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Original Article

A Comparison of the Long-Term Effects of Opium Tincture and Methadone Syrup on Testicular and Prostate Histology in Male Rats

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Abstract

Introduction: A common way to treat opioid dependence is the consumption of opioids, particularly opium and methadone. However, there is little information about its long-term effects on male reproductive health. In this study, rats› male reproductive system›s hormonal and histological parameters were examined in relation to the long-term oral effects of methadone syrup and opium tincture.

Methods: 42 male adult Wistar rats were randomly divided into six equal groups and administered orally with methadone (dose escalation to 50 mg/kg) or opium tincture (dose escalation to 200 mg/kg) for 4 or 8 weeks. At the conclusion of treatment, serum testosterone, the relative weights of the prostate and testes, Johnson's score, and histomorphometric indices such as seminiferous tubule area, germinal epithelium area, and prostatic epithelial height were measured.

Findings: Chronic opium tincture treatment significantly decreased serum testosterone, relative testicular and prostatic weights, and testicular histological indicators ($P<0.05$). Methadone also damaged certain histology measurements but had a lower impact on testosterone. Prostatic epithelial height increased significantly in the 8-week methadone group, whereas all opium groups exhibited notable decreases ($P<0.05$).

Conclusion: Prolonged oral opium tincture causes more detrimental effects on male reproductive function than methadone, whereas extended methadone exposure may lead to prostate-specific structural changes. These findings highlight the need for careful selection and administration of opioid substitution treatments to diminish the risks of reproduction in dependent individuals.

Keywords: Opium tincture, Methadone, Testes, Prostate, Testosterone

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Introduction

Opium, derived from the opium poppy (*Papaver somniferum* L.), is an extremely addictive narcotic[1] with a global prevalence estimated at approximately 30 million individuals, contributing to more than 100,000 opioid overdose deaths each year.[2,3] Among men of reproductive age, the increasing trend in opioid use[4] has become more evident. Due to its shared border with Afghanistan, the world's largest opium producer,[5] Iran has one of the highest per capita opium consumption rates globally, with 28 of every 1,000 individuals over the age of 15 years reported as opium users.[6]

The main alkaloids in opium consist of phenanthrenes (morphine, codeine, thebaine, oripavine) and benzylisoquinolines (papaverine, noscapine)[7], with morphine being the most pharmacologically important. Morphine is widely prescribed for severe and chronic pain, but at high doses, it can carry significant risks, including addiction, respiratory problems, acute toxicity, and death.[8,9] Tincture of opium (laudanum) is an oral aqueous-alcoholic extract containing 10 mg of morphine per milliliter[10,11] and is classified as a narcotic due to its high morphine concentration.[12]

Methadone, a synthetic opioid with analgesic effects comparable to morphine,[13-15] is used as a second-line oral opioid analgesic and a well-known treatment for opioid dependence.[16] Methadone decreases mortality from overdose and injection-related adverse effects, including HIV and hepatitis C transmission.[17]

A higher prevalence of reproductive disorders has been reported in opioid users compared to non-users,[18] with sexual dysfunction affecting up to two-thirds of patients

undergoing opioid treatment and persisting in one-third after treatment.[19] The effect of opioids on spermatogenesis remains uncertain[20], although the hypothesis is that long-term use leads to infertility in men by altering testicular volume and suppressing gonadal hormones.[21] Despite numerous studies on the systemic effects of morphine, the consequences of long-term opium use on the male reproductive endocrine system have not been sufficiently clarified.[4,22]

Despite previous studies on the reproductive toxicity of opioids, the long-lasting comparative effects of oral opium tincture and methadone syrup remain unclear. This study simultaneously evaluated the oral administration of increased doses of both drugs and their impacts on testicular and prostatic histomorphometry in male rats, providing a clearer understanding of their relative reproductive safety in opioid maintenance treatments.

Methods

Animals

Forty-two male adult Wistar rats (8–10 weeks old; 190–240 g) were obtained from the Birjand University of Medical Sciences Animal Center. The animals were randomly assigned to six groups (n = 7) and housed in separate cages under standard laboratory conditions (12 h light/dark cycle, temperature 22 ± 2°C, relative humidity 25–30%), with free access to standard chow (Behpoor Company, Iran) and water. All experimental procedures were conducted at the Birjand Animal House Laboratory Research Center and were approved by the Ethics Committee of Birjand University of Medical Sciences (Ethical code: IR.BUMS.REC.1403.135).

The experimental groups were as follows:

- Control, 4 weeks: Oral gavage with normal saline for 4 weeks.
- Control, 8 weeks: Oral gavage with normal saline for 8 weeks.
- Methadone, 4 weeks: Oral methadone administration for 4 weeks.
- Methadone, 8 weeks: Oral methadone administration for 8 weeks.
- Opium tincture, 4 weeks: Oral opium tincture administration for 4 weeks.
- Opium tincture, 8 weeks: Oral opium tincture administration for 8 weeks.

Administration and Dose Calculation

Methadone syrup (25 mg/5 mL) was obtained from Darou Pakhsh Pharmaceutical Company (Tehran, Iran). Opium tincture (1% solution, equivalent to 10 mg/mL) was purchased from Faran Shimi Pharmaceutical Company (Tehran, Iran).

In the methadone-treated group, animals received oral methadone syrup daily. The initial dose on day 1 was 5 mg/kg, which was gradually increased to 50 mg/kg[23] by day 7 and maintained at this level for the remainder of the experimental period.

Similarly, in the opium tincture-treated group, animals received oral opium tincture syrup daily. The starting dose on day 1 was 50 mg/kg, which was progressively increased to 200 mg/kg[24] by day 7 and maintained at this level until the end of the treatment phase.

Sample Collection

At the end of the treatment period, animals were weighed and anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (5 mg/kg).[25] Blood samples were obtained from the left ventricle for serum testosterone measurement. Testes and prostates were excised, weighed, and fixed in 10% neutral-buffered formalin.

Biochemical Analysis

Serum was obtained by centrifugation at 3000 rpm for 10 min. Testosterone concentrations were measured using a commercial ELISA kit (DiaSorin, Italy/Germany), according to the manufacturer's instructions, and absorbance was read with a Liaison® analyzer (DiaSorin, Italy/Germany).

Histological Processing and Morphometry

Testes and prostates were fixed in 10% neutral-buffered formalin and processed by routine paraffin embedding. Serial sections (5 μm) were cut on a rotary microtome, deparaffinized, and stained with hematoxylin and eosin (H&E). For each animal, three slides were prepared, and 10 randomly selected fields per slide were examined using a calibrated light microscope (Bioblue Euromex, China) equipped with a digital camera, at magnifications of 10x and 40x for testicular sections and 40x for prostatic sections. Morphometric analyses were performed using ImageJ software (NIH, the USA). Testicular parameters included seminiferous tubule cross-sectional area, germinal epithelium area, and the ratio of germinal epithelium area to total tubule area. Prostatic morphometry included measurement of the epithelial height of prostatic acini.

Johnsen's scoring (assessment of spermatogenesis)

Spermatogenic activity was evaluated according to the Johnsen scoring system (Johnsen, 1970). For each animal, at least 10 round cross-sections of seminiferous tubules were selected randomly from three slides (minimum 30 tubules per animal) using 40X magnification. Each tubule was assigned a score from 1 to 10, according to the standard criteria (Table 1).[26-28]

Statistical Analysis

Data were analyzed using GraphPad Prism software (version 8.4.3; GraphPad Software, the USA). The normality of data distribution was assessed with the Shapiro–Wilk test. Variables with normal distribution were compared among groups using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. For non-normally distributed variables, the Kruskal–Wallis test was applied, and pairwise comparisons were conducted using the Mann–Whitney U test when appropriate. All data are presented as mean ± standard deviation (SD), and statistical

significance was defined as $P < 0.05$.

Results

Relative Organ Weights and Serum Testosterone

Relative testicular and prostatic weights, along with serum testosterone concentrations, are presented in Table 2. Rats receiving opium tincture for either 4 or 8 weeks exhibited a significant reduction in relative testicular weight compared with their respective controls ($P < 0.05$ and $P < 0.05$, respectively). Relative prostatic weight was also markedly reduced in both opium groups, with differences observed in the 4-week opium group versus its control ($P < 0.001$) and in the 8-week opium group versus both the 8-week control and methadone groups ($P < 0.001$). Serum testosterone concentrations declined across all treatment groups; however, the reduction reached statistical significance only in the opium tincture group at both 4 weeks ($P < 0.01$) and 8 weeks ($P < 0.05$) compared with their matched control group.

Table 1. Johnsen-like scores for rat spermatogenesis adapted from Johnsen (1970)

Score	Description
10	Full spermatogenesis and normal tubular morphology
9	Many late spermatids and sperm cells
8	Few late spermatids and sperm cells
7	Many early spermatids, no sperm cells
6	Few early spermatids, no sperm cells
5	Many spermatocytes but no spermatids and sperm cells
4	Only a few spermatocytes present, no sperm cells
3	Only spermatogonia present
2	Only Sertoli cells present
1	No cells in the tubular section

Testicular Histology

Seminiferous Tubule Cross-Sectional Area

As shown in Table 3 and Figure 1A, rats treated with methadone or opium tincture for 4 weeks demonstrated a significant reduction in seminiferous tubule cross-sectional area compared with the 4-week controls ($P < 0.001$ for both). Similarly, in the 8-week experimental groups, both treatments resulted in markedly smaller seminiferous tubule areas relative to controls ($P < 0.001$).

Moreover, the 8-week opium group displayed a significantly lower mean value than the 8-week methadone group ($P < 0.001$). No significant differences were observed between the 4- and 8-week intervals within each treatment group, suggesting that the major reductions occur early, and then, stabilize during prolonged exposure.

Histological examination revealed intact seminiferous tubules and normal spermatogenesis in the control groups at both 4 and 8 weeks. Methadone Syrup and Opium Tincture groups showed disrupted seminiferous architecture, loss of germ cell, and vacuolization, which were more noticeable after 8 weeks. The analysis of the Johnsen's score revealed significantly reduced values in both treatment groups compared to the controls, with the lowest scores in the Opium Tincture group at 8 weeks ($P < 0.001$, Figure 1B).

Prostatic Histology

Prostatic Acinar Epithelial Height

As presented in Figure 2A-B and the corresponding data table, rats in the 4-week opium tincture group exhibited a significant reduction in prostatic epithelial height compared with both the 4-week control ($P < 0.001$) and methadone group ($P < 0.01$). No significant difference was observed between the 4-week methadone and control

Table 2. Comparison of testis weight index, prostate weight index, and serum testosterone concentration between the study groups.

Variable Group (n = 7)	Testis weight to body weight (g/g × 100)	Prostate weight to body weight (g/g × 100)	Testosterone (ng/mL)
Control (4 W)	0.71 ± 0.088	0.38 ± 0.03	2.22 ± 0.64
Control (8 w)	0.7 ± 0.086	0.39 ± 0.07	2.60 ± 0.96
Methadone (4 W)	0.67 ± 0.04	0.33 ± 0.06	0.65 ± 0.5
Methadone (8 w)	0.69 ± 0.05	0.42 ± 0.06	0.94 ± 0.66
Opium tincture (4 W)	0.59 ± 0.04*	0.23 ± 0.05**	0.46 ± 0.21*
Opium tincture (8 w)	0.58 ± 0.07$^{\#}$	0.2 ± 0.05$^{\#\#\#,\$\$\$}$	0.5 ± 0.26$^{\#}$

Values are reported as mean ± standard deviation (n = 7). *$P < 0.05$ and **$P < 0.01$: Significant compared with 4-week control group; #$P < 0.05$ and ###$P < 0.001$: Significant compared with 8-week control group; $$$$P < 0.001$: Significant compared with 8-week methadone group

Table 3. Comparison of mean seminiferous tubule area, germinal epithelium area, and the ratio of germinal epithelium to tubule area in the study groups

Variable Group (n = 7)	Tubule area (μm² × 1000)	Germinal epithelium area (μm² × 1000)	Ratio of germinal epithelium to tubule area (%)
Control (4 W)	101.96 ± 3.22	74.61 ± 9.69	73.2 ± 9.33
Control (8 w)	105.5 ± 7.74	73.9 ± 6.81	70.01 ± 2.96
Methadone (4 W)	66 ± 9.77***	41.22 ± 8.08***	62.14 ± 4.69*
Methadone (8 w)	69.84 ± 10.2$^{\#\#\#}$	40.82 ± 7.9$^{\#\#\#}$	58.14 ± 3.46$^{\#}$
Opium tincture (4 W)	56.74 ± 5.43***	31.53 ± 3.73***	55.63 ± 4.86***
Opium tincture (8 w)	52.48 ± 6.66$^{\#\#\#,\$\$\$}$	31.44 ± 5.98$^{\#\#\#}$	59.24 ± 5.92$^{\#}$

Values are reported as mean ± standard deviation (n = 7). *$P < 0.05$ and **$P < 0.01$: Significant compared with 4-week control group; #$P < 0.05$ and ###$P < 0.001$: Significant compared with 8-week control group; $$$$P < 0.001$: Significant compared with 8-week methadone group

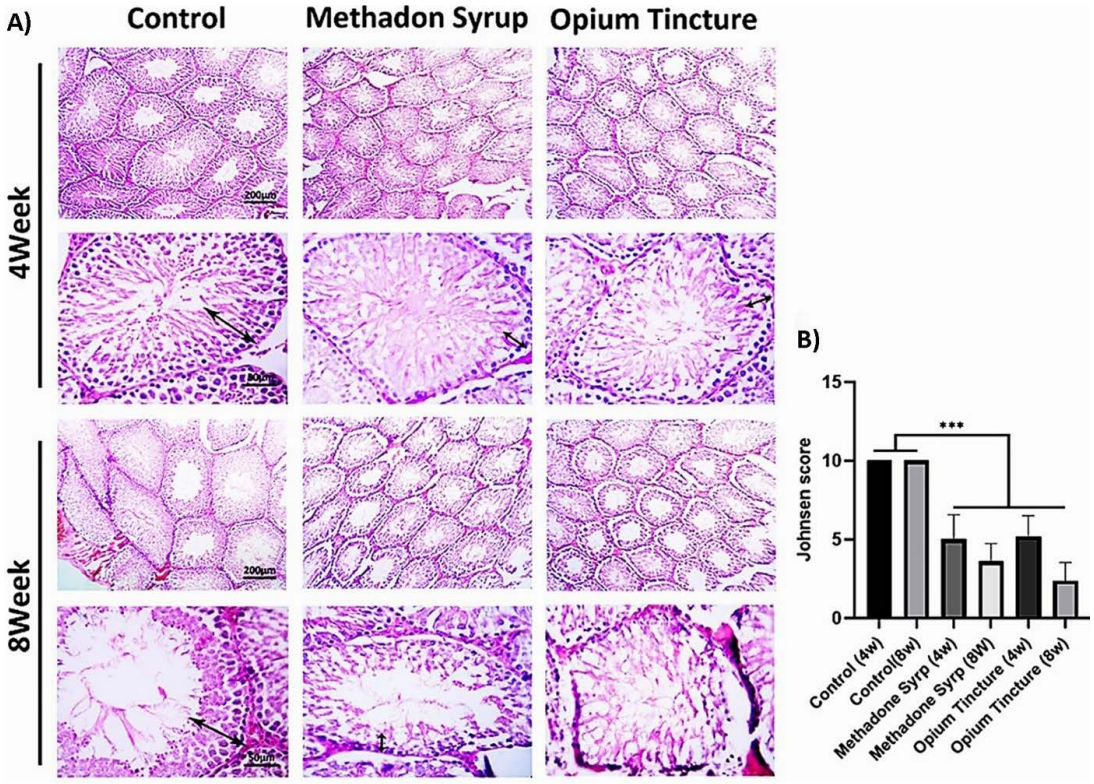

Figure 1. A) Representative histological sections of testicular tissue from control, methadone syrup, and opium tincture groups at 4 and 8 weeks. Hematoxylin and eosin (H&E) staining demonstrates the architecture of seminiferous tubules, germinal epithelium, and spermatogenic cells at 10x and 40x magnifications (scale bars: 200 µm and 50 µm, respectively). Black arrows indicate histological alterations. **B)** Johnsen score analysis of spermatogenesis in the experimental groups. Values are expressed as mean ± SD (n = 7). *** $P < 0.001$ vs. control groups

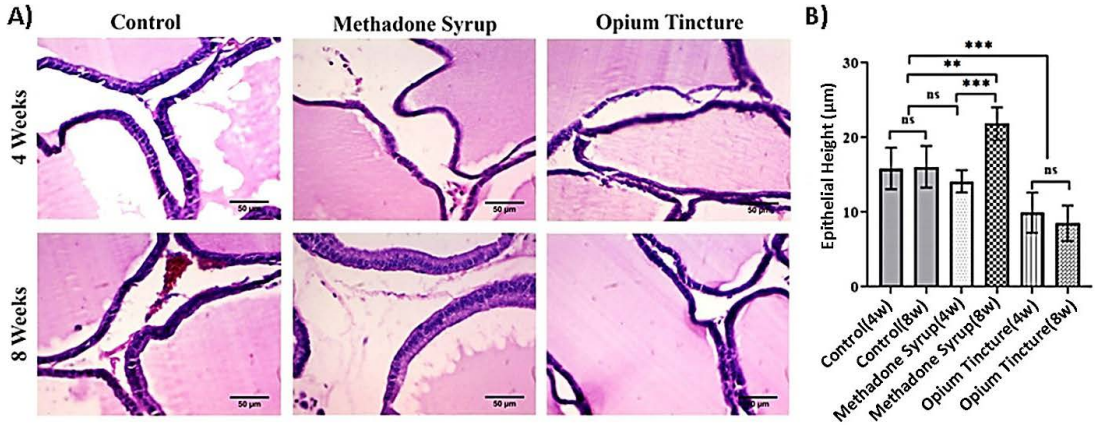

Figure 2. A) Representative histological sections of prostate tissue from control, methadone syrup, and opium tincture groups at 4 and 8 weeks. Hematoxylin and eosin (H&E) staining shows differences in epithelial structure and thickness at 40x magnifications (scale bars: 50 µm). **B)** Quantitative analysis of epithelial height in the experimental groups. Data are expressed as mean ± SD (n = 7). ** $P < 0.01$, *** $P < 0.001$ vs. respective control groups; ns: Not significant

groups ($P > 0.05$). At the 8-week interval, methadone administration resulted in a significant increase in epithelial height relative to the 8-week control ($P < 0.01$), whereas the 8-week opium tincture group showed a marked reduction ($P < 0.001$). Moreover, epithelial height was significantly greater in the 8-week methadone group compared with the 8-week opium tincture group ($P < 0.001$).

Discussion

This study demonstrated that chronic oral administration of opium tincture exerts more pronounced adverse effects on the male reproductive system of rats compared with methadone syrup. Opium tincture caused significant reductions in relative testicular and prostatic weights, serum testosterone concentrations[29], seminiferous tubule cross-sectional area, germinal epithelium thickness, and prostatic epithelial height, indicating extensive gonadal damage and secondary androgen deficiency.[30,31] Similar reductions in testicular weight and seminiferous tubule diameter have been reported in morphine- and methadone-treated rats, highlighting the gonadotoxic potential of chronic opioid exposure.[31-33]

The decline in circulating testosterone observed in the opium-treated groups is consistent with the well-known inhibitory action of opioids on the hypothalamic–pituitary–gonadal (HPG) axis. Opioids suppress hypothalamic GnRH

secretion, leading to the reduced LH and FSH release and impaired Leydig cell function, ultimately resulting in decreased androgen production[31,34]. This endocrine disruption explains the reduced spermatogenic activity and structural alterations of seminiferous tubules observed in the present study, and the findings also reported in rats exposed to morphine, methadone, or buprenorphine.[35,36]

Methadone treatment, while also associated with reductions in some reproductive parameters, appeared less detrimental than opium tincture. This relative difference has been noted in prior comparative studies, where methadone substitution was associated with partial preservation of testicular morphology and higher testosterone levels compared with natural opiates.[35]

These findings suggest that, although methadone exerts suppressive effects on the HPG axis, its impact may be less severe than that of crude opium preparations, possibly due to the differences in pharmacodynamics and the presence of multiple alkaloids in opium.

Consistent with our findings, Jamshidian et al. (2019) reported testosterone suppression following morphine sulfate injection,[21] while Jalili et al. (2016) observed reductions in seminiferous tubule diameter and germinal epithelium thickness after morphine exposure.[37] These changes were attributed to basement membrane thickening, impaired testicular metabolism, germ cell hypoplasia, and seminiferous tubule atrophy.[30] Across these studies, structural alterations such as epithelial thinning, Sertoli cell dysfunction, and germ cell loss were identified as primary mechanisms underlying opioid-induced gonadal damage.[21,37]

Chronic consumption of methadone syrup and opium tincture seriously interfered with spermatogenesis, resulting in the reduced Johnsen's scores and histopathological changes. This effect was time-dependent and more prominent with opium tincture, indicating a stronger gonadotoxic potential compared to methadone. These findings confirm the adverse effects of long-term opioid exposure on male reproductive function.[38]

Both central neuroendocrine and peripheral cellular pathways affect the harmful effects of long-term opioid exposure on the reproductive system. Opioids centrally act on μ-opioid receptors in the hypothalamus[39] to restrict gonadotropin-releasing hormone (GnRH) secretion, resulting in downstream suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from the pituitary gland. This hormonal inhibition interferes with the HPG axis, and consequently, reduction of testicular androgen synthesis and spermatogenic activity.[40]

Peripherally, at the molecular level, opioids bind to μ-opioid receptors expressed in Leydig and Sertoli cells, triggering intracellular cascades that increase reactive oxygen species (ROS) production and mitochondrial dysfunction, leading to oxidative stress-induced apoptotic changes in Sertoli cells, although the severity of damage does not necessarily increase with the duration of exposure, suggesting possible cellular adaptation or downregulation of destructive pathways.[41] The elevated levels of oxidative stress suppress testicular antioxidant defense such as glutathione peroxidase (GPx), resulting in lipid peroxidation and DNA fragmentation in germ cell.[8] Therefore, the significant histopathological and hormonal changes observed in this study can be explained by the synergistic effects of central gonadotropin suppression and local oxidative–apoptotic damage. Similar mechanisms have been described in studies linking opioid exposure to oxidative stress-induced testicular injury and germ cell loss.[29]

Prostatic histology revealed notable differences between opioids. Chronic opium tincture administration (4 and 8 weeks) significantly reduced acinar epithelial height, likely as a consequence of androgen depletion. This observation is consistent with the report of Felix-Patricio et al. (2017), who reported that testosterone reduction after orchiectomy caused epithelial thinning.[42] Conversely, Oliveira et al. (2018) demonstrated that testosterone cypionate treatment increases prostatic epithelial thickness, supporting the androgen dependency of this parameter.[43] Interestingly, methadone exposure for 8 weeks significantly increased prostatic epithelial height in our study, suggesting possible hyperplastic or compensatory responses,[44] which have also been noted in other models of opioid exposure.[30]

The comparisons between short-term (4 weeks) and long-term (8 weeks) exposure indicated that while tissue injury generally persisted, some parameters plateaued without significant further decline. This stabilization may reflect partial repair processes, cellular adaptation, or reduced tissue sensitivity to prolonged opioid exposure, a phenomenon also suggested in prior chronic morphine models.[41]

Conclusion

Chronic oral administration of opium tincture exerts more detrimental effects on male reproductive structure and function in rats than methadone syrup, as evidenced by reductions in serum testosterone, gonadal mass, and key histological indices. Although methadone exposure appeared less detrimental to androgen levels, prolonged treatment was associated with increased prostatic epithelial height, which may reflect compensatory or abnormal proliferative responses.

These findings, consistent with previous reports on morphine and other opioids, underscore the importance of careful consideration in the choice of opioid substitution therapy, including drug type, dose, and duration, with attention to potential long-term reproductive toxicity. Further molecular and translational studies are needed to elucidate the underlying mechanisms to assess the clinical relevance of these observations in humans.

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Competing Interests
The authors declared no conflict of interest.

Ethical Approval
The study was approved by the Ethics Committee of Birjand University of Medical Sciences (Ethical code: IR.BUMS.REC.1403.135).

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