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Article

Effect of the Aqueous Extract of *Luffa Operculata* (L.) Cogn. (Buchinha) Fruit on Reproductive Parameters in Adult Wistar Rats

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ABSTRACT
Medicinal plants are an accessible option for curing or alleviating illnesses. However, they may present toxicity due to their own components, improper handling, or high dosages. Among these plants, *Luffa operculata (L.) Cogn* (buchinha) is popularly used to treat the symptoms of sinusitis and rhinitis, in addition to being used for its abortive properties. Recent studies indicate testicular changes in male rats subjected to an infusion of this plant species in experiments, which directly impairs male fertility and, consequently, reproductive capacity.. However, in the literature, there are few studies investigating this correlation. Given this, the present study aimed to analyze the toxicity of the aqueous extract of *L. operculata (L.) Cogn* (buchinha) on reproductive aspects in adult Wistar rats. To this end, testicular histological analyses, evaluation of sperm count, morphology, and motility, as well as determination of body weight and reproductive organ weights, were employed. Although statistical differences emerged regarding sperm count, daily production, and transit, the relationship between buchinha and male reproductive health requires further in-depth analysis.
Keywords: reproductive health; herbal medicine; Cucurbitaceae.

Introduction

According to the Brazilian Health Regulatory Agency (ANVISA), medicinal plants are all plant species used for therapeutic purposes, generally in teas and infusions, which emerge as an alternative or complement to the use of synthetic drugs, constituting an accessible therapeutic source, especially in peripheral communities, and can provide compounds that, when modified, exhibit greater efficacy and lower toxicity, in addition to the possibility of being used as prototypes for the creation of drugs with similar activity (National Health Surveillance Agency 2011; Turolla & Nascimento 2006). In this sense, when processed to obtain medicines or prophylactic products, they are characterized as herbal medicines, whereas when a specific bioactive compound is isolated for drug development, they are considered phytopharmaceuticals (Cechinel 2020).

Historically, the use of medicinal plants dates back to various ancient civilizations. In Ancient Greece, Hippocrates, considered the father of medicine, already possessed knowledge of the therapeutic properties of plants, as he described numerous plant-based drugs in his work titled *Corpus Hippocraticum*, many of which continue to be used in modern medicine (Simmonds 2023). In Brazil, indigenous and African peoples were responsible for spreading knowledge and cultivating plant species with the potential to cure or alleviate illnesses. Later, this knowledge was incorporated by European colonizers, who established the first apothecaries in Brazilian territory, basing their practice primarily on the biodiversity of native flora, as well as on plant species introduced from Africa and Asia (Saad et al. 2018).

As an example of this scenario, *Luffa operculata* (L.) Cogn. (buchinha), known as buchinha-do-norte or cabacinha, is a plant native to Brazil belonging to the Cucurbitaceae family. It has dark yellow flowers; its fruits, in the early stages, are small, with soft protuberances as they ripen, and after drying, they transform into fibrous and solid structures with a light yellow hue (Badin et al. 2023; Badin & Manaças 2022). It is commonly used to treat nasal and sinus symptoms of rhinitis and sinusitis through infusions of the dried fruit in water, which are

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subsequently administered via inhalation or instilled into the nostrils (Pereira-de-Morais et al. 2025). Although there are published studies in this field, the evidence base remains weak, and the mechanisms of action involved in these respiratory diseases remain insufficiently understood. Nevertheless, various studies report results indicating its efficacy (Pereira-de-Morais et al. 2025; Silva et al. 2018; Salviano 1992; Scalia et al. 2015). In addition, another popular use relates to the abortive properties and inhibition of fertility attributed to the tea made from the dried fruit (Badin et al. 2023; Fajinmi et al. 2022).

However, alongside their benefits, medicinal plants may present toxicity, whether due to their chemical constituents, contaminants during preparation, or overdose, as well as allergic reactions or systemic toxic effects (Al-Khayri et al. 2023; Veiga Junior et al. 2005). Meanwhile, *L. operculata* (L.) Cogn., when used incorrectly, has been associated with cases of fetal malformation, vaginal bleeding, and maternal death; furthermore, nosebleeds have been reported in treatments for respiratory diseases (da Rocha Galucio et al. 2022; Frias et al. 2021). Macroscopic and microscopic changes, including a reduction in Leydig cells, have already been described in experimental models following administration of the plant species (Silva et al. 2018). However, the available studies still exhibit methodological variations and differences regarding the scope of the reproductive parameters evaluated, indicating the need for further investigations into its potential effects on male reproductive function.

Such toxicological effects observed in the male reproductive system with the use of buchinha have also been described in other species of the Cucurbitaceae family. Among them, decreased sperm count and motility, reduced sex hormone levels, and impaired fertility have been reported in rats following administration of the ethanolic extract of *Momordica dioica* and the hydroalcoholic extract of the stem and leaves of *Ecballium elaterium* (Pusuloori et al. 2017; Irisappan & Annamalai 2018).

In view of this, the present study aimed to evaluate the structure of the testes and epididymis, sperm production and quality, as well as the fertility of adult Wistar rats subjected to infusion of the dried fruits of *Luffa operculata* (L.) Cogn., in order to elucidate the effects of the plant on male reproductive parameters.

Materials and Methods

Experimental Group

This experiment utilized animals in accordance with the ethical principles for the use of laboratory animals described by COBEA—the Brazilian College of Animal Experimentation—and the guidelines established by CONCEA - National Council for the Control of Animal Experimentation, in addition to the project's evaluation by the Ethics Committee on the Use of Animals (CEUA) at the Federal University of Grande Dourados (UFGD), under protocol No. 17.2021. Eighteen male Wistar rats (*Rattus norvegicus*) of reproductive age (initial age of 80 days) were used, weighing approximately 300 g, sourced from the UFGD Central Animal Facility and housed in cages at the Technological Animal Facility of the Faculty of Health Sciences under controlled temperature (22°±2°) and lighting conditions (12-hour light/12-hour dark cycle), with *ad libitum* solid and liquid diets.

Luffa operculata extract

The fruits of *Luffa operculata* were collected and identified by the responsible biologist, Dr. Ramão Souza de Deus Júnior. The extract was prepared according to traditional methods, using the dried fruits of the plant, by infusion. In the laboratory, 3 dried fruits were added to 1,020 mL of boiling filtered water and steeped for 10 minutes. Subsequently, the infusion was cooled, filtered, frozen (-70°C), and freeze-dried, resulting in the aqueous extract, designated ELO. This extract was diluted in saline at the time of administration and subsequently used in the experiment described below.

Experiment

Eighteen rats were used for the experiment, divided into 3 groups of 6 rats each, as follows: the TLO group, treated with ELO; the Naive group, which received no treatment; and, finally, the Control group, which received only saline. The extract obtained was diluted in saline and administered at a dose of 1 mg/kg—a dose established in the literature (Alves *et al.* 2018)—via gavage for five consecutive days to the animals in the TLO group, while the other groups received different treatments, as illustrated. All rats were weighed during the five days of the experiment.

Upon completion of the experiment, the animals were euthanized and subsequently decapitated. This method was necessary since blood was collected from the ruptured cervical vessels for subsequent hormonal assays as part of the project to which this article belongs.

The tail of the left epididymis was collected to obtain spermatozoa and for morphological analysis. The head/body region of the left epididymis, along with the right testis, was frozen at -20°C for sperm counting and determination of transit time. The left testis was reserved for histological evaluation. In addition, the reproductive organs were weighed using an analytical balance, including the testis, epididymis, prostate, and seminal vesicles (both full and empty).

Collection of organs for histology

The removed testis had its tunica albuginea sectioned at the poles using small surgical scissors. It was then immersed in ALFAC fixative solution, composed of 80–85% alcohol, 10% formaldehyde, and 5% glacial acetic acid, following the protocol described by Russell et al. (1990). The tissues underwent histological processing, were embedded in paraffin, and cut into 5-μm sections. These sections were stained with hematoxylin and eosin for histological analysis.

Histological evaluation

A histological analysis was conducted by evaluating 100 sections of seminiferous tubules. The state of the epithelium, the lumen contents, and the interstitium of the testes were examined, categorizing morphological abnormalities of the organ according to specifications for toxicological studies (Foley 2001). The tubules were considered abnormal if they exhibited characteristics such as acidophilic cells, multinucleated cells, retained spermatids, cellular degeneration, vacuolization of the epithelium, or cellular desquamation in the lumen. Additionally, the appearance of the testicular interstitium was qualitatively assessed using a Leica® light microscope at magnifications of 100X, 200X, and 400X.

Assessment of sperm production and transit

The right testicles, after being decapsulated and centrifuged, were frozen until the day of homogenization, following the method of Robb et al. (1978) with modifications. The testicular tissue was thawed and homogenized in STM solution (sucrose–Tris–$MgCl_2$), used to preserve cellular and nuclear integrity during tissue processing, according to the weight of the parenchyma, performing two homogenization sessions of 15 seconds each, separated by a 30-second interval on ice. The tissue was then sonicated for 30 seconds. After a 10-fold dilution, a sample was transferred to a Neubauer chamber (4 fields per animal) for counting mature spermatozoa. To calculate daily sperm production (DSP), the number of spermatozoa was divided by 6.1, which represents the number of days in the cycle during which these spermatozoa are present in the seminiferous epithelium. Similarly, the head/body and tail portions of the epididymis were cut into small fragments using scissors and homogenized according to the same protocol used for the testes. Sperm transit time through the epididymis was calculated by dividing the number of sperm in each portion by the DSP.

Sperm motility

Motility was assessed according to the methodology described by Seed et al. (1996), using sperm from the epididymal tail, which were suspended in HTF (*Human Tubal Fluid*). A 10 μL aliquot was placed in a Makler chamber (Irvine, Israel) and examined under a light microscope at 400X magnification. A total of 100 spermatozoa per animal were analyzed, and their motility was categorized as follows: Type A for motile spermatozoa with progressive movement, Type B for motile spermatozoa with non-progressive movement, and Type C for immotile spermatozoa.

Sperm morphology

For this parameter, 100 μl of a solution containing sperm from the epididymal tail was transferred to 900 μl of a 10% formalin solution. Subsequently, smears were prepared on histology slides, which were left to air-dry for 90 minutes. After this period, the smears were examined using a phase-contrast microscope at 400x magnification (Seed et al. 1996). , 200 spermatozoa per animal were analyzed, and the morphological abnormalities found were classified into two categories: a) head abnormalities (including absence of curvature and isolated) and b) tail abnormalities (coiled, broken, and isolated) (Filler 1993).

Statistical analyses

The data were subjected to analysis of variance (ANOVA), and subsequently, the TLO and Naive groups were compared to the Control group using Dunnett's test. The significance level for the analyses was set at $p<0.05$. GraphPad Prism 8 software was used to perform all analyses.

Results and Discussion

L. operculata (L.) Cogn has been the subject of research regarding the use of its infusion for abortive purposes, as well as possible complications in pregnant women (Badin & Manaças 2022). Although there have already been investigations into the effects of *Luffa operculata* on male reproduction, the available studies are relatively limited in terms of an integrated approach to reproductive parameters. Thus, this study aims to complement the existing literature by providing a more comprehensive analysis of reproductive toxicity in males.

The assessment of the toxic effect of plants is performed by observing changes in the weight of animal organs (Table 1), including reproductive organs, particularly when related to the evaluation of reproductive toxicity. In the male reproductive system, a reduction in the weight of these organs—such as the testes, epididymis, and seminal vesicles—which may be associated with decreased fertility, is considered an essential criterion for identifying toxic substances. These changes, along with other associated factors, may indicate exposure to toxic agents and interfere with reproductive activity (Abdu et al. 2023; Silva 2017).

The present study found no differences in final body weight among the experimental groups, with all p-values > 0.05 in the statistical analysis, as shown in Table 1. These results indicate that treatment with *L. operculata* (L.) Cogn. extract, for the period established in this study, did not influence body mass nor did it exhibit an anorexigenic effect.

Additionally, no significant differences were observed in the absolute weights of the reproductive organs between the animals in the TLO group and the other experimental groups. However, the findings of the present study differ from the results described by Alves et al. (2018), who, using the same dosage and administration period, demonstrated effects indicative of toxicity of *Luffa operculata* (L.) Cogn. on testicular weight and relative body weight of the treated animals, showing significant increases compared to the control group.

Despite the results regarding body weight and reproductive organs, a reduction in testicular sperm count was observed, both in daily sperm production and in the count of mature spermatozoa, when compared to groups not exposed to *L. operculata* extract (Table 2).

Regarding the epididymal count, although a significant reduction was observed in the head/body region, daily sperm transit showed no change. On the other hand, in the epididymal tail region, a decrease in sperm count was observed, which influenced sperm transit in this portion of the organ, being higher in rats treated with ELO. This increase may compromise sperm integrity and function due to prolonged exposure to unfavorable conditions, including increased susceptibility to oxidation and oxidative stress

Table 1. Final body weight of rats and weight of reproductive organs

Parameters	TLO	Control	Naive
Final weight (g)	355.90±11.18	361.1±5.76	380.5±6.44
Ventral prostate (mg)	361.90±51.20	389 ± 64.06	265.3±49.50
Relative ventral prostate (mg/100g)	98.73±14.24	106.20 ± 17.64	69.44±12.35
Testis (g)	1.42±0.03	1.50 ± 0.06	1.44±0.07
Relative testis (mg/100g)	384.70±9.15	409.90±20.21	377.00±13.85
Epididymis (mg)	238.3±48.57	169.5 ± 5.75	190.5±9.70

Relative epididymis (mg/100g)	64.61±13.04	46.32 ± 1.54	50.03±2.19
Full seminal vesicle (g)	1.12±0.09	0.97 ± 0.05	1.13 ± 0.10
Relative seminal vesicle weight (mg/100g)	330.30±24.13	265.40±16.45	296.20 ± 24.41
Empty seminal vesicle (mg)	623.3±56.76	526.4 ± 19.98	617.8 ± 57.72
Relative empty seminal vesicle (mg/100g)	169.00±14.90	144.10±6.57	161.90 ± 13.75

Values expressed as mean ± standard deviation, p > 0.05. Analysis of variance (ANOVA) with Dunnet's post hoc test. Source: Compiled by the author

According to Aksak et al. (2022), a decrease in sperm count can significantly affect the quality of seminal composition, not only in terms of quantity but also by interfering with sperm morphology and motility. Furthermore, although organ weights showed no changes, the possibility of damage to gametes cannot be ruled out, making morphological and functional analyses necessary through the structure-function relationship associated with sperm count.

Similarly, faster daily production may result in immature, lower-quality sperm, leading to inadequate selection and allowing sperm with genetic or morphological defects to be ejaculated. On the other hand, slower production, as evidenced by the results of this study, can result in a reduced sperm count, also demonstrated in these results, with these variables possibly influenced by malformation of the seminiferous tubules since they are intrinsically related to the formation of immature spermatids, also leading to functional and morphological difficulties (Simões 2023).

Table 2. Evaluation of daily sperm production and sperm transit

Parameters	**TLO**	**Control**	**Naive**
Testicular sperm count Mature spermatids (x10⁶/test)	82.13±5.47AB	148.8±6.49	135.3±7.89
Mature spermatids (x10⁶/g/test)	59.68±8.89AB	125.30±13.46	149.10±39.73
Sperm production (x10⁶/test/day)	13.46±0.90AB	24.39 ± 1.06	22.18 ± 1.29
Sperm production (x10⁶/test/g/day)	9.78±1.46AB	20.54±2.21	24.44±6.51
Epididymal sperm count Head/body Sperm count (x10⁶/organ)	67.51 ± 7.83AB	113.90±6.09	140.30±17.36
Sperm transit time (days)	5.21±0.86	4.67±0.17	6.68 ± 1.35
Tail Sperm count (x10⁶/organ)	150.60±10.25AB	198.30±10.01	246.10±14.85
Sperm transit time (days)	12.22±1.67AB	8.26 ± 0.67	09.39 ± 1.23

Values expressed as mean ± standard deviation of the mean, A = p ≤ 0.05 compared to the Control group. B = p ≤ 0.05 compared to the Naive group. Analysis of variance (ANOVA) with Dunnet's *post hoc* test. Source: Compiled by the author.

In this context, considering that alterations in spermatogenic dynamics can directly affect sperm functional capacity, sperm motility was evaluated as one of the main indicators of male reproductive competence. The results regarding the different movement patterns are presented in Figure 1.

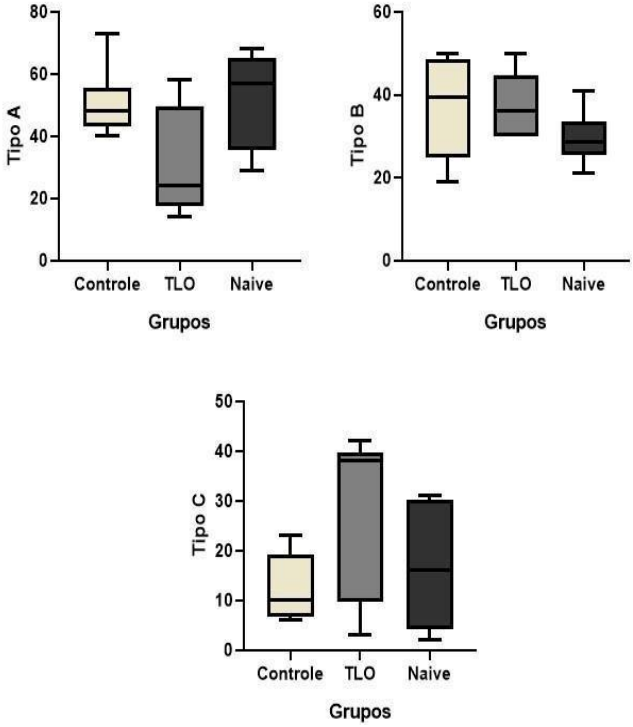


Figure 1. Sperm motility. Values expressed as median (1st quartile–3rd quartile), p > 0.05. Type A = Motile with progressive trajectory. Type B = Motile with non-progressive trajectory. Type C = Immotile. Analysis of variance (ANOVA) with Dunnet's *post hoc* test. Source: Compiled by the author.

Regardless of Table 2, the graphical analyses in Figure 1 show that there were no differences in sperm motility between the group of rats treated with ELO and the groups of rats that were untreated or treated only with serum. Thus, although a numerical decrease was observed, sperm motility was preserved. Considering that the structural integrity of sperm cells is directly related to their functional performance, the morphological characteristics of the sperm were evaluated, with the parameters described in Table 3.

Table 3. Evaluation of sperm morphological characteristics.

Parameters	TLO	Control	Naive
Normal sperm (%)	96.00 (82.75–106.50)	105.00 (98.75–108.00)	107.00 (98.25–114.80)
abnormal sperm (%)			
Head abnormalities	4.00 (2.23–8.25)	2.00 (0.75–3.50)	2.00 (0.00–3.00)
Tail abnormalities	93.50 (88.75–100.00)	100.50 (90.50–114.00)	92.50 (89.25–99.25)
Cytoplasmic droplet	136.00 (92.25–161.00)	58.50 (49.75–89.50)	78.00 (53.75–120.30)

Values expressed as median (1st quartile–3rd quartile), p > 0.05. Analysis of variance (ANOVA) with Dunnet's *post hoc* test. Source: Compiled by the author

The data presented in Table 3 did not show significant differences between the groups regarding sperm morphological analysis, indicating that the structures of the evaluated spermatozoa were not affected by

exposure to the aqueous extract of *L. operculata* (L.) Cogn. No head or tail abnormalities were observed, nor was there an increase in the presence of cytoplasmic droplets, changes that could compromise sperm motility.

The treatment period adopted in this study was established based on the experimental model described by Silva et al. (2018), allowing for methodological comparability among the findings. Considering that the complete spermatogenic cycle in Wistar rats lasts approximately 54–56 days, the evaluated interval covers only part of this biological process, and this aspect should be considered when interpreting the results. Additionally, according to Li et al. (2025), there is a higher probability of impaired sperm motility and morphology when there is a reduction in epididymal transit time. In the present study, however, an increase in transit time was observed, reinforcing this interpretation (Table 2).

Other species of the Cucurbitaceae family, to which the plant under study belongs, also demonstrate a reduction in sperm count and daily sperm production, as well as an increase in epididymal transit time, though with additional impacts on sperm motility and morphology. For example, Pusuloori et al. (2017), when evaluating the effect of the ethanolic extract of *Momordica dioica*, observed a reduction in sperm count similar to that described in this study; however, they also reported morphological and functional changes, differing from the findings presented here. Similarly, Irisappan & Annamalai (2018), when investigating the hydroalcoholic extract of the stem and leaves of *Ecballium elaterium*, reported a reduction not only in sperm count but also in motility in the treated animals, results that partially diverge from those observed in the present study.

A reduced sperm count, even in the absence of morphological or motility changes, may be associated with testicular dysfunction, reflecting impaired sperm production and maturation. Such changes may result from structural modifications in the seminiferous tubules, affecting the organization of germ cells, especially when there is an increase in acidophilic, vacuolated, or multinucleated cells (Machado-Neves 2022). Thus, histological evaluation of the testes becomes essential to investigate tissue integrity and its possible relationship with sperm parameters. The quantitative results of the histological analysis are presented in Table 4.

Table 4. Histological analysis of the testicles.

Parameters	TLO	Control	Naive
Normal cells	82.00 (75.50–88.50)	77.00 (68.00–87.50)	87.00 (76.00–87.00)
Acidophilic cells	13.00 (8.00–15.50)	11.00 (6.60–19.00)	11.00 (8.00–11.50)
Vacuolated cells	5.00 (3.50–9.00)	7.00 (2.75–12.75)	6.00 (3.00–12.50)

Values expressed as median (1st quartile–3rd quartile), $p > 0.05$. Analysis of variance (ANOVA) with Dunnet's *post hoc* test. Source: Compiled by the author.

The qualitative morphological characterization of the testicles can be observed in the representative images shown in Figure 2, which demonstrate the organization of the seminiferous tubules, the arrangement of germinal cells along the seminiferous epithelium, and the preservation of tissue architecture across the experimental groups.

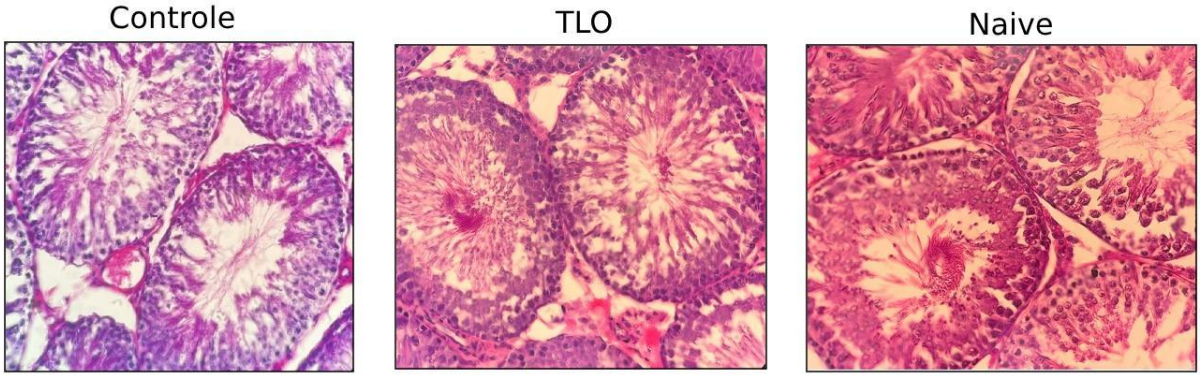


Figure 2. Testicular histological microscopy of the experimental groups. 400x magnification. Source: author's own work.

In this study, no histological abnormalities were identified in any of the experimental groups, as shown in Table 4 and Figure 2. No increase in acidophilic cells or presence of vacuolization was observed, and the histological findings, when present, suggest potential germ cell death and susceptibility to changes resulting from exposure to high levels of toxic agents. These findings contrast with the results obtained by Alves et al. (2018), whose study revealed significant changes, both macroscopically and microscopically, in the tissue structure of the testes of rats treated with the extract, where there was a decrease in the parenchyma of the seminiferous tubules of rats that received the extract of *L. operculata* (L.) Cogn.

Conclusion

Although the results showed a reduction in sperm count and production, as well as an increase in daily transit, it is not possible to state that the aqueous extract of *L.operculata (L.) Cogn.* has any deleterious effect on male fertility. Given the lack of substantial information regarding the male reproductive system, there is a clear need for future research dedicated to evaluating the potential deleterious effects of this plant over the long term.

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