A novel compounded cyproheptadine hydrochloride oral solution: Accelerated stability study and effects on glucose and lipid metabolism in Wistar rats

Uma nova solução oral de cloridrato de ciproeptadina: Estudo de estabilidade acelerada e efeitos sobre o metabolismo da glicose e lipídios em ratos Wistar

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The present study developed a compounded oral solution (COS) containing an active component cyproheptadine hydrochloride (CY) in association with pharmaceutical adjuvants. The main objective is to demonstrate the stability of this solution and the *in vivo* effects on serum and liver parameters of glucose and lipid metabolism. After 180 days of analysis the COS showed physicochemical stability (pH and specific gravity) when submitted to different temperatures (24 °C and 40 °C), but the chemical stability, presented by the content of CY (%) in the formulation, showed that the degradation rate of CY increased 20x when submitted to a stress condition (40 °C). Male Wistar rats were treated with IOS 1.5 (industrial oral solution 1.5 mg.kg^{-1}) or saline for 21 days and IOS 5.0 or COS (5 mg.kg^{-1}) or placebo by gavage for 23 days. Treatment with IOS 1.5 increased liver cholesterol by 30 % without changing liver and body weight in this group. In contrast, treatment with IOS and COS (5 mg.kg^{-1}) increased final body weight by 8 % compared to placebo. Interestingly, the COS group had increased liver glycogen accompanied by reduced food intake compared to IOS. However, COS and IOS 5.0 did not induce changes in adipose tissue weight, liver weight, as well as serum glucose, triacylglycerol and cholesterol levels. This work provides us with data that prove the stability of the compound solution at room temperature, as well as inducing an increase in the final body weight of rats with no alteration in glucose and lipid metabolism.
Keywords: cyproheptadine hydrochloride, stability, metabolism.

O presente estudo desenvolveu uma solução oral magistral (COS) contendo cloridrato de ciproeptadina (CY) em associação com adjuvantes farmacêuticos. O objetivo principal é demonstrar a estabilidade dessa solução e os efeitos *in vivo* nos parâmetros séricos e hepáticos do metabolismo da glicose e lipídios. Após 180 dias, a COS apresentou estabilidade físico-química (pH e densidade relativa) quando submetida a diferentes temperaturas (24 e 40 °C), mas a estabilidade química, apresentada pelo teor de CY (%) na formulação, mostrou que a taxa de degradação de CY aumentou 20x quando submetida a condição de estresse (40 °C). Ratos Wistar machos foram tratados com IOS 1.5 (solução oral industrial 1,5 mg.kg^{-1}) ou salina por 21 dias e IOS 5.0 ou COS (5 mg.kg^{-1}) ou placebo por gavagem por 23 dias. O tratamento com IOS 1.5 aumentou o colesterol hepático em 30 % sem alterar o fígado e o peso corporal neste grupo. Em contraste, o tratamento com IOS e COS (5 mg.kg^{-1}) aumentou o peso corporal final em 8 % em comparação ao placebo. Curiosamente, o grupo COS apresentou aumento do glicogênio hepático acompanhado de redução da ingestão alimentar em comparação à IOS. Entretanto, COS e IOS 5.0 não induziram alterações no peso do tecido adiposo, peso do fígado, bem como nos níveis séricos de glicose, triacilglicerol e colesterol. Este trabalho nos fornece dados que comprovam a estabilidade da solução magistral em temperatura ambiente, além de induzir o aumento do peso corporal final de ratos sem alteração no metabolismo de glicose e lipídios.
Palavras-chave: cloridrato de ciproeptadina, estabilidade, metabolismo.

1. INTRODUCTION

Over the last few years, studies have shown that cyproheptadine hydrochloride (CY) acts safely and effectively in the pediatric treatment of functional gastrointestinal disorders (FGIDs), which comprise functional dyspepsia, functional abdominal pain, abdominal migraine, irritable bowel syndrome, and cyclic vomiting syndrome. These disorders can negatively affect children's quality of life and healthcare costs. CY treatment emerges as a non-invasive and inexpensive option for primary care and gastroenterology practices for children diagnosed with FGIDs [1-5].

CY is known to be both a histamine and a gut serotonin receptor antagonist. Therefore, its antiserotoninergic effect can explain the increase in appetite and weight gain. This also justifies its interest as an appetite stimulant, especially in cases of cystic fibrosis [1, 6, 7]. This drug is also indicated for treatment of allergic conditions such as urticaria, angioedema, rhinitis, conjunctivitis, and pruritic skin disorders [8-10]. Another alternative is combination therapy with tricholine citrate, which is prescribed for hepatobiliary conditions linked with anorexia, leading to weight reduction [11].

After oral administration, CY is absorbed and widely distributed in tissues. It is metabolized by aromatic ring hydroxylation, N-demethylation, heterocyclic ring oxidation, and conjugation with glucuronic acid; the main metabolite found in urine is the quaternary ammonium glucuronide conjugate. About 70 % of the dose is excreted in the urine within 6 days, the remainder is eliminated in the feces. Of the excreted material, ~61 % is conjugated with glucuronic acid, ~10 % is conjugated with sulfate, ~23 % is excreted as polar material not hydrolyzable by sulfatase or glucuronidase, and about 5 % is simply unconjugated [12]. The LD50 orally in mice is 74.2 mg.kg^{-1} [13].

Although there are no reports of relevant adverse events in the literature, the antihistaminergic properties of CY may lead to disturbance of coordination and somnolence. Its anticholinergic properties could cause tachycardia, hyperpyrexia, urinary retention, mydriasis, agitation, and hallucination, among others [14-16]. Accordingly, we highlight a case report of symptomatic pediatric CY toxicity with a confirmatory therapeutic drug level [15]. Recently, a review work gathered evidence that CY can be considered safe [17].

A current systematic review listed studies that investigated biochemical and laboratory alterations related to patients using CY and did not show significant changes in pre-versus post-test results or treatment groups versus placebo. In summary, levels of fasting glucose, plasma insulin, plasma growth hormone, plasma free fatty acids, plasma free amino acids, glucose tolerance, and urea were checked. One study found higher levels of IGF-1 in subjects taking CY. A significant increase in urinary sodium and creatinine excretion was explained by an increase in overall food intake with CY use [18].

Considering that the oral liquid forms available for sale contain CY in association with other active pharmaceutical ingredients, it was necessary to compose a formulation for this study, in which CY is the only pharmacologically active substance. This work aimed to develop a novel compounded oral solution (COS) containing CY. In this search, we evaluated its stability and assessed the effects on serum and liver parameters of lipid and glucose metabolism in Wistar rats.

2. MATERIAL AND METHODS

CY sesquihydrate, $C_{21}H_{21}N \cdot HCl \cdot 1\ 1/2\ H_2O$, was purchased from Pharma Nostra (Campinas, SP, Brazil). EDTA disodium dihydrate was obtained from the Sigma Chemical Company (St. Louis, MO, USA), lactic acid from Proquimios (Rio de Janeiro, RJ, Brazil), methylparaben from Sharon Laboratories Ltd (Ashdod, Israel), propylparaben from Salicylates and Chemicals Pvt. Ltd (Mumbai, India), and propylene glycol from Êxodo Científica (Sumaré, SP, Brazil). The strawberry aroma was kindly donated by Georges Broemmé® (Carmo da Mata, MG, Brazil). Sucrose was from Synth (Diadema, SP, Brazil). Purified water was obtained from the Gehaka® reverse osmosis system model OS10LXE (São Paulo, SP, Brazil). An industrial oral

solution containing CY (0.80 mg.mL^{-1}) and associations (vitamins C, B1, B2, B3 and B6) was purchased from Cifarma batch L3PC45 (Goiás, GO, Brazil) and named IOS. This pharmaceutical product contains sucrose, sodium saccharin, methylparaben, propylparaben, caramel flavor, cherry flavor, propylene glycol, sodium citrate, sodium cyclamate, and purified water as inactive ingredients [19].

2.1 Preparation of compounded oral solution (COS)

An oral solution was prepared consisting of CY (0.80 mg.mL^{-1}), sucrose 65.0 % (w/v), propylene glycol 2.0 % (w/v), methylparaben 0.15 % (w/v), disodium EDTA 0.10 % (w/v), propylparaben 0.05 % (w/v), strawberry aroma 0.05 % (w/v), and purified water to a volume of 100 mL. The pH was adjusted to 3.5 – 4.5 as described in the pharmacopeia [20]. A formulation without CY was also prepared to use as a placebo. The products were stored in amber glass bottles. CY content in oral solution ranges from 90.0 % up to 110.0 % of the labeled content of $C_{21}H_{21}N \cdot HCl$ [20].

2.2 Stability study

The accelerated stability study was carried out for 180 days. The products were stored at 24.0 ± 2.0 ºC and 40.0 ± 2.0 ºC, under 75 ± 5 % relative humidity, and periodically analyzed for organoleptic characteristics, pH, specific gravity, and CY content. All tests were performed at least in triplicate.

The evaluation of organoleptic characteristics was performed in relation to appearance, color, odor, and taste by visual, olfactory, and palatability observation, respectively, according to Zaid et al. (2021) [21]. The determination of pH and specific gravity values was carried out according to Braz. Pharmacopeia 6 (BP6) [22]. For pH, a micro-processed digital pH meter (Tecnopon® model mPA-210 MS, Piracicaba, SP, Brazil) was used. For specific gravity, pycnometrics was employed. The CY content was determined by absorption spectrophotometry at 286 nm [12] using a 1600 Nova Instruments UV-Vis spectrophotometer (Piracicaba, SP, Brazil) after spectrophotometry measurement of CY in an aqueous acid solution (pH 5.0) at 20 µg.mL^{-1} (n = 3) in the range of 260 to 306 nm. Initially, a stock solution containing 1.0 mg.mL^{-1} of CY was prepared, and suitably corrected for the equivalence, moisture, and content factors. For this reason, 55.2 mg of CY sesquihydrate (equivalent to 50 mg of CY) was accurately weighed and diluted in 50 mL of purified water, adjusting the pH to 5.0 ± 0.50. We then evaluated the linearity of the analytical method. The solutions were prepared in triplicate, independently, diluting the stock solution in different concentrations (5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 µg.mL^{-1}) using water at pH 5.0 as a blank, to obtain three analytical curves. The limit of detection (LoD) and the limit of quantification (LoQ) of the employed method was calculated from the standard deviation of the y-intercept and slope of the analytical curves of CY according to the formula given below.

$$LoD = \frac{3.3\,\sigma}{S} \qquad \text{and} \qquad LoQ = \frac{10\,\sigma}{S}$$ Wherein, σ is the standard deviation of the y-intercept and S is the slope of the analytical curve [23].

For the determination of CY in formulation, 1.0 mL of the oral solution was transferred to a 25 mL volumetric flask, which was filled with water at pH 5.0. The estimated concentration of CY in this solution was 17.6 µg.mL^{-1}, which was also measured at 286 nm using the placebo stored in the same conditions as a blank.

Mathematical models were applied to study the degradation kinetics in both storage conditions. The degradation half-life (T_{50}) and shelf life (T_{90}) were subsequently determined at 24 ºC and 40 ºC.

2.3 Evaluation of the *in vivo* effects of CY

Male Wistar rats weighing 50 – 60 g were obtained from the Breeding Center of the Federal University of São João del-Rei and kept in an environmentally controlled room at 23.0 ± 2.0 ℃ under a 12/12 h light/dark cycle with free access to tap water and chow diet (Nuvilab CR1, Nuvital, Brazil). After an adaptation period, with the male Wistar rats weighing approximately 70 g, the experiments were carried out in two steps. Firstly, two groups with four rats each named saline (control group) and IOS 1.5 (industrial oral solution) received 1.5 mg.kg^{-1} of body weight for 21 days. Three other groups (n = 4) named placebo (control group), IOS 5.0 (industrial oral solution), and COS (compounded oral solution) received 5 mg.kg^{-1} of body weight for 23 days. In both assays, the doses were administered by gavage once a day, and body weight and food intake were always measured at 18:00 hrs. The animals were euthanized and tissues and blood were collected between 8:00 and 10:00 am. Blood samples were centrifuged at 826xg for 10 minutes at 4 ℃. Serum glucose, triacylglycerol, and cholesterol were determined by a colorimetric enzymatic method using commercial kits from Bioclin® (Belo Horizonte, MG, Brazil). After lipid extraction with chloroform:methanol [24], followed by chloroform evaporation and lipid resuspension in isopropyl alcohol, the liver triacylglycerol and cholesterol concentrations were determined enzymatically, using commercial kits (Bioclin®). The total glycogen content was quantified by the anthrone assay [25]. Animal protocols received prior institutional approval by the Ethical Committee of the Federal University of São João del-Rei (protocol no. 053/2017).

2.4 Statistical analysis

The results are presented as the arithmetic average of the values and the standard error of the mean ($\overline{x}$ ± S.E.M.). Except for comparisons of the results between IOS 1.5 and saline, which were analyzed using Student's t test, the differences among groups were analyzed using one-way ANOVA followed by the Tukey HSD post-test. The criterion of significance was $p < 0.05$.

3. RESULTS

The IOS used in the present work was stored in an amber glass bottle and exhibited a homogeneous appearance, yellowish color, caramel odor, and slightly sweet taste. Physicochemical analyses showed a pH of 3.91 ± 0.01 and a specific gravity of 1.23 g.mL^{-1}. It was stored at room temperature and protected from light. The COS exhibited the following physical (organoleptic) properties: colorless and homogeneous liquid with a characteristic odor of strawberry flavor. These characteristics did not change during the period of study (data not shown).

Chemical stability was studied from the content of CY (%) in the formulation using a spectrophotometry assay method developed in our laboratory. Initially, it was considered that the CY molecule presents spectrophotometry absorption at 286 nm in an acidic aqueous medium ($A_1^1 = 433a$) and at 284 nm in an alkaline aqueous medium ($A_1^1 = 377a$) by Mofatt et al. (2011) [12]. The UV spectrum obtained for CY in aqueous acid solution (pH 5.0) is shown in Figure 1.
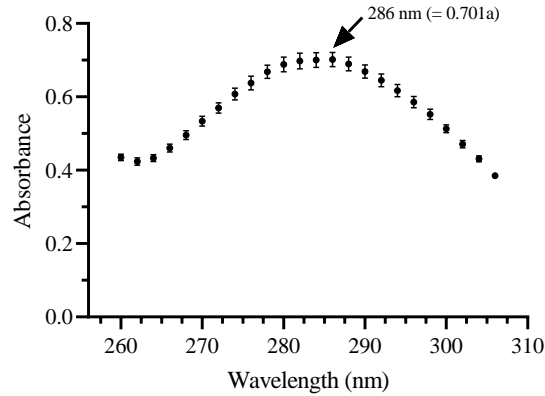
Figure 1: UV spectrum of cyproheptadine hydrochloride (CY) obtained in aqueous acid solution (pH 5.0)
at 20 µg.mL^{-1} (n=3) in the range of 260 to 306 nm.

The spectrophotometry method used to quantify the CY in the COS exhibited linearity in the range of 5.0 to 30.0 µg.mL^{-1}. The analytical curves obtained in pH 5.0 gave rise to the following linear regression equations: y = 0.0312x - 0.0166 (r = 0.9991); y = 0.0322x - 0.0184 (r = 0.9980); and y = 0.0332x - 0.0323 (r = 0.9983). These data allowed the calculation of *LoD* and *LoQ* of CY at 286 nm, respectively 0.88 µg.mL^{-1} and 2.67 µg.mL^{-1}, indicating the sensitivity of the employed method.

Figure 2 shows the results related to the stability study of COS for 180 days. The specific gravity oscillated by only 0.01 units for the COS stored at room temperature (Figure 2a). The pH values were in the range of 3.5 − 4.5 (Figure 2b) as recommended in the pharmacopeia [20]. After eliminating the potential matrix effects, the content of CY in the COS was determined over 180 days of study (Figure 2c). These data suggest that the COS presents physicochemical stability independent of the storage conditions applied in this work.
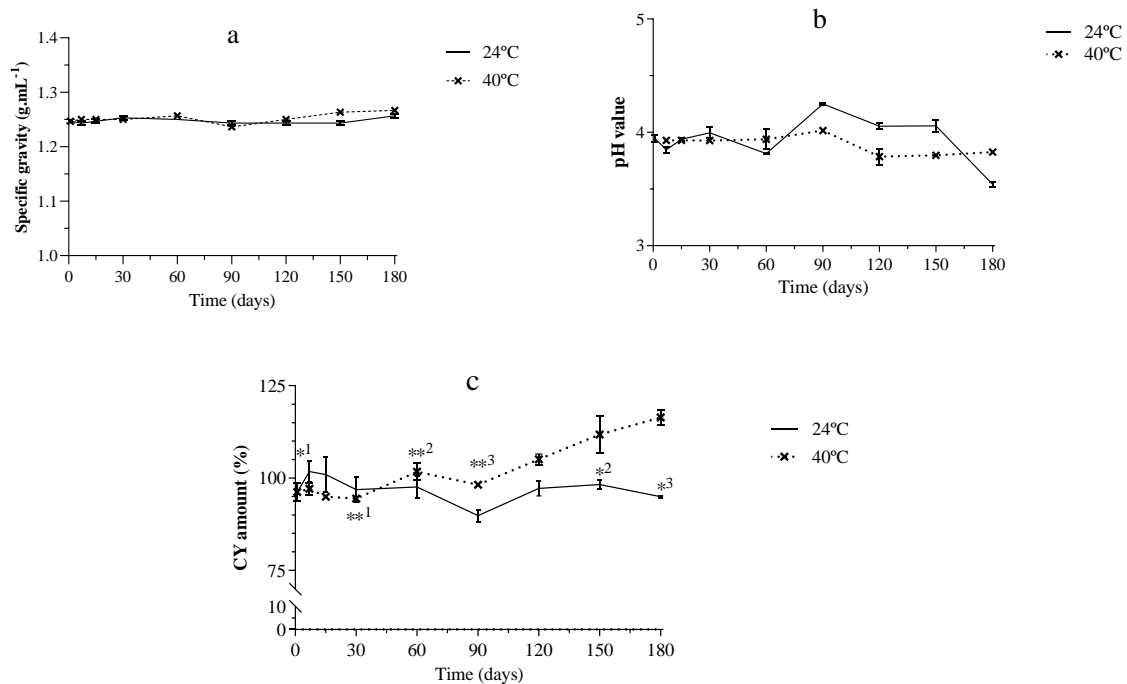

Figure 2: Characteristics of compounded oral solution of CY stored in different temperatures for 180 days, in which (a) Specific gravity; (b) pH value, and (c) CY content (%). Compared to the sample at 40ºC: *[1]p = 0.0093 on the 150th day and p = 0.0002 on the 180th day; *[2]p = 0.0402 on the 150th day; *[3]p < 0.0001 on the 180th day. Compared to the sample maintained at the same

temperature: **[1]p = 0.0024 on the 150th day and p < 0.0001 on the 180th day; **[2]p = 0.0180 on the 180th day; **[3]p = 0.0385 on the 150th day and p = 0.0012 on the 180th day.

The study of degradation kinetics of CY fitted to first-order model (time x log content of CY). From this model, the values found for T_{50} and T_{90} were, respectively, 3465 days and 527 days for the oral solution stored at 24 ℃. In turn, samples stored at 40 ℃ showed T_{50} and T_{90} values, respectively, of 173 days and 26 days.

In addition, the treatment of rats with the IOS 1.5 mg.kg^{-1}, for 3 weeks, increased approximately 30 % the content of liver cholesterol (Table 1). However, IOS 1.5 mg.kg^{-1} did not induce any changes in body weight and food intake; weight of epididymal and retroperitoneal adipose and liver tissues (Table 2); liver content of glycogen and triacylglycerol (Table 1); and serum levels of glucose, triacylglycerol, and cholesterol (Table 3) when compared to saline.

Table 1: Liver weight and liver concentrations of glycogen, triacylglycerol, and cholesterol from rats treated with industrial oral solution (IOS, 1.5 mg.kg^{-1}) and saline or industrial oral solution (IOS, 5 mg.kg^{-1}), compounding oral solution (COS) and placebo for approximately 3 weeks.

	Step 1		Step 2		
	Saline	IOS 1.5	Placebo	IOS 5.0	COS
Liver (g)	12.20 ± 0.26	12.55 ± 0.40	9.00 ± 0.30	10.00 ± 0.40	10.10 ± 0.20*
Glycogen (mg/g liver)	4.15 ± 0.80	1.95 ± 0.60	4.45 ± 1.00	2.50 ± 0.35	6.55 ± 1.10**
Triacylglycerol (mg/g liver)	23.60 ± 6.40	30.07 ± 13.00	14.30 ± 1.30	16.90 ± 4.55	17.50 ± 3.25
Cholesterol (mg/g liver)	26.50 ± 1.35	35.00 ± 1.13*	22.20 ± 1.43	22.45 ± 2.70	24.60 ± 1.80

The data are represented as the average ± S.E.M. of 4 rats in step one and 7 rats in step two. *p<0.05 vs saline or placebo; **p<0.05 vs IOS 5.0.

Table 2: Biometric data from rats treated with industrial oral solution (IOS, 1.5 mg.kg^{-1}) and saline or industrial oral solution (IOS, 5 mg.kg^{-1}), compounding oral solution (COS), and placebo for approximately 3 weeks.

	Step 1		Step 2		
	Saline	IOS 1.5	Placebo	IOS 5.0	COS
Initial body weight (g)	72.50 ± 1.50	72.50 ± 1.71	67.70 ± 3.90	72.40 ± 1.90	70.00 ± 1.80
Final body weight (g)	202.00 ± 4.60	205.30 ± 2.40	192.30 ± 5.30	208.00 ± 3.70*	208.60 ± 4.60*
Food intake (g/100g bw/day)	13.60 ± 0.50	13.10 ± 0.50	12.00 ± 0.24	13.80 ± 0.70*	11.71 ± 0.26**
Epididymal adipose tissue (g/100g bw)	0.98 ± 0.10	1.07 ± 0.10	0.56 ± 0.06	0.57 ± 0.05	0.64 ± 0.06
Retroperitoneal adipose tissue (g/100g bw)	0.71 ± 0.04	0.74 ± 0.05	0.35 ± 0.04	0.37 ± 0.05	0.43 ± 0.04
Liver tissue (g/100g bw)	4.92 ± 0.03	4.98 ± 0.12	4.67 ± 0.04	4.81 ± 0.12	4.77 ± 0.10

The data are represented as the average ± S.E.M. of 4 rats in step one and 7 rats in step two. *p<0.05 vs saline or placebo; **p<0.05 vs IOS 5.0.

Table 3: Serum concentrations of glucose, triacylglycerol, and cholesterol from rats treated with industrial oral solution (IOS, 1.5 mg.kg^{-1}) and saline or industrial oral solution (IOS, 5 mg.kg^{-1}), compounding oral solution (COS), and placebo for approximately 3 weeks.

	Step 1		Step 2		
	Saline	IOS 1.5	Placebo	IOS 5.0	COS

Glucose (mg/dL)	146.42 ± 4.04	153.90 ± 2.54	155.07 ± 5.79	160.62 ± 6.53	154.69 ± 4.12
Triacylglycerol (mg/dL)	105.79 ± 11.13	85.77 ± 5.66	158.74 ± 46.45	168.48 ± 29.74	137.54 ± 29.29
Cholesterol (mg/dL)	83.99 ± 5.49	81.48 ± 4.46	75.51 ± 9.44	94.93 ± 9.96	83.54 ± 12.81

The data are represented as the average ± S.E.M. of 4 rats in step one and 7 rats in step two.

In contrast, treatment of rats with the IOS and COS, both 5 mg.kg^{-1}, for approximately 3 weeks, increased approximately 8 % the final body weight compared to placebo (Table 2). The difference in body weight occurs from 6 days until the end of treatment (Figure 3). Interestingly, the IOS (5 mg.kg^{-1}) induced an increase in food intake (approximately 15 %) compared to placebo, but not the COS (5 mg.kg^{-1}) (Table 2). Indeed, COS 5.0 decreased the food intake compared to IOS (5 mg.kg^{-1}) (Table 2). Additionally, IOS and COS (both 5 mg.kg^{-1}) did not induce changes in the weight of epididymal and retroperitoneal adipose and liver tissues (Table 2), as well as in serum and liver levels of triacylglycerol and cholesterol (Table 1 and 4), and serum levels of glucose (Table 3). However, the COS (5 mg.kg^{-1}) increased the glycogen concentration compared to IOS (5 mg.kg^{-1}), but not to placebo, while liver weight was increased in COS (5 mg.kg^{-1}) compared to placebo (Table 1).
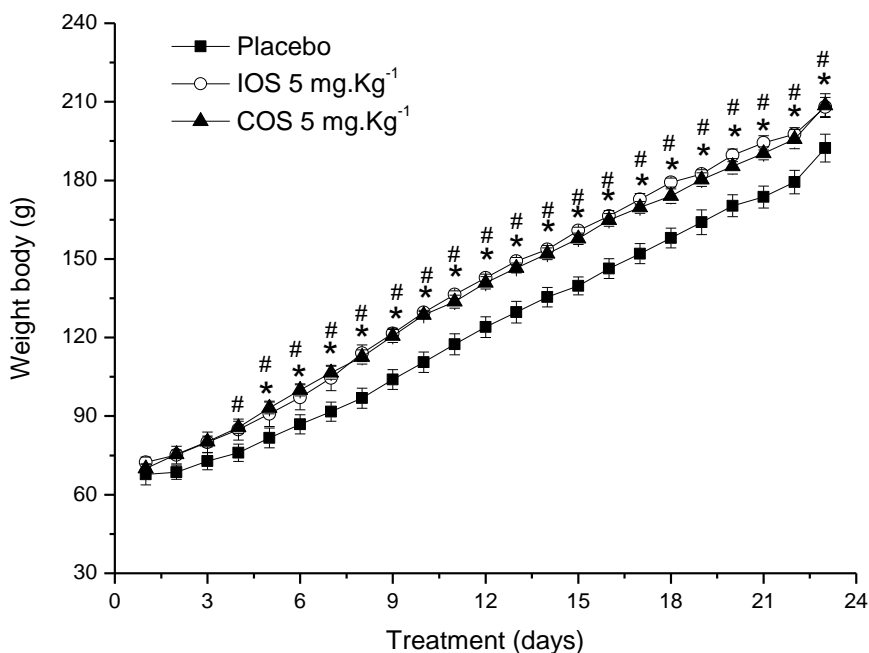

*Figure 3: Effect of industrial oral solution (IOS, 5.0 mg.kg^{-1}) and compounded oral solution (COS, 5.0 mg.kg^{-1}) on body weight from Wistar rats (n: 7 rats). *p<0.05 IOS 5.0 vs placebo; # p<0.05 COS vs placebo.*

4. DISCUSSION

Stability studies aim to provide evidence on how the quality of a drug substance or pharmaceutical form can vary with time under the influence of several environmental factors. Consequently, such studies make it possible to establish recommendations on the storage conditions and half-life of the product, in addition to favoring suggestions related to adjustments in the pharmaceutical formulation [26].

In the present study, the COS was protected from light radiation, although the CY shows evidence of photostability [27]. The physical and physicochemical characteristics of the formulation remained unchanged or within the expected range during the study period for both

temperature conditions (Figures 2a and 2b). This corroborates the results found by Gupta (2007) [28] who studied the stability of CY in a liquid for oral use.

In the study of Gupta (2007) [28], the aqueous solution containing CY, ethanol, glycerin, sucrose, sodium saccharin, anhydrous citric acid, sorbic acid, and raspberry flavor maintained constant both the physical aspect and pH 3.7 for 180 days when stored in an amber glass bottle at a temperature of 25 ℃. In addition, the HPLC-PDA analysis method found no loss of CY within 180 days at room temperature, pointing to the chemical stability of the substance [28]. Noteworthy, these results are comparable with the findings herein obtained by spectrophotometry.

The molecular structure of CY is displayed in Figure 4. It is known that CY is susceptible to oxidative processes as demonstrated by Abdelrahman et al. (2021) [8] when developing a stability-indicative method for determining CY, its impurity, and degradation product. Furthermore, chemical changes in organic molecules can occur given exposure to ultraviolet (UV) light. In this regard, numerous drug substances absorb radiation in this region of the electromagnetic spectrum and this radiation is capable of breaking down the chemical structure [29].
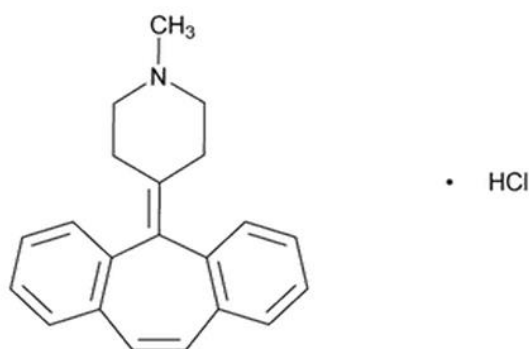

Figure 4: Molecular structure of cyproheptadine hydrochloride (CY). Source: [20].

Abounassif et al. (2005) [27] evaluated the photostability of antihistamine agents of the benzocycloheptane type. These were loratadine, pizotifen malate, ketotifen fumarate, and CY. The authors describe that the exposure of such compounds to UV light could promote $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, which would lead to the cleavage of chemical bonds and hence, the photodecomposition of these substances. However, preliminary photostability investigations tested by a spectrophotometric method demonstrated the photostability of ketotifen and CY. In contrast, the other compounds (loratadine and pizotifen) showed a decrease in absorption at their λ_{max} with subsequent changes in their zero-order absorption curves, being considered photolabile structures. In contrast, in official compendiums, CY is usually recommended to be protected from light [30].

In 2018, Sharaf El-Din et al. [31] exposed the CY to different forced degradation studies. They developed and validated an HPLC methodology coupled with fluorescence detection for the determination of CY in its pure form and tablets. It was demonstrated that CY is highly stable under forced alkaline or acidic degradation conditions. No significant photolytic degradation was observed, even after 48 hours. However, it was noted that there was considerable degradation under oxidative conditions, which depends on the temperature.

Finally, it can be inferred that COS suffered chemical degradation by oxidation when subjected to a stress condition (40 ℃) since the rate of CY degradation increased by 20x (as displayed by the linear regression angular coefficient). Therefore, it is recommended that the COS used in this study be stored at a temperature below 24 ℃. Moreover, the syrup should be protected from freezing [32].

In addition, in 2017, a study proposing the formulation and evaluation of gastroretentive microspheres using mucoadhesive polymers pointed out that CY release may be sustained for 10 hours by optimizing oral therapy for allergic rhinitis [9].

Recently, researchers have been concerned about potential severe hepatic complications, although CY is safe according to the literature [18, 33, 34].

A previous study shows that CY increases the food and water intake in male Wistar BR rats (100 ± 3 g initial body weight) [35]. Weight and food intake were measured twice a day (morning and evening) and CY syrup (0.5-1.5 mg.kg^{-1}) was administered orally in tap water ad libitum for 10 days, while control rats received only syrup or nothing [35]. In that study, although body weight and food intake were always evaluated twice a day, only one graph of each parameter was presented, without clarifying whether it represents the sum of the two daily evaluations and without representing the variability of the data and the statistical test performed.

In our study, CY was administered by gavage, ensuring an accurate dose (1.5 mg.kg^{-1}), and the control was saline, however, the food intake and body weight were similar between the groups at this dose. However, CY administration at a dose of 5 mg.kg^{-1} from IOS or COS increases the body weight accompanied by an increase in food intake in IOS, but not in COS. The effect of both formulations on body weight suggests a direct effect of CY independent of the vitamins (B1, B2, B3, B6, and C) present in IOS. The increase in body weight induced by COS independently of the increase in food intake suggests that the sucrose in placebo and COS (65 % w/v) probably induced greater satiety compared to IOS. The concentration of sucrose in IOS is unknown, but the presence of sodium saccharin and sodium cyclamate, two sweetening agents, suggest a lower concentration of sucrose and lower caloric density. Body weight gain depends on a balance between food intake and energy expenditure.

It has been demonstrated that individuals treated with CY have minimal side effects, the most common being transient drowsiness and headache [36, 37], with no serious adverse events being observed [37]. These events are expected due to the mechanism of action of CY as a histamine antagonist [37]. The most commonly observed side effect was sedation [18]. Considering the lack of effect of CY on food intake, as well as its adverse effects, we can suggest that body weight gain in COS-treated rats at a dose of 5 mg.kg^{-1} is due to lower energy expenditure. The lack of effect of CY on the weight of the adipose tissue and on the serum cholesterol and triacylglycerol corroborates the hypothesis of a reduction in the energy expenditure of the animals (Tables 2 and 3). Individuals treated with CY do not have changes in fasting glucose, free fatty acids, and plasma insulin compared to placebo [38, 39]. Studies investigating the effects of CY on circulating lipids were not found in the literature.

To the best of our knowledge, this is the first study to evaluate the effect of CY on liver lipid and glycogen concentration in rats. Although the administration of IOS (1.5 mg.kg^{-1}) increases the liver cholesterol concentration, a similar effect does not occur with the higher dose (5 mg.kg^{-1}), suggesting that the effect of CY or vitamins is dose-dependent. The IOS has, in addition to CY, vitamin C and complex B, including thiamine (B1). This vitamin is rapidly converted to thiamine pyrophosphate, which is required for the reaction catalyzed by the pyruvate dehydrogenase complex. In this way, we can suggest that greater availability of thiamine may favor the synthesis of acetyl-CoA, a precursor of the synthesis of cholesterol and triacylglycerol.

A single intraperitoneal administration of thiamine (0.1 mg. approximately kg^{-1}) induced an increase in hepatic cholesterol in thiamine-deficient rats when compared to saline rats also deficient in thiamine [40]. In contrast, a single administration of thiamine at a dose of 0.40 mg.kg^{-1} of body weight does not change the hepatic cholesterol content in rats [41]. Such findings suggest that hepatic cholesterol content is regulated by thiamine supply, but this effect appears to be dose-dependent. Additionally, in our study, the IOS 5.0-treated rats (5 mg.kg^{-1}) received 0.75 mg.kg^{-1} of thiamine for approximately 3 weeks.

Our study suggests that CY does not affect the liver glycogen concentration, as both COS and IOS have CY in their composition, but the IOS has vitamins B1, B2, B3, B6, and C in their composition. Among the vitamins present in the IOS, we highlight that pyridoxine, pyridoxamine, and pyridoxal are three isoforms of vitamin B6 converted by the body into pyridoxal phosphate, which covalently binds to a lysine residue and stabilizes glycogen

phosphorylase, thus being an essential component of the key enzyme responsible by glycogen mobilization. The decrease in liver glycogen from IOS-treated rats (approximately 2.50 mg.g^{-1}) seems to be due to a greater glycogen mobilization in comparison with COS-treated rats (approximately 6.55 mg.g^{-1}). Although a previous study has shown that the vitamin B6 vitamers, pyridoxine, pyridoxal, or pyridoxamine (300 mg.kg^{-1}) are capable of promoting the mobilization of hepatic glycogen stores and hyperglycemia in the rat due to the release of adrenomedullary catecholamines [42], our study does not demonstrate an increase in plasma glucose levels, thus the change in liver glycogen seems to be independent of plasma catecholamine levels. Additionally, in our study, the IOS 5.0-treated rats (5 mg.kg^{-1}) received 0.84 mg.kg^{-1} of pyridoxine, thus we believed that this dose was not enough to induce catecholamine release.

A previous study showed that CY at a dose of 2.3 mg.kg^{-1} for 10 days to adult rats weighing 220 g $\pm$ 15 g (n = 8) does not promote changes in serum glucose concentrations, however increasing the dose to 4.6 mg.kg^{-1} promotes a 15 % increase in serum glucose concentration [43]. In our study there was no increase in glycemia, which was expected given the proximity of doses of 4.6 and 5.0 mg.kg^{-1}, however, in addition to the age of rats, there are differences in methodology. While in the previous study, the plasma glucose was evaluated 1 hour after the last CY administration [43], we evaluated the glucose approximately 14 hours after the CY administration. Daily administration of CY at high doses (45 mg.kg^{-1} orally) for 8 days in rats can lead to a significant increase in fasting glycemia. The increase in glucose concentrations induced by CY is reversible because, after 24 hours of CY administration, glycemic values returned to baseline values [44]. Thus, the time between CY administration and glucose measurement had to be considered when comparing different studies.

5. CONCLUSION

The COS presented stability when stored at 24 ºC and exhibited first-order degradation kinetics that can lead to oxidation. Regarding the metabolic effects found in the doses and period of the study, we observed an increase in final body weight in rats with no alteration in glucose and lipid metabolism.

6. ACKNOWLEDGMENTS

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