Evaluation of the antioxidant effects of *Plukenetia volubilis* L. oil, obtained in Cuba, in hepatic injury induced in rats

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ABSTRACT

Oxidative stress and inflammation are physiopathological processes interlinked, that play a fundamental role in different pathological entities. The oil obtained from the fruit of *Plukenetia volubilis* L., commonly known as Sacha Inchi, is used popularly for its antioxidant and anti-inflammatory properties. In this sense, the cultivation of this plant in Cuba has been of interest. The objective of this work is to evaluate the effects of Cuban Sacha Inchi oil (CSIO) on oxidative stress induced by carbon tetrachloride (CCl_4) in rats. For it, CSIO was administered as oral single-dose (25, 50, 100 and 200 mg/kg) one hour before the induction damage. The oral administration of CSIO produced a marked, significant and dose-dependent effect on the increase of oxidative stress indicators (malondialdehyde and oxidized thiol groups in plasma and liver tissue, associated with a stimulation of the activity of catalase (enzyme of the antioxidant endogenous system) in liver tissue. In addition, CSIO prevented the effect on liver damage at the histopathological level, by preventing steatosis, the presence of ballooned hepatocytes, and reducing the lobular infiltration of polymorphonuclear neutrophils. Therefore, CSIO exerts antioxidant effects that contribute to the prevention of acute damage induced by CCl_4 in rats. These effects are also associated with anti-inflammatory properties in the liver. In conclusion, CSIO constitutes a substance with promising effects on acute liver damage in correspondence with its antioxidant and anti-inflammatory properties.

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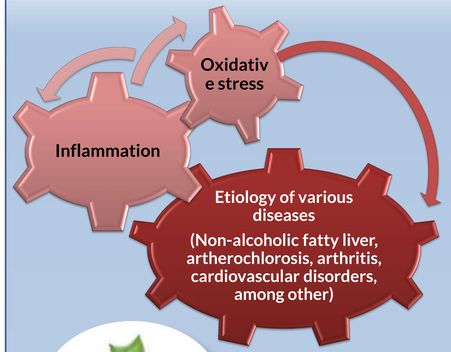
INTRODUCTION



Sacha Inchi (*Plukenetia volubilis* L.)

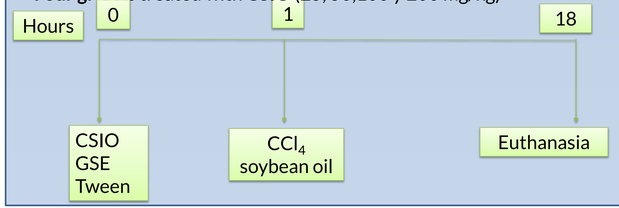
Majority components
α- linolenic-omega (ω)-3, linoleic-ω-6 and oleic-ω-9
Minority components
palmitic, stearic and arachidonic acid

The objective of this work is to evaluate the effects of Cuban Sacha Inchi oil (CSIO) on oxidative stress induced by carbon tetrachloride (CCl_4) in rats.

MATERIALS AND METHODS

The rats were randomly distributed into seven groups (10 rats/group)

- ✓ **Negative control** treated orally with Tween and soybean oil intraperitoneal (ip)
- ✓ Six groups with damage induced by ip administration of CCl_4:
- **Positive control** treated orally with Tween
- **Reference substance** (Grape seed extract-GSE) (250 mg/kg)
- **Four groups** treated with **CSIO** (25, 50,100 y 200 mg/kg)



RESULTS

Fig. 1. Effects of CSIO on oxidized thiol groups levels in liver

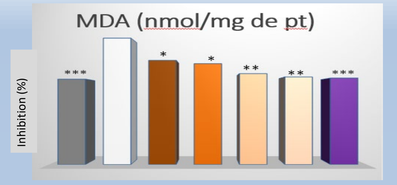


Fig. 2. Effects of CSIO on malondialdehyde levels in liver

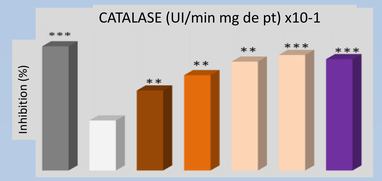
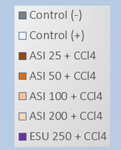
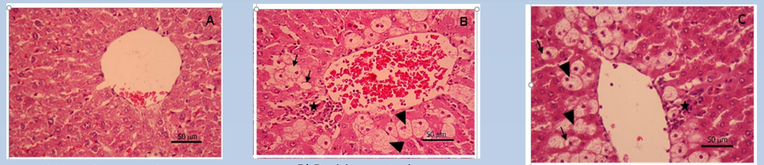


Fig. 3. Effects of CSIO on catalase enzyme activity

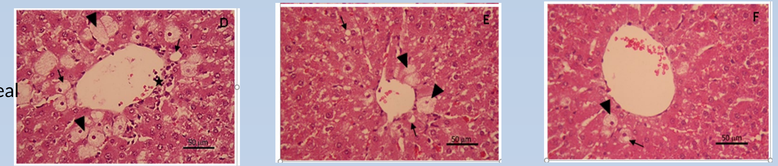
**p<0.05; **p<0.01; ***p<0.001 comparison vs positive control (+) group Mann Whitney U Test*



Similar effects on oxidative variables were seen in plasma



A) Negative control B) Positive control C) *Sacha inchi* extract 25 mg/kg



D) *Sacha inchi* extract 50 mg/kg E) *Sacha inchi* extract 100 mg/kg F) Grape seed extract 250 mg/kg

Fig. 4. Effects of CSIO on histopathological change. Arrows: steatosis, arrowheads: ballooning degeneration, stars: inflammatory infiltrates

The i.p. of CCl4 induced histopathological changes at the level of the hepatic parenchyma (macrovesicular and microvesicular steatosis, ballooned hepatocytes and lobular infiltration of polymorphonuclear neutrophils) unlike the negative control group (without damage), in which the normal histological structure of the liver was observed.

The CSIO (50 – 200 mg/kg) significantly and dose-dependently prevented histopathological changes induced by CCl_4 in liver tissue .

Fig. 5. Effects of CSIO on global liver damage

CSIO decreased serum concentrations of transaminases (ALT and AST) consistent with its protective effects on histopathologic changes.

CONCLUSIONS

The oral administration of CSIO produced a marked, significant and dose-dependent effect on the increase of oxidative stress indicators, associated with a stimulation of the activity of CAT in liver tissue.

The CSIO constitutes a substance with promising effects on acute liver damage in correspondence with its antioxidant and anti-inflammatory properties.

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VALIDATION OF AN ANALYTICAL METHOD FOR DETERMINING 1-OCTACOSANOL AND 1-TRIACONTANOL IN CREAM

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ABSTRACT

Taking into account the anti-inflammatory and antioxidant properties of 1-octacosanol and 1-triacontanol, a cream was recently developed, with these fatty alcohols as active ingredient, for the treatment of damaged skin. Current regulations in Pharmaceutical Industry require that validated analytical methods be available for determining the active ingredients content in all the finished form. Therefore, a Gas Chromatographic analytical method was developed and validated for determining 1-octacosanol and 1-triacontanol in this new cream. The alcohols were extracted with chloroform and analyzed as trimethylsilyl derivatives with a wide-bore capillary column, using 1-eicosanol as internal standard. The specificity study showed that the determination of these alcohols is not interfered by the internal standard or by the other components of the cream, and that no new chromatographic peaks appear in the analysis of samples subjected to stress conditions. The method was found to be linear (correlation coefficient = 1.0) and unbiased (confidence interval of the intercept included the zero value), as well as exact (mean recovery without significant differences with 100%) from 50 to 150% of the nominal concentration. The repeatability and intermediate precision, at the nominal concentration, met the acceptance criteria (CV < 2%), without significant influence of the analyst and the day of analysis on the dispersion of the results. The method, therefore, can be used for quality control and stability studies of this cream.

CONTACT

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INTRODUCTION

The fatty alcohols 1-OCTACOSANOL and 1-TRIACONTANOL, present in sugar cane wax and in beeswax, have demonstrated antiinflamatory and antioxidant properties.

A cream containing these alcohols was developed for taking care of damaged skins.

The objective of this work was to validate an analytical method to determine 1-octacosanol and 1-triacontanol in the cream, which will be used for quality control and stability studies of the cream.

MATERIALS AND METHODS

Test sample preparation

1. Weigh 1 g of cream in a 20 mL vial.
2. Add 5 mL chloroform and 5 mL of PI solution 1-eicosanol (0.4 mg/mL).
3. Heat at 65 °C for 20 min with occasional stirring.
4. Transfer to a separatory funnel and collect the lower phase in a vial.
5. Discard the upper phase and evaporate at 65°C with an air flow.
6. Add 2 mL chloroform and heat at 65°C for 3 min.
7. Take 100 µL and transfer to a test tuve.
8. Add 100 µL N-methyl, N-trimethylsilyltrifluoroacetamide (MSTFA).
9. Heat at 65°C for 30 min.
10. Analyze by Gas Chromatography 1 µL of sample.

Chromatographic procedure: GC-14A chromatograph (Shimadzu) with FID and HP5 column (28 m X 0.53 mm id X 1.0 µm film thickness,). Program: 180°C (1 min. isothermal) to 320°C at 8 °C/min, with 5 min at the final temp. Injector and detector at 320 °C. Carrier gas (H2) at 8 mL/min.

Validation of analytical methodology.

a. Checking the applicability of the system
b. Linearity of the method
c. Accuracy
d. Precision: Repeatability and Reproducibility
e. Specificity

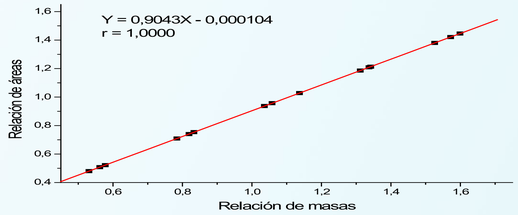
Aplicability of the system

	Limit	Average result
Repeatability	0,5 %	0,44 %
Resolution	2,5	7,68

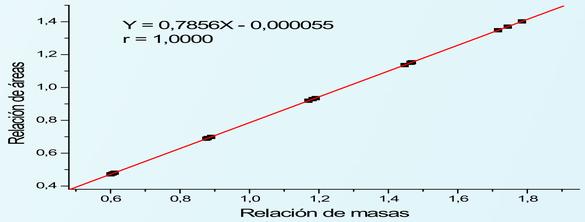
RESULTS

Linearity study results

Alcohol	CVf (%)	CVb (%)
C_{28}	0.12	0.0007
C_{30}	0.01	0.0001
Total	0.01	0.0001
Limit	5.0	2.0



1-Octacosanol linearity



1-Triacontanol linearity

Accuracy study results

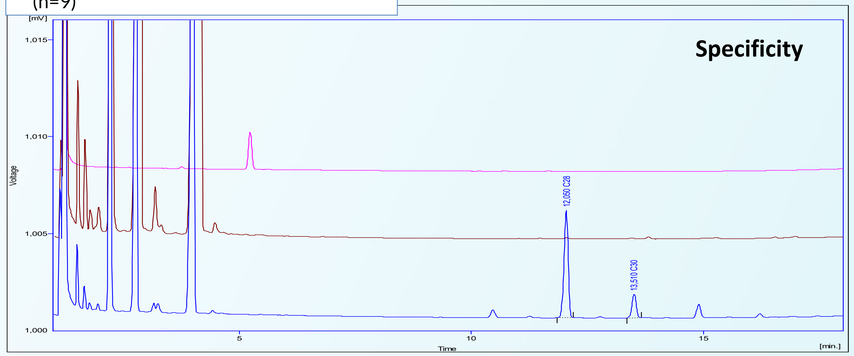
Conc. (%)	Mean recovery (%)	Standard deviation (±)	CV (%)
80	101.9	1.83	1.76
100	100.9	2.00	1.98
120	98.7	1.86	1.88
Total (n=9)	**101.2**	**2.78**	**2.75**

t exp = 1.28 < t tab = 2.306

No significant difference between recovery and 100%.

G exp = 0.62 < G tab = 0.87

Concentration does not influence the variability of results.



Chromatographic profiles of samples: Blue: cream, Brown: placebo and Pink: internal standard.

Chromatographic profiles of cream samples subjected to stress conditions of photolysis, oxidation, basic hydrolysis, acid hydrolysis and thermolysis showed no new extra peak. Then, posible degradation products do not interfere with analytes.

Repeatability and Intermediate precision results

Alcohol	Alcohol content (mg/g cream)	Repeatability CV (%)	Intermediate Precision CV (%)
C_{28}	6.76	1.29	5.15
C_{30}	1.74	2.14	4.29
Total	8.49	1.41	**4.92**

Limit: CV<5.7%

CONCLUSIONS

The analytical method for determining 1-octacosanol and 1-triacontanol in the cream complies with all the validation established requirements. It was accurate, linear and precise between 50 and 150% of the nominal dose, and specific even for samples subjected to degradation conditions. Therefore, it can be used for quality control and stability studies.

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