Laboratory Practices for Health and Environmental Safety"; as well as the specific aspects reflected in the Quality Manual and Standard Work Procedures of the Center for Natural Products belonging to the National Center for Scientific Research (CNIC), Havana, Cuba. The study protocol was reviewed and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the Center for Natural Products.

Male Wistar rats (150-250 g of body weight) were adapted for 7 days to laboratory conditions (25 ± 2 °C, 65 ± 10 % humidity, and 12 h light/dark cycles), with free access to water and food. After completing the quarantine, the rats were randomly distributed into nine experimental groups (10 rats per group) (Table 1). Treatments were orally administered, via intragastric intubation (5 mL/kg), as repeated doses during four weeks.

One hour after the last administration of the treatments, liver damage was induced by a single intraperitoneal injection of CCl_4. Eighteen hours later, the animals were anesthetized in a halothane atmosphere to obtain the corresponding blood and liver samples.

Table 1. Treatment groups

Group	Treatment Doses	CCl_4-induced injury
Negative control	Vehicle (acacia gum/water 1 %)	Vehicle (soybean oil)
Positive control		CCl_4 (suspended in 20 % soybean oil)
AI-FFA	25, 100 and 200 mg/kg	
AI-PSFA		
GSE	250 mg/kg	

AI-FFA: active ingredient-free fatty acids (Plant Production; CNIC, Havana, Cuba); AI-PSFA: active ingredient-potassium salt fatty acids (Plant Production; CNIC, Havana, Cuba); CCl_4: carbon tetrachloride (Merck Darmstadt, Germany); GSE: Grape seed extract (Healing & Medicinal Herbs, Australia).

Biochemical analysis

Blood samples were extracted from the abdominal aorta to obtain serum (for transaminases determination) and plasma (for oxidative variables analyze) by centrifugation at 3000 rpm for 10 min. At the same time, liver tissue samples (0.5 g) were collected and homogenized in 150 mmol/L Tris buffer (pH 7.4).

✓ Transaminases

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations were determined by a colorimetric method (Helfa diagnostic kits, Cuba) at 340 nm wavelength and reported as U/L.

✓ Thiobarbituric acid reactive species

Malondialdehyde (MDA) concentrations in plasma or liver homogenate were determined by spectrophotometric analysis at 534 nm (Ohkawa *et al. 1979*). The colorimetric reaction was produced after the addition of 500 µL of sample, 0.2 mL of sodium dodecyl sulfate (8.1 %), 1.5 mL of acetic acid (20 %, pH 3.5) and 1,5 mL of thiobarbituric acid (0.8 %). After heating the reaction mixture, 5 mL of n-butanol:pyridine (15:1 v/v) was added and immediately the sample were vigorously vortexed and centrifuged at 4 000 rpm for 20 min. MDA values were reported as nmol MDA/mg protein respect to a standard curve of MDA bis (dimethyl acetal).
Protein concentration was determined by the modified Lowry method (Markwell *et al. 1978*).

✓ Oxidized thiol groups

The concentrations of oxidized thiol groups (OTG) were quantified in plasma and liver homogenate using a colorimetric method in a spectrophotometer (412 nm). The sample (200 µL) was mixed with 600 µL of Tris-EDTA buffer 20 mmol/L pH 8.2, 40 µL of 5,5-dithio-bis- (2-nitrobenzoic) 10 mmol/ L and 3.16 mL of absolute ethanol; it was incubated for 20 min at room temperature and centrifuged at 3 000 g for 10 min. The results were calculated taking into account the molar extinction coefficient (13.600 $cm^{-1}M^{-1}$) and were expressed in mmol/L (Hu, *1994*).

✓ Catalase

Catalase (CAT) activity in plasma and liver homogenate was determined by the modified method of Aebi *(1974).* Sample of 10 µL was taken and 2.89 mL of 50 mmol/L potassium phosphate buffer (pH 7.4) was added. The disappearance of H_2O_2 was monitored for 5 min at 240 nm in a spectrophotometer. Catalase activity was calculated by the molar extinction coefficient (43.6 x 10^{-3}) and expressed in IU/min mg of protein x 10^{-1}.

Statistical Analysis

Statistical comparisons between groups were done with the non-parametric Mann-Whitney U-test. A priori, a significance level of $\alpha = 0.05$ was established. Statistical analyzes were performed using the commercial Statistic software for Windows (Release 6.0; StatSoft, Tulsa, OK, USA).
The dose/effect relationship analysis was carried out with the linear regression and correlation method using the Origin 8.0 program (Origin Lab Corporation; USA, Version 8).

RESULTS

Figure 1 shows the effects of AI-FFA and AI-PSFA (25-200 mg/kg) on serum transaminases levels (ALT and AST). The animals of the positive control group (with CCl_4) reached significantly higher values ($p<0.0001$) than those reached by the animals of the negative control group (without damage); while GSE, substance used as reference control, significantly reduced (compared to positive control group) the activity of both enzymes reaching 65.6 and 49.9 % inhibition on ALT and AST, respectively. AI-FFA and AI-PSFA at the dose of 25 mg/kg did not produce significant effects on these variables, although they achieved inhibition percentages of approximately 25 %. Both treatments at doses of 100 and 200 mg/kg significantly, and not dose dependent, reduced the enzymatic activity of ALT and AST, reaching values of inhibition of 60 % and 45 %, respectively.

The results on the oxidative variables in plasma and liver tissue are shown in Tables 2 and 3, respectively. The administration of CCl_4 to the animals of the positive control group produced a significant increase in MDA and OTG concentrations compared to the negative control group (healthy rats) in plasma and liver tissue, as well as it diminished the enzymatic activity of CAT. AI-FFA and AI-PSFA at doses assayed (25, 100 and 200 mg/kg) decreased not dose-dependently the levels of MDA and OTG, being significant and marked at doses of 100 and 200 mg/kg. The major percentages of inhibition achieved was greater than 75 %. Similar effect was observed on the restoration of the enzymatic activity of CAT with all treatments, being only dose-dependently with AI-PSFA (*r=0.999, p=0.015*) which achieved 100 % restoration at major dose assayed. The comparison between similar doses of AI-FFA and AI-PSFA did not show significant differences for all variables analyzed. The GSE (reference substance) markedly and significantly reduced oxidative variables in plasma and in liver tissue, while it restored CAT activity, compared to positive control group (Table 2 and 3).

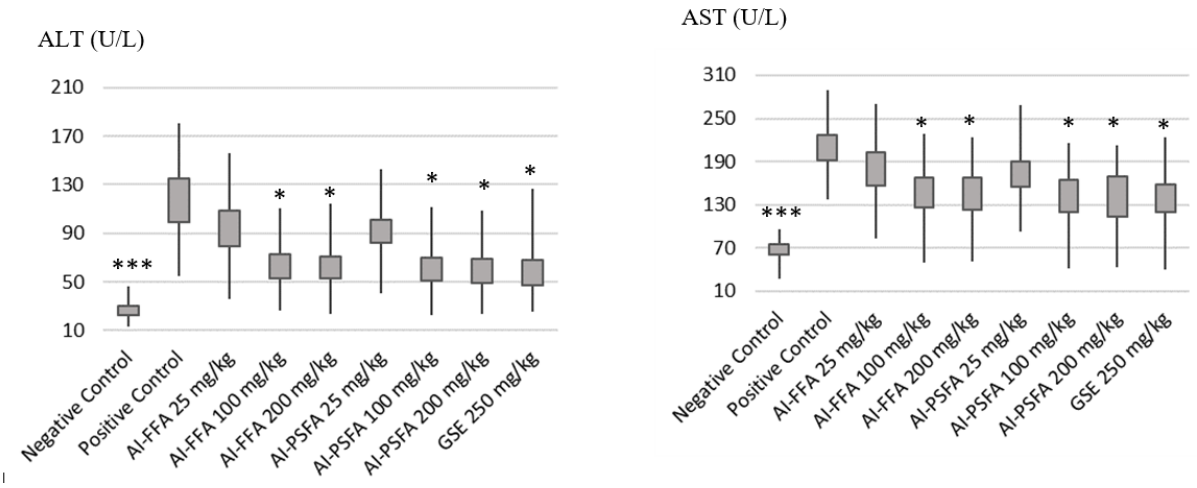

Fig. 1. *Effect of AI-FFA and AI-PSFA on transaminases activity in rats with carbon tetrachloride induced-hepatic injury. AI-FFA: active ingredient-free fatty acids; AI-PSFA: active ingredient-potassium salt fatty acids; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GSE: Grape seed extract. Data as Mean ± SEM (standard error of the mean); *p<0.05, ***p<0.0001 comparison vs positive control group; Mann Whitney U test.*

Table 2. Effect of AI-FFA and AI-PSFA on plasma oxidative variables in rats with carbon tetrachloride induced-hepatic injury

Treatment **Dose** (mg/kg)	**MDA** (mmol/mL)	**I** (%)	**OTG** (mmol/mL)	**I** (%)	**CAT** (UI/min/mg de pt)	**I** (%)
Negative Control	0.42 ± 0.03 **	-	0.45 ± 0.09 ***	-	0.31 ± 0.05 **	-
Positive Control	0.79 ± 0.09	-	1.58 ± 0.19	-	0.14 ± 0.02	-
AI-FFA (25)	0.71 ± 0.06	21.6	1.32 ± 0.20	23.0	0.18 ± 0.03	23.5
AI-FFA (100)	0.51 ± 0.02 **	75.6	0.66 ± 0.07 **	81.4	0.24 ± 0.04 *	58.8
AI-FFA (200)	0.49 ± 0.02 **	81.0	0.54 ± 0.08 **	92.0	0.29 ± 0.04 **	88.2
AI-PSFA (25)	0.69 ± 0.08	27.0	1.29 ± 0.20	25.6	0.18 ± 0.03	23.5
AI-PSFA (100)	0.52 ± 0.05 *	72.9	0.65 ± 0.09 **	82.3	0.27 ± 0.04 *	76.5
AI-PSFA (200)	0.51 ± 0.07 **	75.6	0.52 ± 0.09 **	93.8	0.31 ± 0.05 **	100
GSE (250)	0.52 ± 0.05 **	72.9	0.58 ± 0.09 **	88.5	0.31 ± 0.04 **	100

*AI-FFA: active ingredient-free fatty acids, AI-PSFA: active ingredient-potassium salt fatty acids, CCl4: carbon tetrachloride, GSE: grape seed extract, I: inhibition, MDA: malondialdehyde, OTG: oxidized thiol groups, pt: total proteins. Negative control (without CCl_4), the rest of treatments with CCl_4. Data as Mean ± SEM (standard error of the mean) *p<0.05; **p<0.01; ***p<0.001. Comparison vs positive control group, Mann Whitney U test.*

Table 3. Effect of AI-FFA and AI-PSFA on liver oxidative variables in rats with carbon tetrachloride induced-hepatic injury

Treatment **Dose** (mg/kg)	**MDA** (nmol/mg of pt)	**I** (%)	**OTG** (mmol/mL)	**I** (%)	**CAT** (UI/min/mg de pt)	**I** (%)
Negative Control	2.92 ± 0.34 **	-	1.29 ± 0.08 **	-	1.08 ± 0.10 ***	-
Positive Control	4.57 ± 0.27	-	1.96 ± 0.18	-	0.48 ± 0.06	-
AI-FFA (25)	4.28 ± 0.30	17.6	1.83 ± 0.11	19.4	0.57 ± 0.11	15.0
AI-FFA (100)	3.53 ± 0.27 *	63.0	1.49 ± 0.17 *	70.1	0.76 ± 0.08 *	46.6
AI-FFA (200)	3.26 ± 0.34 *	79.4	1.45 ± 0.12 *	76.1	0.81 ± 0.08 *	55.0
AI-PSFA (25)	4.26 ± 0.33	18.8	1.87 ± 0.10	16.0	0.56 ± 0.08	13.3
AI-PSFA (100)	3.48 ± 0.34 *	66.0	1.41 ± 0.11 *	82.1	0.75 ± 0.06 *	45.0
AI-PSFA (200)	3.37 ± 0.31 *	72.7	1.41 ± 0.15 *	82.1	0.78 ± 0.08 *	50.0
GSE (250)	3.39 ± 0.33 *	71.5	1.38 ± 0.12 *	86.6	0.82 ± 0.06 *	56.6

*AI-FFA: active ingredient-free fatty acids, AI-PSFA: active ingredient-potassium salt fatty acids, CCl4: carbon tetrachloride, GSE: grape seed extract, I: inhibition, MDA: malondialdehyde, OTG: oxidized thiol groups, pt: total proteins. Negative control (without CCl_4), the rest of treatments with CCl_4. Data as Mean ± SEM (standard error of the mean) *p<0.05; **p<0.01; ***p<0.001. Comparison vs positive control group, Mann Whitney U test.*

DISCUSSION

The antioxidant effects of AI of Prevenox® in form of free fatty acids (AI-FFA) and AI in form of potassium salts (AI-PSFA), administered as repeated oral doses, on the increase of lipid peroxidation and protein oxidation and the decrease of CAT enzymatic activity induced by CCl_4 in rats, were demonstrated in this study.

The CCl_4-intraperitoneal administration significantly increased the activity of serum transaminases (ALT and AST) in the animals of the positive control group compared to the animals of the negative control group (healthy rats, vehicles), which demonstrates that there was damage to level of liver tissue that affected its functionality. Furthermore, in correspondence with previous reports that base the relationship between oxidative stress and hepatotoxicity induced by CCl_4 (Favari *et al. 2013;* Adewale *et al. 2014;* Laouar *et al. 2017;* Goodla *et al. 2019*), the positive control group-animals reached high levels of MDA (marker of lipid peroxidation) and OTG (marker of protein oxidation) and a depletion of the enzymatic activity of CAT (marker of the endogenous antioxidant system) in plasma and liver homogenates.

The oxidative stress associated with this model occurs from the generation of the trichloromethyl radical ($CCl_3^{\bullet}$), which reacts rapidly with oxygen forming the trichloromethyl peroxide radical ($Cl_3COO^{\bullet}$) capable of triggering lipid peroxidation of the polyunsaturated fatty acids of the membranes; cause the oxidation of proteins with sulfhydryl groups in their structure, as well as the loss of the activity of antioxidant enzymes (León, *2005*; Favari *et al. 2013*). For this reason, the use of CCl_4 as an oxidative stress inducer constitutes a validated and widely used experimental model to evaluate substances with possible antioxidant effects (Woon *et al. 2015*; Alayunt *et al. 2019*; Ullah *et al. 2020*). Thus, the GSE-reference substance, the gold standard as an antioxidant, significantly prevented the oxidative stress induced by CCl_4, achieving an antioxidant efficacy greater than 50 % inhibition on the analyzed variables (MDA, OGT y CAT), thus corroborating its antioxidant and hepatoprotective effects demonstrated in previous studies (Belviranli *et al. 2015*; Hassan *et al. 2016*; Khazri *et al. 2016*; Bilgic *et al. 2018*). These results confer validity to the experimental model and to the findings obtained in this study of the antioxidant effects of AI-FFA and AI-PSFA.

AI-FFA and AI-PSFA, administered as single oral doses (25-200 mg/kg), significantly reduced the increase in serum ALT and AST enzymatic activity, demonstrating their efficacy in preventing CCl_4-induced damage at the level of liver tissue, improving its functionality. Also, AI-FFA and AI-PSFA prevented oxidative stress by decreasing lipid peroxidation (MDA) and protein oxidation (OGT), as well as by stimulating the endogenous antioxidant defense system (CAT).

The pharmacological efficacy exerted by AI-FFA on oxidative stress variables in rats with liver damage induced by CCl_4 was in correspondence with a previous report in the model of liver damage induced by paracetamol (Pérez *et al. 2007*). It should be noted that Prevenox® has been registered as an antioxidant nutritional supplement since 2004. Thus, its antioxidant effects have been demonstrated in experimental and clinical studies (Castaño *et al. 2002*; Castaño *et al. 2003*; Pérez *et al. 2008*). Taking into account this result and that the statistical comparison between similar doses of AI-FFA and AI-PSFA did not show significant differences between both treatments, we can affirm that the technological modifications carried out on an industrial scale to obtain AI-PSFA, which contains the fatty acids present in AI-FFA but in the form of potassium salts, do not affect the pharmacological efficacy of this new substance.

On the other hand, it has been shown that salification (formation of an organic salt) can have an advantageous impact on the pharmaceutical industry, mainly because the chemical structure of the active substance is not altered; the therapeutic effect is the same as the base free, the results using this technique are relatively fast, greater solubility in aqueous solutions compared to their respective neutral species, which influences the absorption of the substance and therefore the therapeutic effect (Ortiz & Balderrabano*, 2017*).

CONCLUSIONS

The AI in form of potassium salts (AI-PSFA) exerted similar pharmacology efficacy as an antioxidant to that of AI with its free FA (AI-FFA).

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