Natural Resources for Human Health

visaga publishing house

Original Research

View Article Online

Check for updates

Received 21 June 2022
Revised 29 July 2022
Accepted 29 July 2022
Available online 14 September 2022

Edited by Onur Bender

KEYWORDS:
Indigo
toxicity
body composition
forced swimming test
endurance

Natr Resour Human Health 2023; 3 (1): 86-93
https://doi.org/10.53365/nrfhh/152508
eISSN: 2583-1194
Copyright © 2023 Visagaa Publishing House

Effects of *Indigofera suffruticosa* Miller (Wild indigo) leaves aqueous extract subacute supplementation in mice

Rebeca Xavier Cunha [1], Weber Melo Nascimento [1], Joao Ricardhis Saturnino Oliveira [1], Alvaro Nobrega Melo Madureira [1], Ana Paula Sant'Anna Silva [1], Vera Lucia Menezes Lima [1,*]

[1]Laboratório de Lipídios e Aplicações de Biomoléculas em Doenças Prevalentes e Negligenciadas. Departamento de Bioquímica, Centro de Biociências, Universidade Federal de Pernambuco, Avenida Professor Moraes Rego, 1235, CEP 50670-90 Recife, PE, Brazil

ABSTRACT: Supplementation with plant-based products is becoming more popular recently. This change in the supplementation market is related to larger benefits found in natural products, and fewer risks for chronic side effects found in synthetic products. Extracts rich in antioxidant properties and plants used by folk medicine are the main target to become a botanical supplement. *Indigofera suffruticosa* leaves aqueous extract (IsAE) was supplemented to mice diet for eleven days (50 mg/kg and 100 mg/kg, p.o.) to assess subacute toxicity and potential benefits. Body parameters were evaluated before and after supplementation. On the tenth day, mice were submitted to a forced swimming test to verify endurance. Lipids and glucose metabolism were also evaluated. During supplementation, no toxicity was observed. A mild decrease in body fat and weight was observed. There was no difference in swimming time among groups, but a significant increase was found for group 100 mg/kg after normalizing time by body weight. IsAE induces mild decrease (not statistically significant) in lipids and glucose levels. Thus, low doses of IsAE seem to be safe for usage, in the tested condition. In addition, 100 mg/kg of IsAE has the potential to improve endurance and might modulate fat and lipid concentrations of healthy mice in long-term supplementation.

1. INTRODUCTION

Supplements have gained the attention of those that are trying to improve their quality of life through nutrition (Bailey et al., 2003). Global market of dietary supplements were estimated at USD 140.4 billion in 2020 (GVR, 2021a), and 81 % of U.S. people over 55 years of age reported dietary supplement usage in the same year (CRN, 2020). Although there is no global consensus on how to define and regulate dietary supplements, they are products that are meant to supplement diets, and some of them may also have health-improving effects because they are not regarded to be medications and are not intended to treat illnesses (Dwyer et al., 2018). Each country has specific components that can be included in dietary food supplements, but in general they can be vitamins, minerals, carbohydrates, plant nutrients, bioactive substances, enzymes, and probiotics (ANVISA, 2018).

Among dietary supplements, botanical dietary supplements (BDS) are awakening a great interest, reaching USD 5.26 billions in market value in 2017, globally GVR (2021b), and 44 % of Americans reported supplementation with botanical or herbal components in 2020 (CRN, 2020). Often, BDS is presented as plant products in the form of fluid extract, dry extract powders, decoctions, infusions, tinctures, syrups, herbal tea bags, plant powders, granules, capsules, tablets, and oils (WHO, 2018). Since there may be antagonism between bioactive components in mixtures, isolated molecules from plants can occasionally be sold in place of a complex mixture such as an extract, but they may overlook potential synergisms (Caesar & Cech, 2019).

BDS have different health-related potential and nutritional benefits that depend on the daily intake and the amount of the botanical bioactive compound ingested, which provide different degrees of effectiveness and can influence their safety (Krochmal et al., 2004). Therefore, these dietary supplements should be scientifically tested to determine their safety and quality as well as their health benefits (Van Breemen et al., 2008). According to the National Toxicology Program of the U.S. Department of Health and Human Services, six botanical dietary supplements are now the subject of investigations; one of them is the *Usnea*

* Corresponding author.
E-mail address: lima.vera.ufpe@gmail.com (Vera Lucia Menezes Lima)

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

lichen, which is frequently used for weight loss. Twelve more have been the subject of study; one substance is the popular anxiety-relieving herb Kava Kava (NTP, 2020). Obesity (Choi et al., 2020), oxidative stress (Hung et al., 2012), abnormal lipid profiles and glycemia (Eleazu et al., 2018), fatigue (C.-S. Chen et al., 2016), and inflammation (Rosillo et al., 2012) are a few health-related disorders that the ongoing use of BDS seems to resolve.

Indigofera suffruticosa Mill., popularly known as "wild indigo", "anil" or "anileira", is a member of Fabaceae family, and was originally found in Tropical America regions (Matos et al., 2011; Paulino et al., 2010). In Brazil, it is found in all states (Do, 2021). *I. suffruticosa* is used in popular medicine as antispasmodic, antiepileptic, purgative, antipyretic, sedative (Hastings, 1990), in colic and kidney diseases (Matos, 1999), as a stomach enhancer and diuretic (Agra et al., 2007). Studies proved that it has anti-inflammatory activity (Leite et al., 2003; Nascimento et al., 2022), antioxidant (Arriaga et al., 2013), chemopreventive (C.C. Chen et al., 2013), and gastroprotective (Luiz-Ferreira et al., 2011). Although there is scientific proof for some of the health advantages of *I. suffruticosa*, studies on the impact of supplementing a regular diet are lacking (Campos et al., n.d.). Thus, this study aimed to evaluate the subacute toxicity and behavior of health-related parameters in mice supplemented with *I. suffruticosa* leaves aqueous extract for 11 days.

2. MATERIALS AND METHODS

2.1. Collection of plant and preparation of *I. suffruticosa* aqueous extract (IsAE)

Indigofera suffruticosa Miller was collected in São Caetano, Pernambuco, Brazil (Latitude: 8° 19' 33" S; Longitude: 36° 04' 21" W). Plant was identified by Dr. Marlene Carvalho de Alencar Barbosa from the Department of Botany - UFPE (Federal University of Pernambuco) and a specimen was deposited in the herbarium UFP - Geraldo Mariz (number 45,217), at UFPE. In addition, there is a registration in the National System to Traditional Knowledge and Genetic Heritage (SisGen, Brazil) under the identification A149769. Leaves were separated and dried in a hot-air oven at 38 ° C for 72 hours. After drying, leaves were crushed in an industrial blender (Metalurgic Vithory Ltda., Brazil) to obtain the powder. *I. suffruticosa* leaves powder (50g) was mixed with 200 ml of distilled water (1:4), and left under constant stirring for 16 hours. These were then filtered with the assistance of a vacuum pump (Exipump, Brazil) and concentrated by freeze-drying (Liotop, Brazil).

2.2. Oral supplementation

The supplementation test was performed according to the previous supplementation methodology with some modifications (Araújo et al., 2016) and approved by the UFPE Animals Use Ethics Committee (CEUA) (protocol: 23076.042305/2017-67). Three experimental groups of six adult mice male albino Swiss (12-14 weeks) were obtained from Laboratório de Imunopatologia Keizo Asami (LIKA). After the one-week acclimatization period, they orally received 100 μl of water containing 50 mg/kg of IsAE, 100 μl of water containing or 100 mg/kg of IsAE, or just 100 μl of water (control group) for eleven days with the support of gavage needle (p.o.). This dose was established according to previously unpublished works regarding the acute toxicity of *I. suffruticosa* performed in our laboratory. During this period, animals were in a vivarium under ideal conditions of temperature (22 $\pm$ 2 °C), light cycles of 12h light/ 12h dark, and suitable humidity (55 $\pm$ 5 %), besides water and standard rodent food (Presence, Neovia group, Brazil) ad libitum.

2.3. Subacute toxic analysis

During eleven days of supplementation, mice behavior and any signal of toxicity or death were observed accordingly to Organization for Economic Co-operation and Development (OECD, 2001). Motor alterations, reflex loss, breathing alterations, contortions, piloerection, bleeding, edema, aggressiveness, or enervation were observed and recorded on all days for all groups.

2.4. Body parameters

Before administrations, animals were weighed with an analytical digital balance (BEL Engineering® s.r.l., Italy) and measured their nose to root tail length with a measuring tape (expressed in centimeters). On the tenth day of supplementation, data was collected again. With the initial and final weight and length of each animal, body mass index (BMI) was calculated as following the formula below and expressed in grams per cubic centimeter (g/cm^2).

$$BMI = \frac{Mass}{Length\ (nose\ to\ root\ of\ tail)^2}$$

2.5. Forced swimming test

Mice were put through a forced swimming test on the tenth day, an hour after receiving oral IsAE, following a previously published methodology with some modifications, following weight evaluation (Can et al., 2012). Mice's tails accounted for 10 % of their entire body weight. Individual mice were placed on a recipient that measured 35 centimeters in height by 25 centimetres in diameter and filled with water to a depth of 18 centimeters. The swimming time was recorded until the mice could not return to the water's surface for 5 seconds. At this point, mice were eliminated from the recipient and dried in a warm towel. We estimated the mean forced swimming time (minutes) and standardized it to final body weight.

2.6. Blood collection and biochemical analysis

Blood samples were collected via retro-orbital plexus using a capillary without anticoagulant after 24 hours of the forced swimming test. After the collection, blood was centrifuged at 550xg for 10 minutes (FANEM® Ltda., Brazil) and the serum was separated for biochemical quantification (B.S. Santos et

al., 2009). Parameters analyzed were total cholesterol (TC), triglycerides (TG), cholesterol HDL (HDL-c) and non-HDL-c cholesterol [LDL cholesterol (LDL-c), and cholesterol VLDL (VLDL-c)] and glucose by commercial in vitro diagnostic kit (Labtest Diagnóstica S.A., Brazil) and expressed in mmol/l. VLDL-c was calculated using TG values, and LDL-c according to Friedewald formula, as follows:

$$VLDL - c = \frac{TG}{5}$$

$$LDL - c = TC - (HDL - c + VLDL - c)$$

Values of LDL-c, total cholesterol, and HDL-c were used for calculating Castelli index 1 and 2 as follows in the formulas below:

$$Castelli\ 1 = \frac{TC}{HDL - c} \quad Castelli\ 2 = \frac{LDL - c}{HDL - c}$$

2.7. Analysis of body fat quantity

Anesthetized animals were submitted to surgery, in which the perirenal, peritoneal, and gonadal fats were removed and weighed in an analytical digital balance (BEL Engineering® s.r.l., Italy). After this procedure, animals were euthanized. Besides the mean of quantity of this body fats, the percentage of body fat in respect to the final body weight was also calculated.

2.8. Statistical analysis

All the result were presented as a mean ± SEM. GraphPad PRISM® 5 (GraphPad Software Inc., USA) was used to assess the significance between the groups using one-way analysis of variance (ANOVA), followed by Bonferroni post-test. For statistical significance, $p < 0.05$ was considered.

3. RESULTS AND DISCUSSION

Supplementation of aqueous extract of *I. suffruticosa* leaves for 11 days can also assess its subacute toxicity. During these days, none of the animals showed any visible signs of toxic effects, and death was not recorded, which shows that 50 mg/kg and 100 mg/kg of leaves aqueous extract by oral administration does not have subacute toxicity, or lethality.

Botanical dietary supplements are emerging as a viable natural option in the daily dietary complement, due to their widespread reputation for being safer, more affordable, and having a huge diversity of phytochemicals that benefit health (Raskin et al., 2002; Van Breemen, 2015). *I. suffruticosa* is known for its toxicity when the whole plant is ingested, or when its aerial parts (*in natura*) are served ad *libitum* or orally forced in bovines, sheeps and goats. The most reported signals of toxicity are anemia, hemoglobinuria, and kidney and liver damage (Assis et al., 2009; Figueiredo et al., 2012; Neto et al., 2001; I. Salvador et al., 2010; D.M. Silva et al., 2006). Aqueous fruit extract, intraperitoneally administered, showed liver damage with a significant number of cells with aberrations in Balb C mice (Ribeiro et al., 1991). Leaves and branches (*in natura*) daily administered to guinea pigs (for 15 days) in all the animals presented apathy and blue urine and anemia. Necropsy revealed liver damage and authors associated the haemolityc anemia due to a possible presence of aniline (I.S. Salvador et al., 2011).

However, when intraperitoneally administred (for 7 days), IsAE not only treated sarcoma 180 infected mice, but also during the treatment time did not affect renal and hepathic morphology, indicating its safety use as a anticancer agent (Santana et al., 2015; I.B. Silva et al., 2014; Vieira et al., 2007). In liver, leaves methanolic extract demonstrated a hepatoprotective effect on paracetamol-induced liver damage and also indigan (a purified compound from *I. suffruticosa*) do not showed toxicity to liver when administred intraperitoneally (Lima et al., 2019, 2014).

The security of IsAE as a potential BDS has been assessed and its subacute toxicity results corroborated with previous *in vitro* toxicity tests that showed that different extractions types and parts of the plant *I. suffruticosa* did not demonstrate high toxicity, and *in vivo* tests demonstrated only low signs of toxicity, but when intraperitoneally administered, which suggested that some possible toxic compounds are metabolized during digestion (T.M.S. Silva et al., 2019; Vieira et al., 2007). As BDS are commonly ingested, the present and previous studies add important information on the safe use of IsAE for its multiple purposes.

Results of initial and final body weight of the mice in supplementation with aqueous extract of *I. suffruticosa* are shown in Table 1. There is no significant difference in weight in these two moments. Mice Body Mass Index (BMI) in the two moments also does not show a significant difference between groups.

As shown in a study that administered *Rhodiola rosea* aqueous and fermented extract for 15 days at a concentration of 1.5 g/kg body weight (Kunming mice), the results of which were different from those of this study in that they showed an increase in body weight over time, but there was no statistically significant difference between the groups (Kang et al., 2015). Another study evaluated a 20 days supplementation (Kunming mice) with aqueous extract of *Millettia speciosae* rhizomes, which also showed an increase in the body weight over time in doses of 500, 1,000, and 2,000 mg/ kg, but not statistically significant when compared to the control group. This indicates that unlike *I. suffruticosa* aqueous extract, aqueous extract can increase body weight (Zhao et al., 2015). In a longer supplementation time (28 days), it was seen a gain of weight after administration of *Anisomeles indica* whole plant aqueous extract, however, this was also not statistically significant when compared to the control group in doses of 125 mg/ kg, 250 mg/ kg, and 500 mg/ kg (C.-S. Chen et al., 2016). Even in administrations for longer periods and at higher doses, the weight of the animals seems to not significaty change, however, these studies did not evaluate the animals body fat and also did not determine the fat quantity percentage, as can be seen in the previous review, methodological and study coverage differences to assess body weight reduction can reveal different levels of potential (Ríos-

Table 1
Initial and final body weight and BMI of mice inIsAE oral supplementationfor 10 days.

	Control (n=6)	50 mg/kg IsAE (n=6)	100 mg/kg IsAE (n=6)	p-Value
Initial body weight (g)	38.75 ± 1.31	38.67 ± 2.97	40.67 ± 1.82	0.289
Final body weight (g)	38.00 ± 1.15	37.08 ± 2.19	38.00 ± 1.22	0.738
Initial BMI (g/cm^2)	0.35 ± 0.01	0.38 ± 0.02	0.35 ± 0.01	0.564
Final BMI (g/cm^2)	0.39 ± 0.01	0.41 ± 0.03	0.42 ± 0.03	0.330

Data expressed as mean ± SEM. Statistical analysis was performed with two-way ANOVA followed by Bonferroni's test.

Table 2
Fat concentration in mice after IsAE supplementation for 11 days (p.o).

	Control (n=6)	50 mg/kg IsAE (n=6)	100 mg/kg IsAE (n=6)	p- Value
Perirenal fat (mg)	649.33 ± 52.80	606.17 ± 98.85	473.33 ± 63.00	0.367
Peritoneal fat (mg)	1105.17 ± 212.47	1452.17± 221.68	1142.17 ± 183.37	0.915
Gonadal fat (mg)	160.50 ± 28.58	61.33 ± 15.82*	59.00 ±8.08*	**0.040**
Fat quantity (%)	5.12 ± 0.85	5.60 ± 0.64	4.34 ± 0.59	0.700

Data expressed as mean ± SEM. *compared to control, statistical analysis was performed with two-way ANOVA followed by Bonferroni's test.

Hoyo & Gutiérrez-Salmeán, 2016).

Body fats analysis is shown in Table 2, no significant difference between the treated groups and control was found for perirenal and peritoneal fat, only gonadal fat weight had a significant decrease for both dosages. Besides that, when the fat percentage was calculated, it was not possible to perceive a significant difference between the groups. Even if there is no significant difference, the results show a mild decrease in all fat counts (Table 2).

During the supplementation period with aqueous extract of *I. suffruticosa*, body weight, body fat, and fat quantity percentage could be gradually decreased (not significant, except for gonadal fat), this may be due to an increase in the energy expenditure, to an appetite suppressant, to lipase inhibitor effect, or due to a regulation in lipid metabolism and the differentiation of adipocytes, as previously summarized in a review as the possible mechanisms of anti-obesity (Sun et al., 2016). It is observed in this study that a reduction in body weight and fat is reflected in an increase in swimming time, especially when this increase is normalized by the animals final weight.

As shown in Fig 1a, there is an increase in swimming time as IsAE concentration increases, but this difference was not significant. However, when the swimming time was normalized by the final animal weight as shown in Fig.1b, significant differences were found between the control group and the animals in the 100 mg/kg IsAE group.

Many studies did not find prolonged swimming times even when the administration period and the dose were greater or when the weight placed in its tail was smaller. Administering aqueous and fermented extract of *R. rosea* (1.5 g/ kg body weight for 15 days) and submitting the mice to swimming (7 % of body weight in the tail), researchers found 197.2 ± 41.85 s (approximately 3.29 minutes) as the longest swimming time. The antifatigue effect was associated with the antioxidant activity, increase in serum lactate dehydrogenase and decrease in blood urea, nitrogen, and lactic acid observed, mainly for the fermented extract (Kang et al., 2015). Another study, revealed swimming time similar (81.3 ± 8.3 min) to our dose of 100 mg/ kg on the 28th day of supplementation with ethanolic extract of *Rubus coreanus* in a dose of 1 g/ kg and performing the swimming test without load (ICR mice). A decrease in lactate, better use of energy sources like glycogen, increase in fat use, and oxidative stress protection were the main factors associated with this activity (You et al., 2015). And another study did not find a swimming time (with 5 % or 10 % body weight in the tail in ddY mice) bigger than 300 seconds (5 min) for 10 % of load and 25 minutes for 5 % load in doses of 150 and 300 mg/ kg body weight of *Trigonella foenum* graecum (Fenugreek) seed ethanolic extract in 4 weeks of administration. The increase in fatty acids utilization as an energetic source and increase in hepatic and muscle glycogen were the main explanations for this effect (Ikeuchi et al., 2006). In twenty days of supplementation with *M. speciosae* aqueous extract, it was not found swimming time (with 5 % body weight in the tail of Kunming mice) was higher than 60 minutes in any doses (500, 1,000, and 2,000 mg/ kg body weight). *M. speciosae* aqueous extract supplementation showed to decrease triglycerides, improve fat metabolization, delay blood urea nitrogen (BUN) accumulation, decrease creatine phosphokinase (CK), and improvement of glycogen in the muscle (Zhao et al., 2015). The highest swimming time value for the highest dose (500 mg/ kg) of another study was 692.5 ± 96.8 s (approximately 11.54 minutes) in 28 days of administration of *A. indica* aqueous extract for Balb/c female mice. Their results are attributed to the decrease in plasma triglycerides and ammonia levels and an increase in liver and muscle glycogen (C.-S. Chen et al., 2016). However, no study performed normalization for the forced swimming time by the animal final weight, which is important because the animal weight influence the swimming performance, and this influence is discounted after normalization (Bogdanova et al., 2013).

In this study, specific exercise-related analytes or pathways were not tested, but the trend for reduction in lipids and glucose, even though not significant, and the reduction in the fat quantity (significant for gonadal fat) may be a signal that IsAE supplementation can improve exercise endurance in the forced swimming test through consuming of energy sources to sustain the extensive exercise, as seen in previous studies (Ikeuchi et al., 2006; You et al., 2015). *I. suffruticosa* already demonstrated antioxidant activity (Arriaga et al., 2013) and anti-inflammatory properties (Leite et al., 2003), these two activities are essential to enhance exercise performance as reported by other botanical supplements (Harty et al., 2019). Thus, IsAE supplementation can be acting scavenging reactive species and controlling the exercise-related inflammation generated during strenuous exercise as reported for other botanical supplements, attenuating muscular fatigue and improving animal performance during swimming.

Evaluating the lipid and glucose metabolism, significant differences in lipid profile and glucose levels between the groups tested were not observed. Only a mild lowering activity (not significant) in triglycerides, total cholesterol, and glucose was observed. Determination of Castelli 1 and 2 indexes also corroborate with those results (Table 3).

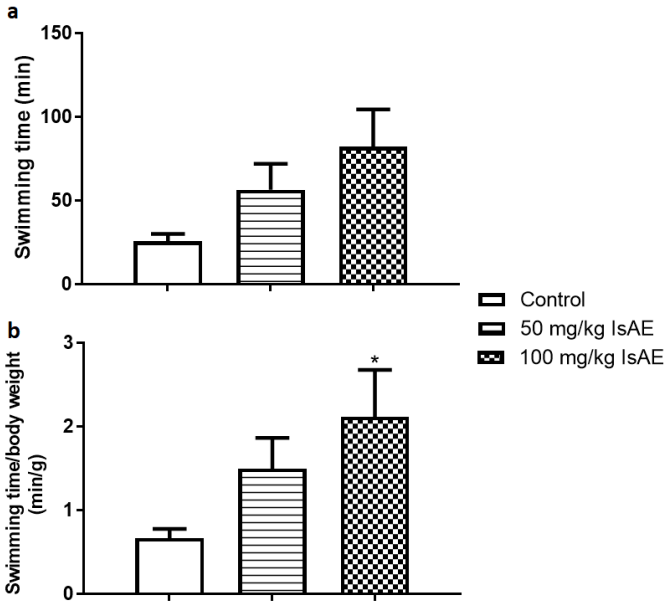

Figure 1. Results of swimming forced test of mice after IsAE oral administration for 10 days. (a) crude values; (b) time of swimming normalized for the body weight of mice. Results are expressed as mean ± SEM, n = 6. *p<0.05 compared to control, one-way ANOVA followed by Bonferroni's test.

Evaluation of biochemical indicators following daily dosing did not appear to significantly affect the animals metabolism, indicating safety. A mild lowering activity (not significant) in triglycerides, total cholesterol, and glucose was observed. Similar results occurred in previous studies, in Wistar rat supplementation for 21 days with *Cornus mas* hydro-methanolic extract intraperitoneally administered (doses of 50, 200, and 400 mg/kg) found a not significant decrease in the same analytes of the present study and a mild increase and decrease (also not significant) in the HDL-c and TC, respectively (Abdollahi et al., 2014). In a more recent study supplementing for 28 days with the ethanol extract of boiled *Treculia africana* seed there was not significant difference in the lipid profile of Wistar rats supplemented with 100 and 200 mg/kg of the extract, additionally was found an increase in the LDL-c in only the dose of 100 mg/kg and no glycemic status improvement (Eleazu et al., 2018). In a high-cholesterol diet supplemented for 4 weeks with *Ajuga iva* boiled aqueous extract (0.5 % in the diet) a significant improvement in the lipid profile and antioxidant status was observed (Chenni et al., 2007). Also, significant improvement in lipid, glucose and antioxidant status was found in supplementation with 1% of *Lepidium meyenii* (Maca) in a high sucrose diet (hereditary hypertriglyceridemic rats) for 2 weeks Večeřa et al. (2007). There is a clear relationship between antioxidant properties and the improvement of glucose and lipid metabolism that these botanical supplements have, combating oxidative stress, with some of them even testing the antioxidant status to determine this relationship as seen in some studies that tested antioxidant enzymes and lipid peroxidation status (Chenni et al., 2007). Večeřa et al. (2007).

The not significant changes in weight (Table 1), visceral fats (Table 2), and biochemical parameters (Table 3) show that IsAE is not toxic when daily administered for a short period of time. It is possible that IsAE optimized energy expenditure and maintainance of life basic processes, because of IsAE seemed to not add significant caloric deposits, nor glycemic elevations as can be seen in Table 3, but improved exercise (Figure 1) possibly due to the improvement of the glycogen metabolism and, as the forced-swimimng time is an extenuant exercise test, IsAE might enhance lipid pathways to provide energy (Hanhineva et al., 2010; Pérez-Torres et al., 2021).

Indigofera suffruticosa has already some of its phytochemical constituents clarified, showing different classes of fatty acids found in leaves ethanolic extract (Vijisaral Elezabeth & Arumugam, 2014), acyclic diterpene alcohol (phytol), methyl linoleate, monoterpenes, sesquiterpenes, phenylpropanoids as main constituent in leave oil (Arriaga et al., 2013; T.M.S. Silva et al., 2019), alkaloids, flavonoids, phenylpropanoids, triterpenoids and volatile oils in different degrees of presence in ether, chloroform and acetone leaves extracts (A. Santos et al., 2015), aqueous and ethanolic extracts that showed presence of various phenolic compounds (C.-S. Chen et al., 2016) , and also saponins, triterpenes, coumarins and flavonoids (Govín et al., n.d.). Some of these phytochemicals are well known to directly or indirectly influence in the same health-related activities observed in this study.

4. CONCLUSIONS

Results of this study suggest that low doses of IsAE are safe for usage. IsAE seems to have the potential to diminish fat in a short period, and body weight in a longer supplementation period,

Table 3
Lipid andglucose profile of mice after IsAE oral administration for 11 days.

	Control	50 mg/kg IsAE	100 mg/kg IsAE	p-Value
Cholesterol total (mmol/l)	1.64±0.06	1.96±0.17	1.57±0.17	0.079
Triglycerides (mmol/l)	1.00±0.09	1.02±0.19	0.97±0.03	0.958
HDL-c (mmol/l)	1.12±0.11	1.16±0.11	0.91±0.17	0.387
Non- HDL-c (mmol/l)	0.40±0.08	0.80±0.14	0.66±0.10	0.060
Castelli 1	1.47±0.12	1.73±0.19	2.77±1.15	0.471
Castelli 2	0.29±0.12	0.55±0.16	1.28±0.85	0.471
Glucose (mmol/l)	8.03±0.41	7.92±0.43	7.43±0.47	0.602

Data expressed as mean ± SEM. Statistical analysis was performed with two-way ANOVA followed by Bonferroni's test.

and this does not disturb the homeostasis of analytes related to energetic metabolism, but pathways are still not clear. For muscle function, IsAE enhances muscle capacity for endurance tasks, and this might be due to the reduction of oxidative stress. Therefore, future studies should be focused on determining the precise mechanism of body weight and fat reduction. Thus, besides its popular medicinal use, IsAE seems to be a promising supplement.

CONFLICTS OF INTEREST

The authors declare no competing interests.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES)-Finance code 001. Also, we thank Dr. Marlene Carvalho de Alencar Barbosa for giving support during the protocol for plant identification, and Albérico Real do Espírito Santo Filho for his technical help in this study.

ORCID

Rebeca Xavier Cunha	0000-0003-2550-4259
Weber Melo Nascimento	0000-0001-8839-7310
Joao Ricardhis Saturnino Oliveira	0000-0002-5024-8253
Alvaro Nobrega Melo Madureira	0000-0001-5664-7442
Ana Paula Sant'Anna Silva	0000-0003-4189-5253
Vera Lucia Menezes Lima	0000-0003-2581-9218

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ETHICAL APPROVAL

All the experiments had approbation by UFPE Committee ofEthics and Research with animal (Protocol: 23076.042305/2017-67).

AUTHOR CONTRIBUTIONS

R. X. da C., performed the experiments, designed the project and wrote the manuscript; W. M. N., J. R. S. de O. and A. N. de M. M. performed the experiments; A. P. S da S. and V. L. de M. L. designed the project and revised the final manuscript.

REFERENCES

Abdollahi, B., Abbasi, M.M., Milani, P.Z., Nourdadgar, A.S., Khojasteh, S.M.B., Nejati, V., 2014. Hydro-methanolic extract of *Cornus mas* L. and blood glucose, lipid profile and hematological parameters of male rats. Iranian Red Crescent Medical Journal. 16, e17784. https://doi.org/10.5812/ircmj.17784

Agra, M.F., Baracho, G.S., Nurit, K., Basílio, I.J.L.D., Coelho, V.P.M., 2007. Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. Journal of Ethnopharmacology. 111, 383–395. https://doi.org/10.1016/j.jep.2006.12.007

ANVISA., 2018. Instrução Normativa - IN no 28, de 26 de julho de 2018. http://antigo.anvisa.gov.br/documents/10181/3898888/IN_28_2018_COMP.pdf/db9c7460-ae66-4f78-8576-dfd019bc9fa1. Date accessed: 2021-07-09

Araújo, E.S., Garcia, R.S., Dambrós, B., Pieniz, S., Schneider, A., Abib, R.T., 2016. Impact of vitamin C supplementation on lipid peroxidation and reduced glutathione levels in the liver tissue of mice with cyclophosphamide-induced immunosuppression. Revista de Nutrição. 29, 579–587.

Arriaga, A.M.C., Lemos, T.L.G., Santiago, G.M.P., Andrade-Neto, M., Braga, M.A., Almeida, M.C.S., De, Gomes, T.B.M., Rodrigues, F.E.A., Vasconcelos, J.N., Alves, P.B., 2013. Chemical composition and antioxidant activity of *Indigofera suffruticosa*. Chemistry of Natural Compounds. 49, 150–151. https://doi.org/10.1007/s10600-013-0539-7

Assis, T.S., Medeiros, R.M.T., Araújo, A.S., De, Dantas, A.F.M., Riet-Correa, F., 2009. Intoxicações por plantas em ruminantes e equídeos no Sertão Paraibano. Pesquisa Veterinária Brasileira. 29, 919–924. https://doi.org/10.1590/S0100-736X2009001100010

Bailey, R.L., Gahche, J.J., Lentino, C.V., Dwyer, J.T., Engel, J.S., Thomas, P.R., Betz, J.M., Sempos, C.T., Picciano, M.F., 2003. Dietary supplement use in the United States, 2003-2006. The Journal of Nutrition. 141, 261–266. https://doi.org/10.3945/jn.110.133025

Bogdanova, O.V., Kanekar, S., D'anci, K.E., Renshaw, P.F., 2013. Factors influencing behavior in the forced swim test. Physiology and Behavior. 118, 227–239. https://doi.org/10.1016/j.physbeh.2013.05.012

Caesar, L.K., Cech, N.B., 2019. Synergy and antagonism in natural product extracts: When 1 + 1 does not equal 2. Natural Product Reports. 36, 869–888. https://doi.org/10.1039/C9NP00011A

Campos, J.K.L., Araújo, T.F., Da, S., Brito, T.G., Da, S., Silva, A.P.S., Da, Cunha, R.X., Da, Martins, M.B., Silva, N.H., Da, Santos, B.S., Silva, C.A., Da, Lima, V.L., De, M., n.d..

Can, A., Dao, D.T., Arad, M., Terrillion, C.E., Piantadosi, S.C., Gould, T.D., 2012. The mouse forced swim test. Journal of Visualized

Experiments. 59, 1–5. https://doi.org/10.3791/3638
Chen, C.C., Liu, C.S., Li, C.C., Tsai, C.W., Yao, H.T., Liu, T.C., Chen, H.W., Chen, P.Y., Wu, Y.L., Lii, C.K., Liu, K.L., 2013. *Indigofera suffruticosa* Mill extracts up-regulate the expression of the π class of glutathione S-transferase and NAD(P)H: Quinone oxidoreductase 1 in rat Clone 9 liver cells. Food and Chemical Toxicology. 59, 610–617.
Chen, C.-S., Wang, M.-L., Liu, R., Chen, S.-P., Lu, T.-M., Tsai, W.-Y., Huang, C.-F., Yang, C.-C., Tzeng, Y.-M., 2016. Anti-fatigue effect of aqueous extract of *Anisomeles indica* (L) Kuntze in mice. Tropical Journal of Pharmaceutical Research. 15, 489–495. https://doi.org/10.4314/tjpr.v15i3.9
Chenni, A., Yahia, D.A., Boukortt, F.O., Prost, J., Lacaille-Dubois, M.A., Bouchenak, M., 2007. Effect of aqueous extract of *Ajuga iva* supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high-cholesterol diet. Journal of Ethnopharmacology. 109(2), 207–213. https://doi.org/10.1016/j.jep.2006.05.036
Choi, H.-I., Lee, D.-H., Park, S.-H., Jang, Y.-J., Ahn, J., Ha, T.-Y., Jung, C.H., 2020. Antiobesity effects of the combination of *Patrinia scabiosaefolia* root and *Hippophae rhamnoides* leaf extracts. Journal of Food Biochemistry. 44(6), e13214. https://doi.org/10.1111/jfbc.13214
CRN., 2020. 2020 CRN Consumer survey on dietary supplements: Consumer intelligence to enhance business outcomes. https://www.crnusa.org/resources/2020-crn-consumer-survey-dietary-supplements-consumer-intelligence-enhance-business. Date accessed: 2021-07-09
Do, B.F., 2021. *Indigofera suffruticosa* Mill. in Flora do Brasil 2020 under construction. http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB22980. Date accessed: 2021-08-21
Dwyer, J.T., Coates, P.M., Smith, M.J., 2018. Dietary supplements: Regulatory challenges and research resources. Nutrients. 10, 41–41. https://doi.org/10.3390/nu10010041
Eleazu, K., Maduabuchi, P., Eleazu, C., 2018. Effect of ethanol extract of boiled breadfruit (*Treculia africana*) seed on the oral glucose tolerance, lipid profile, and body weight of normoglycemic albino rats. Food Science and Nutrition. 6, 904–911. https://doi.org/10.1002/fsn3.626
Figueiredo, A.P.M., Medeiros, R.M.T., Dantas, F.P.M., Leite, A.L.D., Fighera, R.A., Riet-Correa, F., 2012. Intoxicação experimental por *Indigofera suffruticosa* em caprinos e ovinos. Pesquisa Veterinária Brasileira. 32, 126–130. https://doi.org/10.1590/S0100-736X2012000200006
Govín, E.S., Lamas, A.M.P., Figueredo, D.C., Ferradá, C.A.R., Guerrero, M., Arias, M.R., n.d..
GVR., 2021a. Dietary supplements market size, share &trends analysis report by ingredient (Vitamins, proteins &amino acids), by form, by application, by end user, by distribution channel, and segment forecasts, 2021 - 2028. https://www.grandviewresearch.com/industry-analysis/dietary-supplements-market. Date accessed: 2021-07-09
GVR., 2021b. Herbal supplements market size, share &trends analysis report by product (Turmeric, Echinacea), by formulation (Capsules, powder and granules), by consumer (Pregnant women, adults), and segment forecasts, 2018 - 2025. https://www.grandviewresearch.com/industry-analysis/herbal-supplements-market. Date accessed: 2021-07-09
Hanhineva, K., Törrönen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkänen, H., Poutanen, K., 2010. Impact of dietary polyphenols on carbohydrate metabolism. International Journal of Molecular Sciences. 11, 1365–1402. https://doi.org/10.3390/ijms11041365
Harty, P.S., Cottet, M.L., Malloy, J.K., Kerksick, C.M., 2019. Nutritional and supplementation strategies to prevent and attenuate exercise-induced muscle damage: Abrief review. Sports Medicine - Open. 5, 1–17. https://doi.org/10.1186/s40798-018-0176-6
Hastings, R., 1990. Medicinal legumes of Mexico: Fabaceae, Papilionoideae, part one. Economic Botany. 44, 336–342. https://doi.org/10.1007/BF03183915
Hung, G.D., Li, P.C., Lee, H.S., Chang, H.M., Chien, C.T., Lee, K.L., 2012. Green tea extract supplementation ameliorates CCl4-induced hepatic oxidative stress, fibrosis, and acute-phase protein expression in rat. Journal of the Formosan Medical Association. 111, 550–559. https://doi.org/10.1016/j.jfma.2011.06.026
Ikeuchi, M., Yamaguchi, K., Koyama, T., Sono, Y., Yazawa, K., 2006. Effects of fenugreek seeds (*Trigonella foenum* greaecum) extract on endurance capacity in mice. Journal of Nutritional Science and Vitaminology. 52, 287–292. https://doi.org/10.3177/jnsv.52.287
Kang, D.Z., Hong, H.D., Kim, K.I., Choi, S.Y., 2015. Anti-fatigue effects of fermented *Rhodiola rosea* extract in mice. Preventive Nutrition and Food Science. 20, 38–42. https://doi.org/10.3746/pnf.2015.20.1.38
Krochmal, R., Hardy, M., Bowerman, S., Lu, Q.Y., Wang, H.J., Elashoff, R., Heber, D., 2004. Phytochemical assays of commercial botanical dietary supplements. Evidence-Based Complementary and Alternative Medicine. 1, 305–313. https://doi.org/10.1093/ecam/neh040
Leite, S.P., Silva, L.L.S., Da, Catanho, M.T.J.A., Lima, E.O., Lima, V.L., De Meneses., 2003. Atividade Antiinflamatória do extrato de *Indigofera suffruticosa*. Revista Brasileira de Ciências da Saúde. 7, 47–52.
Lima, I.R., Silva, I.B., Lima, R.M.L., Silva, T.M.S., Maia, M.B.S., Leite, S.P., 2019. Hepatoprotective efficacy of methanolic extract of *Indigofera suffruticosa* (Mill) on paracetamol-induced liver damage in mice. Arquivos de Gastroenterologia. 56, 333–338. https://doi.org/10.1590/s0004-2803.201900000-62
Lima, I.R., Vieira, J.R.C., Silva, I.B., Leite, R.M.P., Maia, M.B.S., Leite, S.P., 2014. Indican from Añil (*Indigofera suffruticosa* Miller) as an herbal protective agent to the liver. Analytical and Quantitative Cytopathology and Histopathology. 36, 41–45.
Luiz-Ferreira, A., Cola, M., Barbastefano, V., Farias-Silva, E., Calvo, T.R., De Almeida, A.B.A., Pellizzon, C.H., Hiruma-Lima, C.A., Vilegas, W., Souza-Brito, A.R.M., 2011. *Indigofera suffruticosa* Mill as new source of healing agent: Involvement of prostaglandin and mucus and heat shock proteins. Journal of Ethnopharmacology. 137, 192–198. https://doi.org/10.1016/j.jep.2011.05.006
Matos, F.J., 1999. Plantas da medicina popular do nordeste: Propriedades atribuídas e confirmadas. EUFC,.
Matos, F.J., De, A., Lorenzi, H., Santos, L., De, F.L., Matos, M.E.O., Silva, M.G., De V, Sousa, M.P., De., 2011. Plantas tóxicas: Estudo de fitotoxicologia química de plantas brasileiras,. Instituto Plantarum, Nova Odessa, São Paulo, p. 256.
Nascimento, W.M., Oliveira, J.R.S., Cunha, R.X., Gambôa, D.S.R., Silva, A.P.S., Lima, V.L., De, M., 2022. Evaluation of the treatment of fever, pain and inflammation with *Indigofera suffruticosa* Miller leaves aqueous extract. Journal of Ethnopharmacology. 287, 114958. https://doi.org/10.1016/j.jep.2021.114958
Neto, J.D.B., Oliveira, C.M.C., Peixoto, P.V., Barbosa, I.B.P., Ávila, S.C., Tokarnia, C.H., 2001.
NTP., 2020. Botanical dietary supplements. https://ntp.niehs.nih.gov/whatwestudy/topics/botanical/index.html. Date accessed: 2020-04-22
OECD., 2001. Test guideline 425: Acute oral toxicity-up-and-down procedure, Guideline for Testing of Chemicals 1–26. . https://doi.org/10.1787/9789264071049-en
Paulino, J.V., Pessine, E., Teixeira, S., De, P., 2010. Estudos

morfoanatômicos da semente e da plântula de espécies de Anileiras (Indigofera L., Leguminosae). Acta Botanica Brasilica. 24, 1–7. https://doi.org/10.1590/S0102-33062010000100001

Pérez-Torres, I., Castrejón-Téllez, V., Soto, M.E., Rubio-Ruiz, M.E., Manzano-Pech, L., Guarner-Lans, V., 2021. Oxidative stress, plant natural antioxidants, and obesity. International Journal of Molecular Sciences. 22, 1786. https://doi.org/10.3390/ijms22041786

Raskin, I., Ribnicky, D.M., Komarnytsky, S., Poulev, A., Borisjuk, N., Brinker, A., Moreno, D.A., Ripoll, C., Yakoby, N., 'neal, J.M.O., Cornwell, T., Pastor, I., Fridlender, B., 2002. Plants and Human health in the twenty-first century. Trends in Biotechnology. 20, 522–531. https://doi.org/10.1016/s0167-7799(02)02080-2

Ribeiro, L.R., Bautista, A.R.P.L., Silva, A.R., Sales, L.A., Salvadori, D.M.F., Maia, P.C., 1991. Toxicological and toxicogenetic effects of plants used in poular medicine and in cattle food. Memórias do Instituto Oswaldo Cruz. 86, 89–91. https://doi.org/10.1590/s0074-02761991000600022

Ríos-Hoyo, A., Gutiérrez-Salmeán, G., 2016. New dietary supplements for obesity: What we currently know. Current Obesity Reports. 5, 262–270. https://doi.org/10.1007/s13679-016-0214-y

Rosillo, M.A., Sánchez-Hidalgo, M., Cárdeno, A., Aparicio-Soto, M., Sánchez-Fidalgo, S., Villegas, I., Lastra, C.A.D.L., 2012. Dietary supplementation of an ellagic acid-enriched pomegranate extract attenuates chronic colonic inflammation in rats. Pharmacological Research. 66, 235–242. https://doi.org/10.1016/j.phrs.2012.05.006

Salvador, I., De, S., Medeiros, R.M.T., Pessoa, C.R.M., Dantas, A.F.M., Júnior, G.S., Riet-Correa, F., 2010. Intoxicação por *Indigofera suffruticosa* (Leg. Papilionideae) em bovinos. . Pesquisa Veterinária Brasileira. 30, 953–957.

Salvador, I.S., Medeiros, R.M.T., Pessoa, C.R.M., Oliveira, D.M., Duarte, A.L.A., Fighera, R.A., Riet-Correa, F., 2011. Experimental poisoning of guinea pig (*Cavia porcellus*) with *Indigofera suffruticosa*. Toxicon. 57, 927–931. https://doi.org/10.1016/j.toxicon.2011.03.007

Santana, M.A.N., Silva, I.B., Da, Barbosa, J.A.P., Lima, I.R., Vieira, J.R.C., Lima, R.M.P.L., Leite, S.P., 2015. Histomorphometric analysis in kidney tissue of mice treated with *Indigofera suffruticosa* Mill. International Journal of Pharmacy and Pharmaceutical Sciences. 7, 505–506.

Santos, A., dos, T., Araújo, da Silva, L., Silva, C., Oliveira, A., Araújo, J., Correia, M., Lima, V., 2015. Organic extracts from *Indigofera suffruticosa* leaves have antimicrobial and synergic actions with erythromycin against *Staphylococcus aureus*. Frontiers in Microbiology. 6, 1–7. https://doi.org/10.3389/fmicb.2015.00013

Santos, B.S., Junior, M.R., De, M., Paiva, M.H.S., Filho, A., De, A.P., Araújo, T.F., Da, S., Florêncio, E.G., Amâncio, A.P., Mota, C.R.F., De, C., Batista, C.B., Miranda, M.D.F., De, A., Costa, C.J., Da, S., Lima, V.L., De, M., 2009. Comparative analysis of lipid profile in men from Pernambuco State, according to III and IV Brazilian Dyslipidemias Guidelines. Revista Brasileira de Análises Clínicas. 41, 295–297.

Silva, D.M., Da, Riet-Correa, F., Medeiros, R.M.T., Oliveira, O.F., De., 2006. Pesquisa Veterinária Brasileira. 26, 223–236.

Silva, I.B., Da, Lima, I.R., Santana, M.A.N., Leite, R.M.P., Leite, S.P., 2014. *Indigofera suffruticosa* Mill (Fabaceae): Hepatic responses in mice bearing sarcoma 180. International Journal of Morphology. 32, 1228–1233. http://dx.doi.org/10.4067/S0717-95022014000400017

Silva, T.M.S., Sobreira, R.C.B., Silva, J.A.G., Costa, M.A.S., Lima, M.I.A., Silva, A.A., Silva, W.A., Pontes, W.J.T., Leite, S.P., 2019. Erythrocyte osmotic fragility and cytotoxic activity of the aqueous extract of leaves of *Indigofera suffruticosa* Mill (Fabaceae). Revista Virtual de Química. 11, 1802–1809.

Sun, N.N., Wu, T.Y., Chau, C.F., 2016. Natural dietary and herbal products in anti-obesity treatment. Molecules. 21, 1351–1351. https://doi.org/10.3390/molecules21101351

Van Breemen, R.B., 2015. Development of safe and effective botanical dietary supplements. Journal of Medicinal Chemistry. 58, 8360–8372. https://doi.org/10.1021/acs.jmedchem.5b00417

Van Breemen, R.B., Fong, H.H.S., Farnsworth, N.R., 2008. Ensuring the safety of botanical dietary supplements. American Journal of Clinical Nutrition. 87, 509–513. https://doi.org/10.1093/ajcn/87.2.509s

Večeřa, R., Orolin, J., Škottová, N., Kazdová, L., Oliyarnik, J., O and Ulrichová, Šimánek, V., 2007. The influence of maca (*Lepidium meyenii*) on antioxidant status, lipid and glucose metabolism in rat. Plant Foods for Human Nutrition. 62, 59–63. https://doi.org/10.1007/s11130-007-0042-z

Vieira, J.R.C., Souza, I.A., De, Nascimento, S.C., Do, Leite, S.P., 2007. *Indigofera suffruticosa*: An alternative anticancer therapy. Evidence-based Complementary and Alternative Medicine. 4, 355–359. https://doi.org/10.1093/ecam/nel102

Vijisaral Elezabeth, D., Arumugam, S., 2014. GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* . Journal of Chemical and Pharmaceutical Research. 6, 294–300.

WHO., 2018. WHO expert committee on specifications for pharmaceutical preparations, fifty-second report. WHO Technical Report Series No. 1010.

You, Y., Kim, K., Jeong, H., Park, J., Kim, O.K., Jun, W., 2015. Ethanolic extract from *Rubus coreanus* enhanced swimming capacity in mice. Food Science and Biotechnology. 24, 2213–2217. https://doi.org/10.1007/s10068-015-0295-5

Zhao, X.N., Wang, X.F., Liao, J., Bin, Guo, H.Z., Yu, X.D., Liang, J.L., Zhang, X., Su, Z.R., Zhang, X.J., Zeng, H.F., 2015. Antifatigue effect of *Millettiae speciosae* Champ (Leguminosae) extract in mice. Tropical Journal of Pharmaceutical Research. 14, 479–485. https://doi.org/10.4314/tjpr.v14i3.17