

# Synergistic effect of the hydroalcoholic extract from *Lepidium meyenii* (Brassicaceae) and *Fagara tessmannii* (Rutaceae) on male sexual organs and hormone level in rats

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## ABSTRACT

**Background:** *Lepidium meyenii* is a plant, which has been used in folk medicine to treat infertility and to increase sexual desire. However, few reports have investigated the administration of this plant with other plants having the same properties. **Objective:** The present investigation was designed to evaluate whether the combination of *Lepidium meyenii* and *Fagara tessmannii* can improve spermatogenesis and testosterone level in rats. **Materials and Method:** Twenty male rats were treated daily for 2 weeks with the hydroalcoholic extract of *Fagara tessmannii* and *Lepidium meyenii* (Fag + MN) as follow: (vehicle), (0.01 g + 0.5 mg), (0.1 g + 5 mg) and (1 g + 50 mg)/kg BW. **Results:** At doses Fag 0.01 g/MN 0.5 mg and Fag 0.1 g/MN 5 mg, the weight of seminal vesicle, prostate, and testis significantly decreased ( $P < 0.05$ ) while at dose Fag 1 g/MN 50 mg, the weight of epididymis and testis significantly increased ( $P < 0.05$ ) when compared to the control. We noticed a significant increase of the number of spermatids/test ( $P < 0.05$ ), epididymis sperm count ( $P < 0.05$ ), and DSP/test of the rats at dose Fag 1 g/MN 50 mg while at dose Fag 0.01 g/MN 0.5 mg and Fag 0.1 g/MN 5 mg, sperm count was reduced in male organs, particularly in vas deferens ( $P < 0.05$ ) and epididymis ( $P < 0.001$ ). The serum testosterone concentration significantly decreased ( $P < 0.05$ ) at lowest dose Fag 0.01 g/MN 0.5 mg. However, at highest dose Fag 1 g/MN 50 mg, the serum testosterone concentration increased significantly ( $P < 0.05$ ). The length of stage VII-VIII and IX-I of the seminiferous tubule significantly ( $P < 0.05$ ) increased while the length of stage II-VI significantly ( $P < 0.05$ ) decreased. **Conclusion:** The results indicated that the combination of *Lepidium meyenii* (Black Maca) with *Fagara tessmannii* can improve male reproductive organs activities.

**Key words:** *Fagara tessmannii*, *Lepidium meyenii* (Maca Negro), spermatogenesis, testosterone level.

## INTRODUCTION

*Lepidium meyenii*, well-known in South America as “Maca,” is an Andean crop, which is found only in a very restricted area of central Peru, in the agro-ecological zone between 4000 and 4500 m. There are three varieties of Maca, recognizable through their external color (Maca Negro or Black Maca, Yellow Maca, and Red Maca).<sup>[1]</sup> Maca Negro

has been described to possess many medicinal properties in traditional herbal medicine. The root of Maca Negro contains alkaloids, steroids, and chemicals compounds as glucosinolates, isothiocyanates, macamides, and alkamides.<sup>[2-4]</sup> Published clinical studies of Maca Negro seem to be related to its property of improving sexuality and fertility.<sup>[5]</sup> Some studies suggest that secondary metabolites found in Maca Negro extracts are important constituents responsible for its physiological effects.<sup>[6]</sup> The tuber of the plant is used for human consumption because of its nutritional value and for energy.<sup>[7]</sup> Despite of all these finding, few studies has been done to evaluate the effect of Maca Negro with other plants having the same biological properties, since the combination

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of plants can enhance the needed biological effect.<sup>[8]</sup> *Fagara tessmannii* is a tree from the family of Rutaceae, which grows in tropical and subtropical regions and locally known in the littoral region of Cameroon as “Ewounga” or “Bongo.” Aerial parts and roots of this tree are used in the folk medicine for the treatment of infertility, fibroma, and sexual weakness. Recently, we have found that *Fagara tessmannii*, which is used in the folk medicine to enhance sexuality in men, negatively impaired reproductive organ activities.<sup>[9]</sup> Previous phytochemical investigations of *Fagara tessmannii* and its genus yielded terpenes, methylnitidine chloride, nitidine, fagaramide, flindersine, and lignans.<sup>[10-14]</sup> Because they are crude drugs, it is known that plants have slight differences concerning the component and composition depending on where they are grown and where they are harvested.<sup>[15]</sup> So, this work was done to investigate whether the combination of *Lepidium meyenii* Maca Negro (Peruvian plant) and *Fagara tessmannii* (African plant) can improve spermatogenesis and testosterone level in rats.

## MATERIALS AND METHODS

### Preparation of hydroalcoholic extracts of *Lepidium meyenii* (Maca Negro)

Hydroalcoholic extract of Maca Negro was prepared with aqueous ethanol (60%, v/v) by percolation at room temperature for 24 h and concentrate at low pressure to constant weight. The extract was prepared by Eng. Alfonso Higa from Agroindustrial Chanchamayo (Lima, Peru). A sample with 100 g of dried Maca Negro hypocotyls produced 7.6 g of hydroalcoholic Maca Negro. This extract was further dissolved in distilled water to obtain various concentrations. Samples of 1 mL were placed in small vials and kept at 4°C until used.

### Preparation of hydroalcoholic extracts of *Fagara tessmannii*

1000 g of the dried pulverized stem bark of *Fagara tessmannii* was macerated with 5 L ethanol at room temperature for 2 weeks. After filtration, the solvent was removed and placed under reduced pressure. About 30 g of the hydroalcoholic extract was obtained. This extract was further dissolved in distilled water to obtain various concentrations. Samples of 1 mL were placed in small vials and kept at 4°C until used.

### Screening of the plants

Previous studies in our laboratory have demonstrated that *Fagara tessmannii* up to 2 g/kg body weight is not toxic.<sup>[9]</sup> Maca Negro extract can be considered safe in doses up to 5 g extract/kg, corresponding to some 11 g dry hypocotyls/kg.<sup>[16]</sup>

### Animals

A total of 20 -old male Holtzman rats aging of three-month and weighing  $308.06 \pm 18.16$  g, from the animal house of

the Universidad Peruana Cayetano Heredia, were used. They were housed in groups of five, in Plexiglas cages, in acclimatized colony rooms (22°C; 60% humidity) on a 12 h light/dark cycle, with lights off at 07:00 h. Animals were housed under standard conditions (12 h light/12 h dark, 22°C). Food in pellets (Purina Laboratory) and tap water were available *ad libitum*. Housing conditions and experimentations were in accordance with the Institutional Review Board from the Universidad Peruana Cayetano Heredia. The rats were divided into the following four groups and orally treated with (vehicle), (0.01 g + 0.5 mg), (0.1 g + 5 mg), (1 g + 50 mg)/kg BW per day of *Fagara tessmannii* + Maca Negro respectively (abbreviated as control; Fag 0.01 g/MN 0.5 mg; Fag 0.1 g/MN 5 mg; Fag 1 g/MN 50 mg). According to Chung, (2005),<sup>[16]</sup> length of stage VII-VIII did not increase at doses of Maca higher than 1 g/kg BW. The hydroalcoholic extract or vehicle was administered by gavage with intubations needle no. 18 (Fisher Scientific, Pittsburgh, PA). One day after the treatment, the animals were sacrificed and blood sample was collected for hormone assay (testosterone). Testes, seminal vesicle, epididymis, and ventral prostate were removed and cleared of attached fat and connective tissue and weighed. The seminiferous tubules were prepared for transillumination assessment, testes, vas deferens, and epididymis for sperm count.

### Epididymal sperm count

Homogenization-resistant epididymal sperm from non-perfused rats were counted as described previously<sup>[17]</sup> with some modifications. Modifications consisted to measure separately caput/corpus to the cauda epididymis. Homogenization was performed in 5 mL of saline (NaCl 0.9%). Homogenates were kept refrigerated at 4°C for 24 hours to allow sperm to be released from the walls. Then, 10 mL of the refrigerated homogenate was added to 70 mL of eosin (2%), and a sample was placed in a Neubauer chamber. Head sperms were counted in 25 squares for four times. The average sperm count of each rat was multiplied by 0.06 (sperm  $\times 10^6$ /mL) and then by 5 mL (sperm  $\times 10^6$  per caput/corpus or cauda epididymis). Data are referred as sperm per caput/corpus or cauda epididymis.

### Vas deferens sperm count

The vas deferens was cut in two parts corresponding to the large and thick one. Each part was homogenized with 1 mL of saline. An aliquot was diluted with two parts of eosin (0.2%). Homogenization-resistant sperm heads were counted in the 25 squares of the Neubauer chamber. Four chambers were measured in each sample, and they were averaged. Results from the large and the thick part were multiplied by 0.03 and defined as sperm  $\times 10^6$  per part of vas deferens. Data were expressed as the total amount of

sperms in vas deferens (sperm count in the large and the thick part of vas deferens).

### Daily sperm production

The capsule of the left testis was removed, and the parenchyma was homogenized in 10 mL of 0.9% saline-0.05% (v/v) Triton X-100 solution for 1 minute by a homogenizer, and then diluted into 1/10.<sup>[18]</sup> The number of homogenization-resistant elongated spermatid nuclei per testis was determined with a hemocytometer. Counts for four hemocytometer chambers were averaged. The DSP and its efficiency (DSP per weight of testis) were determined by division of the elongated spermatid count per testis and spermatids per gram of testis by 6.3, the duration of steps 17 to 19 spermatids in the seminiferous epithelial cycle for Holtzman rats.<sup>[19]</sup> The epididymal sperm transit rate was calculated by dividing the cauda epididymal sperm number by the DSP.<sup>[20]</sup>

### Assessment of stages of rat seminiferous cycle

Assessment of the seminiferous epithelium tubules stage length was made by transillumination under an inverted stereomicroscope at 40× magnification as previously described.<sup>[21]</sup> For each rat, a total length of 1000 mm was assessed. The stages assessed were I, II-III, IV-V, VI, VII, VIII, IX-XI, XII, XIII-IV as described originally by Parvinen, (1982)<sup>[22]</sup> and then pooled as stages II-VI, VII-VIII, and IX-I.

### Serum testosterone assay

Serum testosterone concentration were measured by radioimmunoassay using commercial kits (Diagnostic Products Co., Los Angeles, USA) in rats treated for 14 days with vehicle, and *Fagara tessmannii*. The hormone labeled with iodine-125 was used as radioactive marker. Samples were run in the same assay to avoid inter-assay variation. The intra-assay variation was 5.5%, and sensitivity was 4 ng/dl

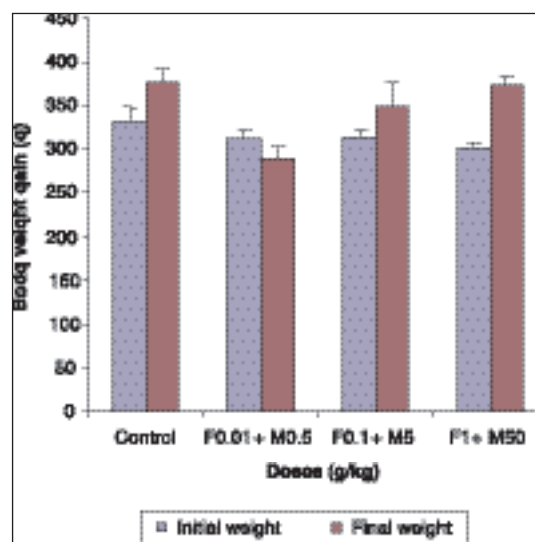
### Statistical analysis

Data were presented as mean  $\pm$  SEM and analyzed using Student's *t*-test and analysis of variance (one way) when more than two groups were compared.  $P < 0.05$  was considered significant.

## RESULTS

### Effect of *Fagara tessmannii* and *Lepidium meyenii* (Maca Negro) on body weight changes and relative reproductive organs weight

Fourteen days following administration of Maca Negro and *Fagara tessmannii*, there were a relative changes in body weight gain in treated animal, excepted at dose Fag 0.1 g/MN 5 mg where body weight decreased [Figure 1]. This changes included too the reproductive organs [Table 1]. At dose of Fag 0.01 g/MN 0.5 mg and (Fag 0.1 g/MN 5 mg), we noticed a significant ( $P < 0.05$ ) weight loss of seminal vesicle ( $0.43 \pm 0.06$  g), prostate ( $0.25 \pm 0.02$  g), and testis ( $1.04 \pm 0.05$  g) when compared to the control ( $1.01 \pm 0.09$  g), ( $0.378 \pm 0.01$  g), and ( $1.43 \pm 0.07$  g), respectively. However, at dose of (Fag 1 g/ MN 50 mg), the weight of epididymis ( $0.52 \pm 0.02$  g) and testis ( $1.71 \pm 0.03$  g) increased significantly ( $P < 0.05$ ) when compared to the control ( $0.45 \pm 0.17$  g) and ( $1.43 \pm 0.07$  g), respectively. Regression analysis showed that the weight of testis was significantly related with the epididymis sperm count ( $R^2 = 0.8$   $P < 0.01$ )



**Figure 1:** Effect of *Fagara tessmannii* and *Lepidium meyenii* (Maca Negro) on body weight

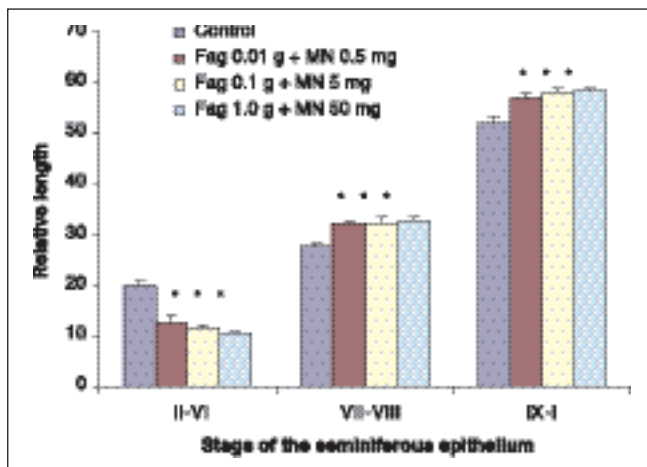
**Table 1: Organ weight (g) of male rats after 14 days of treatment with *Fagara tessmannii* and *Lepidium meyenii* (Maca Negro)**

| Parameters n=5            | Dose (g/kg)     |                      |                   |                   |
|---------------------------|-----------------|----------------------|-------------------|-------------------|
|                           | Control         | Fag 0.01 g/MN 0.5 mg | Fag 0.1 g/MN 5 mg | Fag 1 g/ MN 50 mg |
| Weight of epididymis      | 0.46 $\pm$ 0.02 | 0.41 $\pm$ 0.03      | 0.39 $\pm$ 0.02   | 0.52 $\pm$ 0.02*  |
| Weight of seminal vesicle | 1.11 $\pm$ 0.08 | 0.43 $\pm$ 0.06*     | 0.94 $\pm$ 0.06   | 1.08 $\pm$ 0.07   |
| Weight of prostate        | 0.39 $\pm$ 0.01 | 0.25 $\pm$ 0.02*     | 0.32 $\pm$ 0.01   | 0.38 $\pm$ 0.03   |
| Weight of testes          | 1.51 $\pm$ 0.06 | 1.37 $\pm$ 0.09      | 1.04 $\pm$ 0.05*  | 1.81 $\pm$ 0.03*  |

Value are mean $\pm$ SE; n=5; \* $P < 0.05$  with respect to control; Weight of testes/Epididyme sperm count ( $R^2=0.8$ ,  $P < 0.01$ )

### Effect of *Fagara tessmannii* with *Lepidium meyenii* (Maca Negro) on sperm count

According to Table 2, after the treatment of animal with the combination of Maca Negro and *Fagara tessmannii*, we noticed a significant increase of number of spermatids/test ( $P < 0.05$ ), epididymis sperm count ( $P < 0.05$ ), and DSP/test of the rats at dose Fag 1 g/MN 50 mg while at dose Fag 0.01 g/MN 0.5 mg and Fag 0.1 g/MN 5 mg, sperm count was reduced in male organs, particularly in vas deferens ( $P < 0.05$ ) and epididymis ( $P < 0.001$ ). Regression analysis showed that spermatids was significantly related to daily sperm production ( $R^2 = 1$   $P < 0.01$ ) and weight of testis ( $R^2 = 0.65$   $P < 0.51$ ). Epididymis sperm count was significantly related to weight of testis ( $R^2 = 0.81$   $P < 0.01$ ).



**Figure 2:** Effect of *Fagara tessmannii* and *Lepidium meyenii* (Maca Negro) on seminiferous tubule

### Effect of *Fagara tessmannii* with *Lepidium meyenii* (Maca Negro) on serum testosterone concentration

The serum testosterone concentration dose-dependently tended to increase following 14 days of treatment with *Fagara tessmannii* in combination with Maca Negro, leading in the high augmentation at dose Fag 1 g/MN 50 mg (351.46 (232.86-490.36) ng/dl). However, at lowest dose (Fag 0.01 g/MN 0.5 mg), the serum testosterone concentration decreased significantly ( $P < 0.05$ ) when compared to the control group [Table 3]. Regression analysis showed that testosterone concentration was significantly related to the weight of seminal vesicle ( $R^2 = 0.75$   $P < 0.01$ ) and epididymis ( $R^2 = 0.76$   $P < 0.01$ ).

### Effect of *Fagara tessmannii* with *Lepidium meyenii* (Maca Negro) on spermatogenesis

According to Figure 2, daily administration of the extract for 14 days increased significantly ( $P < 0.05$ ) the length of stage VII-VIII and IX-I when compared to their control groups, whereas the length of stage II-VI reduced significantly.

## DISCUSSION

Nowadays, the incidence of sexual inadequacy in human males, including sexual impotence, and the concern that it causes in the affected subjects, are indirectly indicated by the great number of available treatments based in the use of combination of drugs in order to improve their biological effect. So, some investigators used extract of *Aloe buettneri*, *Justicia insularis*, *Hibiscus macranthus*, *Dicliptera verticillata* in

**Table 2: Sperm count ( $\times 10^6$ ) of male rats after 14 days of treatment of *Fagara tessmannii* with *Lepidium meyenii* (Maca Negro)**

| Parameters n=5         | Dose (g/kg) |                      |                   |                  |
|------------------------|-------------|----------------------|-------------------|------------------|
|                        | Control     | Fag 0.01 g/MN 0.5 mg | Fag 0.1 g/MN 5 mg | Fag 1 g/MN 50 mg |
| Spermatids/test        | 98.9±13.94  | 96.15±4.79           | 52.25±9.73        | 141.10±8.27*     |
| Spermatids/g test      | 68.19±7.88  | 68.82±7.81           | 49.19±6.91        | 84.81±6.47       |
| Deferens sperm count   | 5.53±0.77   | 6.33±1.17            | 3.10±0.47*        | 4.54±0.48        |
| Epididyme sperm count  | 135.36±6.66 | 118.45±4.40          | 62.95±6.28**      | 156.20±8.82*     |
| DSP/test               | 15.69±2.21  | 15.26±0.76           | 8.29±1.54         | 22.39±1.34       |
| DSP/W.testis           | 10.82±1.25  | 10.92±1.24           | 7.80±1.04         | 13.46±1.03       |
| Sperm transit in cauda | 3.46±0.26   | 3.88±0.73            | 3.41±0.72         | 5.04±0.84        |

Value are mean±SE; n=5; \* $P < 0.05$ ; \*\* $P < 0.01$  with respect to control, spermatids/DSP ( $R^2 = 1$ ,  $P < 0.01$ ); Spermatids/Weight of testes ( $R^2 = 0.65$ ,  $P < 0.5$ ); DSP=Daily sperm production

**Table 3: Blood concentration of testosterone of male rats after 14 days of treatment of *Fagara tessmannii* with *Lepidium meyenii* (Maca Negro)**

| Parameters n=5       | Dose (g/kg)  |                      |                           |                           |
|----------------------|--------------|----------------------|---------------------------|---------------------------|
|                      | Control      | Fag 0.01 g/MN 0.5 mg | Fag 0.1 g/MN 5 mg         | Fag 1 g/MN 50 mg          |
| Testosterone (ng/dl) | 131.76±43.36 | 68.29±33.76*         | 257.15±44.67 <sup>a</sup> | 351.46±75.02 <sup>b</sup> |

The data are analyzed by the Mann-Whitney test; \* $P < 0.05$  with respect to control; <sup>a</sup> $P < 0.05$  with respect to control and to Fag 0.01 g/MN 0.5 mg; Testosterone/Weight of seminal vesicle ( $R^2 = 0.75$ ,  $P < 0.01$ ); Testosterone/Weight of epididyme ( $R^2 = 0.76$ ,  $P < 0.01$ )

order to improve male reproductive function.<sup>[23]</sup> Other researchers' study showed the efficiency of mixture *Hibiscus macranthus* and *Basella alba* on testosterone production.<sup>[24]</sup> One study showed that the combination of *Hypericum perforatum* and *Passiflora incarnate* enhanced anti-depressive therapeutic effects.<sup>[25]</sup> Maca Negro is a well-known Peruvian plant, which previous studies demonstrated the traditional fertility-enhancing properties in rodents and in men.<sup>[26-28]</sup> Recently, we have found that although *Fagara tessmannii* is used to enhance sexuality, this plant negatively impaired reproductive organs activities.<sup>[9]</sup> The present study was attempted to assess the biological effect of Maca Negro with *Fagara tessmannii* obtained from littoral region of Cameroon (Central Africa).

In the present work, the use of *Fagara tessmannii* and Maca Negro presented a relative body weight loss in low dose (0.01 g/0.5 mg). It is well-known that androgen plays an important role in the regulation of growth of the mammalian prostate gland. Testosterone can stimulate proliferation and inhibit death of the glandular epithelial cells.<sup>[29,30]</sup> Androgen action is also believed to be involved in the development of prostatic disease.<sup>[31]</sup> On the other hand, it is proved that androgens are steroids having anabolic properties.<sup>[32,33]</sup> So, the body weight loss could be related to the decrease of testosterone in that dose.

The present study also shows that the combination of *Fagara tessmannii* with Maca Negro at low dose (0.01 g/0.5 mg) significantly reduced the weight of prostate and seminal vesicle while in the high dose (1 g/ 50 mg), the weight of epididymis and testis enhanced significantly. We have recently demonstrated in our lab that the weight of those organs decreased particularly at low doses after *Fagara tessmannii* treatment.<sup>[9]</sup> So, one can conclude that the two plants has synergistic effect on prostate and seminal vesicle at low dose and at the high dose, Maca Negro can improve the weight of epididymis and testis. The weight loss and weight gain could be attributed respectively to the critical decrease and increase of testosterone level at those doses, since testosterone level was significantly related to the weight of seminal vesicle ( $R^2 = 0.75$ ,  $P < 0.01$ ) and epididymis ( $R^2 = 0.76$ ,  $P < 0.01$ ). The mechanism by which Maca Negro modulates the release of testosterone is not clear. One work has been done to show how Maca Negro interacts with its target *in vivo* and *in vitro*.<sup>[34]</sup> Presently, it is not clear whether the effects of Maca Negro are due to direct activities of its components on selected cells or indirectly on the components of *Fagara tessmannii*. Some researchers have shown that oral administration of Maca Negro improves sexual performance without changing reproductive hormone levels.<sup>[35]</sup> Other study demonstrated that Maca Negro does not exert direct androgenic activities.<sup>[36]</sup> On the other hand, *Fagara tessmannii*

dose-dependently increased the level of testosterone in treated male rats.<sup>[9]</sup> Therefore, one can speculate that the mechanism of action which leads to androgen secretion could be due to interaction between active components of the two plants. Nevertheless, we must notice that it is not recommended to use those plants in low doses when an effect on fertility is required.

The present study also shows that at the high dose, the number of spermatids per testis and Daily Sperm Production (DSP) significantly ( $P < 0.05$ ) increased when compared to the treatment with *Fagara tessmannii* alone,<sup>[9]</sup> confirming that Black Maca can modulate processes related to DSP and sperm count in epididymis.<sup>[37]</sup> This modulation is probably related to the potential antioxidants activities present in the Maca Negro's extract since antioxidants increase spermatogenesis,<sup>[38,39]</sup> or particularly to the bioavailability of testosterone.<sup>[40]</sup> It has been reported that testosterone withdrawal lead to cause DNA fragmentation by stimulating activation in Sertoli cells *in vitro*, which indicated that decreased testosterone levels can stimulate apoptotic pathways.<sup>[41]</sup> Other research study showed that low serum testosterone levels can adversely affect the structure, weight, and functions of the testis, epididymis, and prostate gland.<sup>[42,43]</sup> So, the increase of testosterone level in this work played an important role in the improvement of the sperm count.

The present work also showed that the relative length of stage VII-VIII and IX-I increased significantly while the length of stage II-VI reduced significantly when compared to control. Well, the length of stage VII-VIII did not change after *Fagara tessmannii* treatment alone when compared to control.<sup>[9]</sup> These suggest that Maca Negro can set up the release of elongated spermatids from Sertoli cells into the tubule lumen. This explains why the number of spermatids, epididymis sperm count, and DSP increased significantly in this work when compared to treatment with *Fagara tessmannii* alone<sup>[9]</sup> where those parameters had decreased. Spermiation is the final step of spermatogenesis and is a multi-step process commencing at the beginning of stage VII and ending during stage VIII in the rats.<sup>[44]</sup> Spermatids at step 19 are observed in stages VII and VIII of the seminiferous epithelium.<sup>[45]</sup> It has been postulated that 42 days are required for a complete restoration of advanced spermatids (steps 17-19).<sup>[46]</sup> Therefore, the slight increase (31 mm vs. 27 mm) of the length of stage VII-VIII observed in the present study is likely due to the reduced period (14 days) of treatment applied in this work. On the other hand, the combination of *Fagara tessmannii* and *Lepidium meyenii* have also shown that this extract could promote the progression of spermatogenesis, since the length of stages IX-I also increased significantly, although at the beginning of the formation of the sperm cells, we

noticed a down regulation of spermatogenesis of that combination, which was related to the decrease of the length of stage II-VI (side-effect).

In conclusion, according to our data, Maca Negro can really improve activities of male reproductive organs. Complementary works are needed to elucidate the mechanism of action related to these properties. Nowadays, the use of extract of plants as an alternative for treatment of the infertile man has recently been emphasized.<sup>[47]</sup> Although the potency of Maca Negro with another plant on male reproductive system has been demonstrated, it is not recommended to mix them together in order to avoid some negative side-effects.

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