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# Effects of Artepelin-C Supplementation Present in Propolis Related to Inflammatory Processes in Physically Active Individuals

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#### **ABSTRACT**

The scientific literature shows that propolis has both anti-inflammatory and antibacterial activities and is widely used in phytotherapic therapy. In this context, its main objective is to evaluate the inflammation recovery process in physically active individuals, from two groups, with or without propolis intake. Volunteers had their food, blood and pain parameters evaluated with or without propolis intake. The trial used seven male volunteers undergoing specific training in two 30day protocols, one using placebo and another using propolis which contains Artepelin-C (chemically 3,5-diprenyl-p-coumarin acid, which is one of the main phenolic acids present in the green propolis extract). Participants between 18 and 35 years old under no medication should have had at least a 6-month workout. Performance physical tests were applied, body composition measurements and blood collection were taken and a 24-hour food recall and food frequency questionnaire were carried out at São Judas Tadeu University. All volunteers were asked to register their food intake during the 30-day protocol and data were analyzed by using ANOVA and Students T-test for paired samples at <0.05 significance level set. A significant lymphocytes increase during placebo protocol and a significant reduction of these cells during propolis protocol were observed. Concerning propolis and placebo protocols, volunteers showed less pain during the former, which means that propolis is highly beneficial for those practicing intense physical exertion. Artepelin-C present in propolis protocol and having a modulator effect in inflammatory processes enables a higher intense physical exertion more easily, due to its property to minimize pain.

**Keywords:**Physical activity;Inflammation process;Body composition; Propolis; Artepelin-C; Blood tests; Pain perception and evaluation.

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#### 1. INTRODUCTION

Inflammatory processes are extremely serious in athletes' lives, as they have elevated biomarkers of oxidative stress, which promotes inflammation along with muscle damage during periods of intense training and competition [1]. Intense training during competitions are associated with pain, fatigue and the possibility of injury [2]. If recovery after training during the competition season is not adequate, there may be elevation in the levels of pro-inflammatory markers which has serious professional and personal impacts [1,3,4]

In Brazil, propolis is classified in 13 groups, based on its organoleptic and physical-chemical characteristics [5]. This material has attracted the attention of countless researchers for the effectiveness in the different pharmacological properties which can bring benefits to human health, such as antimicrobial, antioxidant and anti-inflammatory activities [6].

Artepelin-C is a phenolic bioactive present in the propolis extract, which is responsible for the pharmacological anti-inflammatory activities attributed to the herbal products. For the development of this study, propolis, a product commercially available was used in form capsules, as it contains the biomarker Artepelin-C (chemically 3,5-diprenyl-p-coumarin acid which is one of the main phenolic acids present in green propolis extract).

#### 2. OBJECTIVE

To elucidate the effects of Artepelin-C, using the propolis on inflammatory processes induced in volunteers practicing physical activity. This will evaluate the body composition by means of parameters measured by bioimpedance, by application of food registry, by blood tests in the period before treatment and on the last day of treatment. Also maximum physical exertion test and application of the validated pain questionnaire Brief Pain Questionnaire (BPQ), Visual Analogue Scale (VAS) and Effort Perception Scale (BORG) were done.

#### 3. HYPOTHESIS

It is believed that the physical activity group with inflammation undergoing treatment with Artepelin-C will have better results for the variables of body composition, blood parameters, pain and inflammation reduction when compared to the placebo protocol.

#### 4. METHODS

This is a double blind crossover experiment in which the volunteers after leaving the placebo protocol, start a 30-day rest, and a week after that, begin the propolis protocol (intervention).

This work was approved by the Ethics and Research Committee of São Judas Tadeu University (USJT), São Paulo City, SP, Brazil, number 2,593,564. All participants agreed, after a thorough explanation, to sign the informed consent form before starting the study (Appendix 3). All the questionnaires are identified as: Appendix 1 (24 h Nutritional Recordatory), Appendix 2 (Food frequency questionnaire), Appendix 3 (Free Informed Consent Form), Appendix 4 (Pain scale – Brief Pain Inventory), Appendix 5 (Visual Analogue Scale - VAS), Appendix 6 (Borg scale) and Appendix 7 (Training program)

#### Sample size

The sample size consisted of seven men volunteers for both protocols, placebo and propolis. The participants were selected and recruited from the research team personal contacts.

#### **Monitoring training**

Research volunteers (male gender) had to maintain their minimum frequency of 3 training sessions per week during 30-day period at their own training site. It was also necessary to attend São Judas Tadeu University only on the initial and final collection of each protocol.

The volunteers were given the validated questionnaires for the evaluation of BPI (Brief Pain Inventory), VAS (Visual Analogue Scale) and the Borg scale for Effort Assessment Perception, which were completed according to the days trained, respecting the minimum frequency established. At the end of each week,

the calendar and the answered questionnaires were sent to responsible research team.

Volunteers were instructed to neither alter their diet during the monitored training period, nor ingest other supplements such as herbs, stimulants and medications, except for propolis during the 30-day propolis protocol.

#### **Propolis protocol**

Propolis was offered in 70 mg capsules per day, for 30 days, and it was named as "propolis protocol" (which contains Artepelin-C - chemically 3,5-diprenyl-p-coumarin acid, one of the main phenolic acids present in the green propolis extract)

The placebo was offered in capsules with the same physical and excipients characteristics (magnesium stearate, colloidal silicon dioxide, lactose monohydrate mesh 200 and microcrystalline cellulose) and named as "placebo protocol".

#### **Trials carried out**

Tests were performed for body composition, such as weight, height and bioimpedance before and after the experiments. A 1 RM protocol test for strength training on the bench press and leg press 45 exercises were carried out. Food registration forms were also provided for the 30-day protocol, being completed by the volunteers of the survey for weekly delivery. In addition, blood samples were collected in heparinized tubes and EDTA (4 ml / tube) for analyzes during two days for each protocol and training. Daily follow-up via messengers and electronic mails were sent on the first and the last day of the experiment for each protocol.

#### **Body build**

Body build was measured in InBody® model InBody120® bioimpedance scale, in which the volunteers were instructed to keep a 4-hour water fasting. The tests were performed according to Perini, de Oliveira et al. (2005) [8].

The height was estimated using a stadiometer and performed according to Lohman, Roche and Martorell (1988) [9].

#### Strength training 1RM

The 1RM strength test consists of performing repeated exercises with the maximum load that the individual can withstand. Warm up is required from 5 to 10 repetitions, weight lifting between 40% and 60% of the estimated 1 RM, followed by one-minute interval with stretching exercises. Then, 3 to 5 more warm up replicates are initiated, with weight lifting between 60% and 80% of the estimated 1RM, followed by two minute intervals. After that, the volunteer will attempt to perform a repetition of weight lifting he/she will be able to withstand. If the results are successful, the volunteer should have a 3 to 5-minute rest and afterwards resume with a heavier weight lifting to determine 1RM [10].

#### **Training program**

This training starts with warm-up on a treadmill with 8 to 12 cycles running, 60 maximum intensity seconds of exercise, followed by a 75 second rest, according to the Gibala protocol [11]. After that, a super series training followed with 8 to 12 repetitions, using the descending pyramid method (decreasing in each series the load to be lifted). The decreasing pyramid method was used as a training strategy so that the volunteer could begin training with a heavier load in the first two grades in which there is tendency to decrease the load due to muscle fatigue caused by high intensity. For this training, a 65% to 75% load of the estimated 1RM was used for each volunteer [12].

## Daily dietary reference intakes (DIR)

The daily dietary record is a method that does not depend on the memory of the participant, so it can be considered the most valid method to measure the intake for a certain period. The individual should record all food and beverages as well as their respective amounts during the period requested by the nutritionist [13].

The volunteer is expected to write a detailed report about the portion consumed, such as: a slice of cheese, a medium banana, a candy, a packet of cookies, and so on [14].

The information obtained by this method is influenced by the volunteers' ability to accurately record their food consumption. This ability varies according to age, gender, level of education, among other factors. Elderly and people with some kind of cognitive impairment represent the factor that most influences responses as caregiver are required to record the information.

#### **Blood collection**

The volunteers were submitted to perforation of the brachial vein. Aliquots of heparinized blood and EDTA were used, the latter being used for hemogram and leukogram in an automated device. Data was precise because the volunteers fasted for 8 hours.

## Brief Pain Inventory (BPI) and Visual Analogue Scale (VAS)

Brief Pain Inventory (BPI), shown in Appendix 4, is a multidimensional instrument that assigns scores of 0-10 to measure the items intensity, pain interference in walking ability, daily activities of the patient, at work, social activities, mood and sleep. The pain evaluation performed by the patient consists of the pain observed at the time of the questionnaire, the most and least intense and also the average pain during the last 24 hours [15].

BPI, in its reduced form, allows the evaluation of pain in several aspects: location, intensity, and comparison between different intensities, treatment evaluation and the relief brought by the treatment which impacts in the patient daily life, as well as age and gender [15].

Visual Analogue Scale (VAS) (Appendix 5) consists of a line numbered 0 (no pain) and 10 (unbearable pain) and the patient evaluation of pain intensity [15].

These pain assessment instruments were used to identify the type and intensity of pain, in both groups, generated by the inflammatory process suffered by the volunteers while training during the treatment.

#### Perception of effort scale

The perception of effort (PE) is a multifactorial event, where different sensations are present,

being affected by physiological and psychological mechanisms [16]. PE refers mainly to intense muscular work, being related to the intensity exercise concept which is defined as the subjective intensity of exertion, tension, discomfort or fatigue experienced during physical exercises, which can be aerobic and / or force [17].

Scales were developed for measuring the effort perception with a specific classificatory method, establishing a relationship between a stimulus and a response [16].

Borg scale, shown in Appendix 6, consists of a table with a list of values from 0 (rest) to 10 (exhaustive) effort.

#### Inclusion and exclusion criteria

Men between 18 and 35 years old practicing at least six months of physical activity at least three times a week, willing to use propolis during 30 days, and a further 30 days with placebo were Included in the research.

Volunteers having allergy, injuries or using ergogenic pharmacological agents and anti-inflammatory drugs; those who did not maintain the appropriate frequency of at least 3 training sessions per week during a 30-day period and also those who did not use the herbal remedy properly were excluded from the survey.

#### **Research location**

The 1RM tests; blood collection; bioimpedance; evaluation of body composition were carried out in the Laboratory of Human Movement and academy of the University São Judas Tadeu.

## Data analyzes

Data were submitted to the Shapiro-Wilk normality test and after its confirmation, the other analyzes were performed. In sequence, they were compared using two-way ANOVA and Student's t-test for independent samples, with a significance level set at p <0.05. The program used for data analysis was the Statistical Package for the Social Sciences (SPSS) version 20.0 produced by IBM® [18].

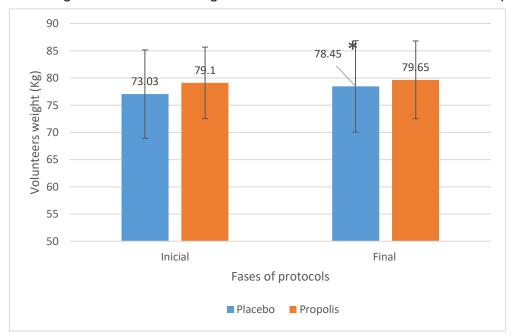
#### 5. RESULTS

The results are derived from the data obtained from the volunteers who participated in both protocols of training placebo and propolis, being evaluated in the initial and final period of each protocol.

#### **Body built**

#### **Volunteers** weight

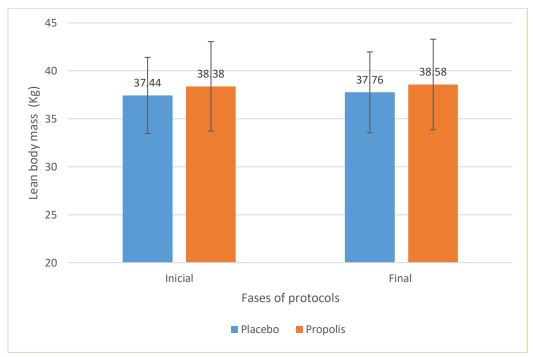
Graph 1 – Volunteers weight mean values in kg at the initial and final moments of each protocol



\*p< 0.05 There was a significant increase in weight between the initial and final moments in placebo protocols (Initial =  $77.03 \pm 1.88$ , Final =  $78.45 \pm 1.98$ , p = 0.045).

#### Lean body mass

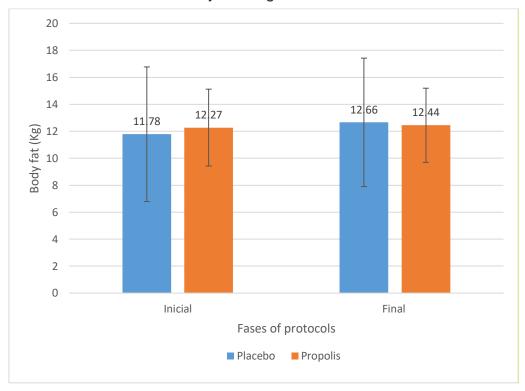
Graph 2 – Volunteers mean lean body mass values in kg at the initial and final moments of each protocol.



There was no significant increase in lean mass among the volunteers in any of the protocols.

#### **Body fat**

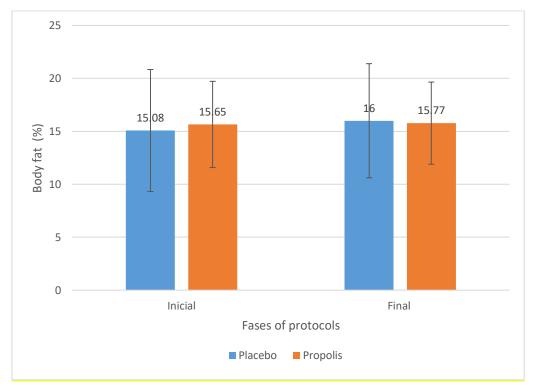
Graph 3 – Volunteers mean values of body fat in kg at the initial and final moments of each protocol.



\*p< 0.05 No significant increase was observed.

#### **Body fat percentage**

Graph 4 - Volunteers mean values for body fat percentage at the initial and final moments of each protocol.

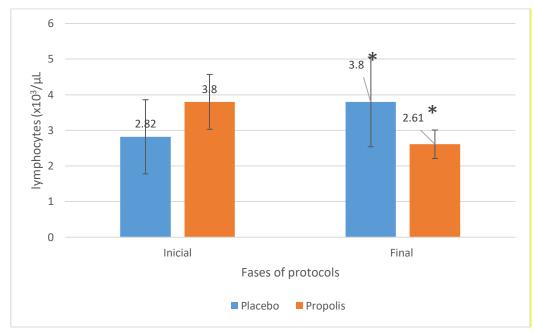


No significant results were observed.

#### **HEMATOLOGICAL PROFILE**

#### Lymphocytes

Graph 5 – Volunteers lymphocytes serum mean values at the initial and final moments of each protocol.

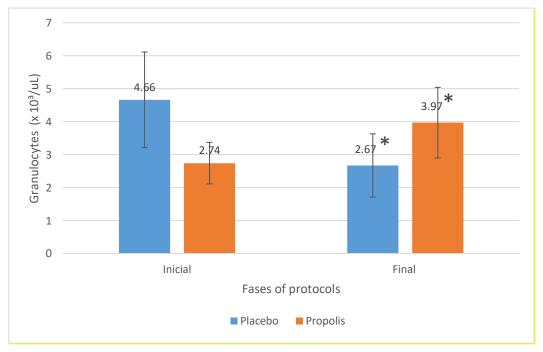


\*p< 0.05

There was a significant decrease in lymphocytes in the propolis protocol occurred at the final moment. (Final =  $2.61 \pm 0.376$ , p = 0.033).

#### Granulocyte

Graph 6 – Volunteers granulocytes serum mean values at the initial and final moments of each protocol.

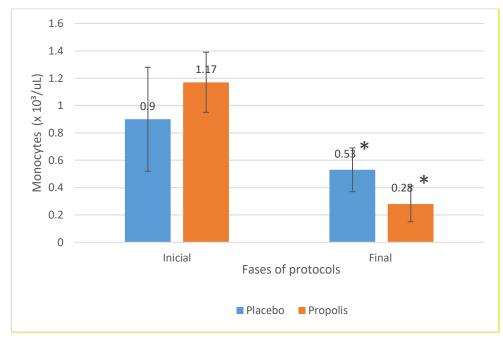


\*p< 0.05

There was a significant decrease at the end of the protocol where placebo was used (Initial =  $4.66 \pm 0.39$ , Final =  $2.67 \pm 0.33$ , p = 0.006). In the propolis protocol it was possible to observe a significant increase (Initial =  $2.74 \pm 0.440$ , Final =  $3.97 \pm 0.38$ , p = 0.024).

#### **Monocytes**

Graph 7 – Volunteers monocytes serum mean values at the initial and final moments of each protocol.



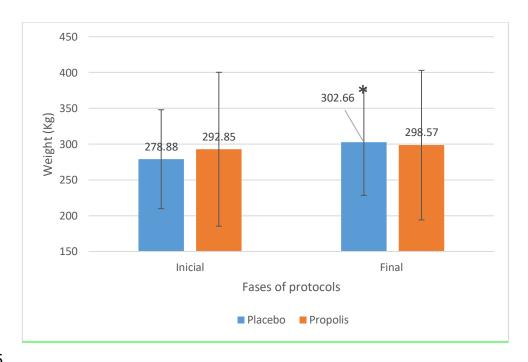
\*p< 0.05

There was also a statistically significant difference at the final moment in both protocols.

#### Strength test (1RM)

#### **1RM Leg Press**

Graph 8 – Volunteers in the maximal resistance test (1RM), in the leg press exercise, showed mean values of weight erected (kg) in the maximal resistance test (1RM) at the initial and final moments of each protocol.

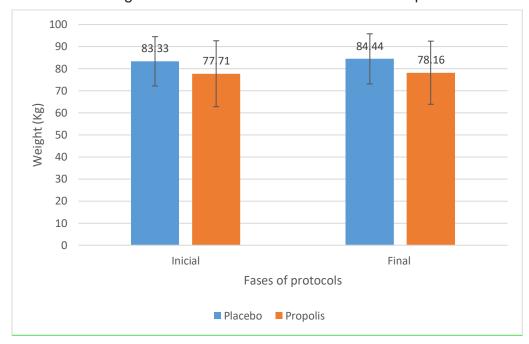


\*p< 0.05

In the 1RM variable for the leg press exercise, there was a significant increase between the initial and final moments for placebo protocols.

#### 1RM Supine (bench press)

Graph 9 - Volunteers lifting erected weight (kg) in the maximal resistance test (1RM) in the supine exercise presented mean values of kg at the initial and final moments of each protocol.

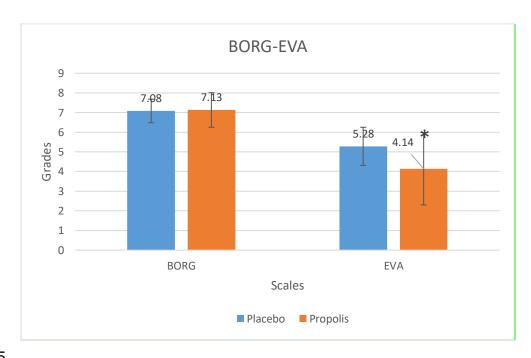


There was no statistically significant result.

#### **Pain Scales**

#### Effort and pain perception scales (BORG-EVA)

Graph 10 - Mean values for the perception of effort and pain related to the training sessions performed by volunteers in placebo and propolis protocols.

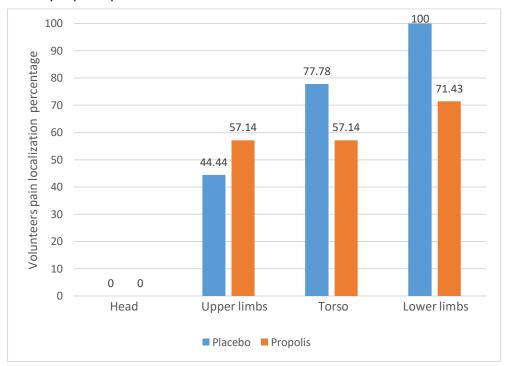


\*p< 0.05

There was no statistically significant difference in the effort perception variable (Borg). However, in the variable pain perception there was a statistically significant reduction in the average pain perception of volunteers in the propolis protocol compared to the mean pain perception of volunteers in the placebo protocol (Placebo =  $5.28 \pm 0.97$ , Propolis =  $4.14 \pm 1.84$ , p = 0.004).

#### **Brief Pain Inventory (BPI)**

Graph 11 - The percentages for pain location related to the training sessions performed by the volunteers in placebo and propolis protocols.

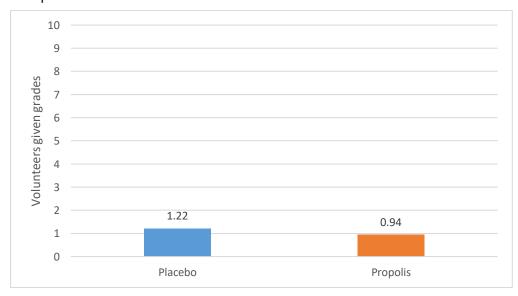


Volunteers have not had headache in any protocols; 44.44% of volunteers felt pain in the upper limbs during the placebo protocol and 57.14% felt pain in the same region during propolis protocol.

77.78% of the volunteers felt pain in the trunk during the placebo protocol, and 57.14% experienced pain in the same region during the propolis protocol.

A 100% of the participants felt pain in their lower limbs during the placebo protocol, while 71.43% felt pain in the same region during the propolis protocol.

Graph 12- Averages of pooled values for pain interference related to training sessions in daily activities during the two protocols.

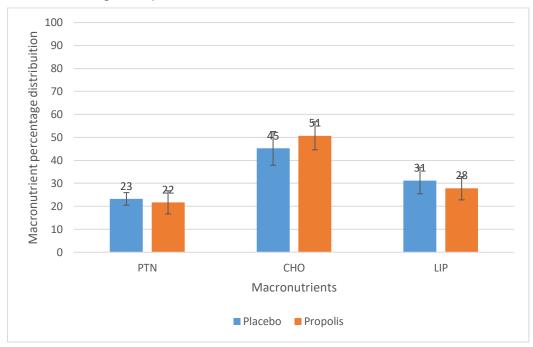


In both protocols, there was a low pain interference in the daily activities.

During the placebo protocol, the pain interference average was 1.22 while during the propolis protocol, the pain average was 0, 94, on a scale of 0 to 10.

### Daily food records

Graph 13 – Mean values and standard deviation of the macronutrient percentage distribution records among volunteers during both protocols.



Legend: PTN = protein / CHO = carbohydrates / LIP = lipids

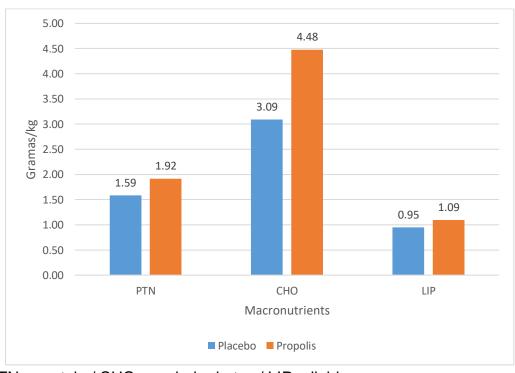
There was a little difference between the macronutrients percentage distribution in both protocols. During the placebo protocol, there was 23% of protein consumption compared to the propolis protocol

in which there was 22% of protein consumption.

There was an average of 45% of the total carbohydrates calories intake during the placebo protocol and 51% of carbohydrates intake during propolis protocol.

There was an average of 31% of lipid distribution during placebo protocol and 28% of total calories during the propolis protocol.

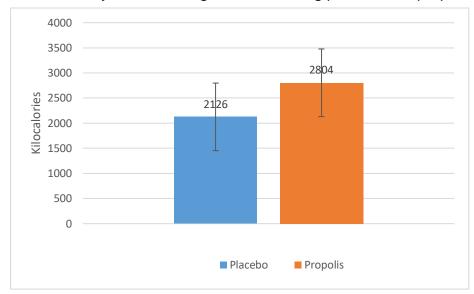
Graph 14 - Volunteers dietary intake records of macronutrient (g/kg) during placebo and propolis protocols.



Legend: PTN = protein / CHO = carbohydrates / LIP = lipids

The mean protein intake was 1.59g / kg and 1.92g / kg for the placebo and propolis protocols, respectively. The mean carbohydrate intake was 3.09g / kg (placebo protocol) and 4.48g / kg (propolis protocol). The mean intake in grams per kilogram of lipids was 0.95 (placebo protocol) and 1.09 (propolis protocol).

Graph 15 – Volunteers caloric dietary intake average records during placebo and propolis protocols.



The caloric intake of the volunteers was 2126 Kcal/day and 2804 Kcal /day during placebo and propolis protocols, respectively.

#### 6. DISCUSSION

Seven volunteers were recruited for this research performing all the proposed tests and significant differences were observed in some of the evaluated parameters. For instance, there was a significant increase in body weight and fat during the placebo protocol, but no statistically significant difference in these results during the propolis protocol was observed.

During 1RM exercise test for the 45° leg press exercise, an average increase of 13.9kg (p <0.05) was observed during the placebo protocol. According to França et al., (2015) [19], the muscles of the lower limbs adapt to a training protocol more easily, due to larger size and greater number of muscles in this region, and also a greater recruitment of muscle fibers in high training intensity, generating more strength and increased muscle tissue. Due to this, the adaptation favored loads increase in 1RM test for the lower limbs in the placebo protocol, which was the first to be performed by volunteers, and no differences were observed in this parameter in propolis protocol.

Pain is linked to the inflammatory process generated by muscle injury during the protocols. However, there was a statistically significant difference concerning pain perception reported daily by volunteers in the visual analogue scale (VAS). It was lower during the propolis protocol, which is, most likely, related to the reduction of the inflammatory process caused by propolis intake.

Lymphocytes are cells of the immune system which circulate continuously in the bloodstream and tissues [20]. Concerning leukocytes values, there was a significant increase in serum lymphocytes during the placebo protocol but a decrease in the concentration of these cells in propolis protocol.

These results are due to the fact that lymphocytes have an inflammatory function, showing that there was an increase in the inflammatory process during the placebo protocol and a decrease in the inflammatory process during the propolis protocol [21].

Also, there was a significant decrease in serum monocytes in both protocols. The reduction of

monocytes during propolis protocol can be explained by the decrease of the inflammatory process [22]. The decrease of monocytes during the placebo protocol might have been due to the inflammatory process increase in this protocol, which increased the need of these cells in the inflamed muscle tissue.

There was a significant decrease in granulocytes during the placebo protocol, which may be explained by the increased neutrophils need in inflamed muscle tissue [20,23].

During the propolis protocol, a significant granulocytes increase was observed in the volunteers. Basophils, eosinophils and mast cells make up the granulocyte group and are related to the allergic response from the immune system, acting in conjunction with IgE antibodies [23].

Despite the fact that Artepelin-C, present in propolis potential, may trigger off an allergenic response, granulocyte levels have remained within the reference studies.

The other results did not present significant differences.

#### 7. CONCLUSION

According to the results presented after a 30 day protocols with training and supplementation (propolis protocol), we concluded that Artepelin-C is beneficial to practitioners in intense physical activities as it has a modulating effect on mediators of inflammatory processes, which results in a lower perception of pain, making it possible to ease and maintain high-intensity training.

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## Appendix 1 - 24h Nutritional Recordatory

Meal Schedule Food / Preparations Amount (Usual mean	e:	( years old)	
	Meal Schedule	Food / Preparations	Amount (Usual measure)

## Appendix 2 – Food frequency questionnaire

				Consum	ption			Preparation (baked,	
	Nourishment			W		Е	N	Types	cooked, grilled, fried
	1.	D	1-2x	3-4x	5-6x	_	.``		and on)
	bread								
es	rice								
mng	pasta								
<u>)</u>	fillied wafer								
Cereals and legumes	crackers								
sals	cookie								
Cere	cakes								
O	beans								
	legumes								
"	vegetebles								
Vegetables	legumes								
etal	potato/manioc								
/eg	Examples:								
	fruits								
	papaya								
Fruit	banana								
ᇤ	orange								
	Examples:								
	milk								
	yogurt creamy cheese								
Milk	cheese								
2	Examples:								
	•								
	beef								
<u>v</u>	pork								
999	poultry								
Meet and eggs	fish ham								
a a	chicken giblets								
Μe	cold cuts								
	eggs								
	fritters								
ō	margerine								
anc	butter								
ner	mayonese olive oil								
ete	candy								
we	bubble gum								
S, S	chocolat								
Sugar, candies, sweetener and oil	chips								
car	snacks								
јаг,	sugar								
ìns	sweetener								
	coffee								
<b>6</b> 3	juice								
Beverage	processed juices								
ÿ∨er	soft drinks								
Be	water								
	alcoholics aily frequence) / W: wee								

D: Daily (daily frequence) / W: weekly / E: eventual / N: never

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#### **Appendix 3 - Free Informed Consent Form**

EFFECTS OF ARTEPELIN-C SUPPLEMENTATION PRESENT IN PROPOLIS RELATED TO INFLAMMATORY PROCESSES IN PHYSICALLY ACTIVE INDIVIDUALS

#### Dear volunteer:

I would like to invite you, physical activities practioneer using or not Artepilin-C, to voluntarily participate in a study in which your relevant aspects (inflammatory, nutritional and bloody profiles) will be investigated.

We are available to clarify any questions you may have related to the study during its development. You will participate in this study as subject to the research.

Before expressing your consent for participation, you must read the instructions below. This document is composed of two identical forms, being one with you and another with the researchers. The forms must be signed and initialed in case you consent in the participation.

#### 1. Study Objectives

To investigate relevant aspects of inflammatory, nutricional and bloody profiles in physical activities practioneers using or not Artepelin-C.

#### 2. Evaluation proceedings

After agreeing and signing the form, the following questionnaire will be applied: nutritional recordatory at the first day of the research and another one, which will evaluate your 24-hour diet. EVA questionnaire to evaluate pain and also the recommended training prescription of the stablished protocol must be filled in.

Additionally, some measures related to weight, height, bioimpedance and venous blood collection will be performed by professionals in safe places.

#### 3. Research performance

The research is being conducted by a team of professionals and undergrads in Nutrition, Pharmacy and Physical Education oriented by Professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="mailto:prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinaldi@usjt.br">professors Dr. André Rinaldi Fukushima and Dr. Érico Chagas Caperuto (<a href="prof.rinal

#### 4. Voluntary participation without financial support

This research does not imply in any financial commitment between you and Universidade São Judas Tadeu.

#### 5. Research risks and benefits

The volunteers will not receive any material gain or payment.

You will get as benefits, a complete Artepelin-C treatment (30 capsules) and the anthropometric and bloody diagnostic of your nutritional status.

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The involved risks are minimal considering that the proposed activities, always monitored by the researchers, are not so invasive (blood exams).

Any injury happening with you will be treated at the nearest public hospital. You can give up at any time, not being harmed, no matter the reason.

#### 6. Freedom of refusal and quitting

You can either deny the consent or give up at any time during the research with no harm.

#### 7. Used material for data collection

You will have approximately a two-hour participation. Data collected will be stored under the researchers' responsibility.

#### 8. Guarantee of confidentiality

The results of the study will be used in scientific Works and can be presented during scientific events without volunteers' identification.

#### 9. Material storage and destruction

The collected and analyzed data will be stored and filed for five years, before being destroyed, in a safe place to assure information confidentiality.

In case of participating of the study you must fill in the text below and sign.

I Private Individual Registration number declare having been informed about
the study proceedings and proposals "EFFECTS OF ARTEPELINA-C SUPPLEMENTATION PRESENT IN
PROPOLIS RELATED TO INFLAMMATORY PROCESSES IN PHYSICALLY ACTIVE INDIVIDUALS" and my
signature and participation are of my free will. I am aware of the utilization of the study results in
further publications.
I confirm having received a signed form of this document according to recommendations from
Comissão Nacional de Ética em Pesquisa (CONEP).
(City, and date),
Vonlunteer Signature Researcher signature

## Appendix 4 - Pain scale

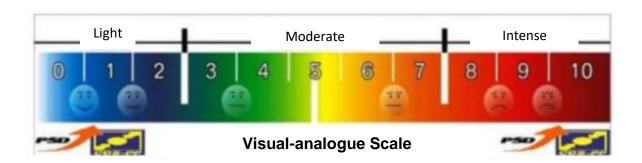
## **Brief pain inventory - BPI**

1-) Most people sometimes have pain (headache, toothache, among others).												
Have you had a different pain today?												
1- ( ) yes 2. ( ) No												
2-) Highlight with an X where you feel pain and where it is more intense												
			fro	ont						hac	:k	
	right	5 34	2 . 6 . 10 . 15	7 8 11 13 14 16 37 17 39 00 41	9 355	eft		lef	1 22 2 42/2	17 18 9 /20 23 24 17 30 9 32 44 46	21 26	right
3-) Circle the I	numk	oer c	orres	spoi	ndin	g to	the	wor	st pa	ain y	ou ha	ave felt the last 24
hours				-		-			•	•		
	0	1	2	3	4	5	6	7	8	9	10	strongest pain
4-) Circle the	num	ber v	vhic	h be	st d	esci	ribes	s the	e we	aker	pain	you have felt the
last 24 hours												
no pain	0	1	2	3	4	5	6	7	8	9	10	strongest pain
5-) Circle the r	numb	er th	at re	pre	sent	s th	e av	erag	je of	you	r pair	1
no pain	0	1	2	3	4	5	6	7	8	9	10	strongest pain
6-) Circle the	numl	ber w	hich	n sh	ows	the	pair	ı yoı	ı are	fee	ling a	t the moment
no pain	0	1	2	3	4	5	6	7	8	9	10	strongest pain

name of the treatment / medicine						ıular requ					Start date	
				frequency								
8-) What improv	emei	nt w	as p	orov	rided	by	trea	tme	nts	or m	edica	ations in the last
24 hours? Circle	e the	per	cen	tage	e wh	ich k	est	imp	rov	ed y	our a	lleviation
no alleviation 0%	10%	20%	6 30°	% 4	0%	50%	60%	6 70	0%	80%	90%	100% total alleviation
9-) In the last 24	hours	s, cir	cle t	he r	numk	er w	hich	bes	t de	scrib	es at	what extent the pair
interfered in your	:											
General Activition	es											
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Humor Mood												
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Walking ability												
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Job												
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Relating with pe	ople											
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Sleepness												
no interference	0	1	2	3	4	5	6	7	8	9	10	total interference
Life enjoyment												

## Appendix 5 – Visual Analogue Scale (VAS)

## Please, you must inform the intensity of the pain according to Visual Analogue Scale



Intensity =		
/		

Date

Signature and doctor's stamp

## Appendix 6 - Borg Scale adapted. Perceived exertion is correct



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## **Appendix 7 – Training Program**

## High intensity training program

Workout	Weight	Series	Repetitions	INTERVAL	Frequency/No Frequency
race		8 a 12	60" seconds	75" seconds	3 times
Barbell Training	75% ↓	5	15 a 6		5
Tríceps head bar	75% ↓	5	15 a 6		5
Inclined bench press (machine)	75% ↓	5	15 a 6		5
Side elevation (halter)	75% ↓	5	15 a 6	2 minutes	5
Leg curl	75% ↓	8 a 12	15 a 6		5
Squat	75% ↓	8 a 12	15 a 6		5
Leg press	75% ↓	8 a 12	15 a 6		5
Leg Extension	75% ↓	8 a 12	15 a 6	2 minutes	5