

Tolerability and toxicity of lipidosterolic extract of american dwarf palm *Serenoa repens* in Wistar rats: well-known extract, new insight

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Abstract. – Background and Objectives: *Serenoa repens* extracts (SrE) have been used for centuries in the treatment of benign prostatic hyperplasia (BPH). According to recommendations that each product should be examined separately, including its tolerability and toxicity, we conducted this study in order to broaden the current cognition about tolerability and toxicity of SrE, in particular of German brand Prostamol-unoR.

Materials and Methods: Twenty-four adult male Wistar rats were randomly distributed into 4 groups of 6 animals. The first control group (O) received water (1 ml/kgBW) and second control group (OO) received olive oil (1 ml/kgb.w.) every day for 30 days. The third and fourth group of rats (SR5 and SR10) were treated with SrE (150 and 300 mg/kgb.w. daily) dissolved in olive oil. Tolerability and toxicity of SrE were estimated on the basis of daily monitoring of behavior, body weight gain (BWG), relative weight of liver, left kidney, prostate and left testis, and values of general biochemical parameters. Total liver proteins (TLP) and glutathione content in hepatocyte suspension were also determined.

Results: BWG was significantly unchanged in SR5 and SR10 compared to both controls in all intervals of measurement and at the end of treatment ($p > 0.05$). LW/BW ratio was significantly higher in SR10 compared with O ($p < 0.01$). Creatinine and potassium were significantly higher in SR5 compared to O ($p < 0.05$), but in SR10 were significantly higher compared to both control groups ($p < 0.01$). TLP content was significantly higher in SR5 compared to OO ($p < 0.01$). The content of glutathione in homogeneous suspension of hepatocytes didn't alter significantly.

Conclusions: Obtained results have expanded the current state of knowledge about the tolerability and toxicity of SrE, in particular of Prostamol-unoR. For the adoption of a more pre-

cise conclusion about its tolerability and toxicity, it should be excluded possible limiting factors that we identified in this study.

Key Words:

Serenoa repens, Tolerability, Toxicity, Wistar rats.

Introduction

Extracts of *Serenoa repens* (SrE) derived from partially dried American dwarf palm fruit Saw palmetto (family Arecaceae), which grows mainly in the southeast United States, from South Carolina to Florida. They are widely available and their uses in the treatment of patients with benign prostatic hyperplasia (BPH) are rising throughout of the world¹.

It is assumed that the major pharmacologically active substances are free fatty acids: lauric, oleic, myristic and palmitic²⁻⁴. Flavonoids and sterols exert anti-inflammatory effects that could also have an impact on the overall improvement of lower urinary tract symptoms (LUTS)⁵⁻⁶. Although the precise mechanism of action has not been clarified yet, it is found that SrE exerts antiandrogenic, proapoptotic and anti-inflammatory effects⁷.

Exact data related to the pharmacokinetics of different SrE have not been known till now. Yale et al⁸ revealed that SrE acts as strong inhibitor of CYP2D6, CYP3A4 and CYP2C9 liver enzymes that participate in metabolism of many drugs that potentially can lead to significant interactions. Markowitz JS et al⁹ did not find the impact of

multiple dose of SrE on the CYP2D6 and 3A4 liver enzymes. Some other Authors¹⁰ came to the same conclusion. Singh ZN et al¹¹ evaluated the influence of SrE on rat liver function by measuring its effects on several enzymes and formation of malondialdehyde (MDA), a byproduct of lipid peroxidation. Applied doses of 9.14 and 22.86 mg/kgb.w./day didn't cause a significant difference in animal body weight, enzyme activity, or MDA formation at the end of two and four weeks treatment.

The majority of adverse effects of SrE is mild, infrequent and reversible, and includes abdominal pain, diarrhea, nausea and fatigue, headache, decreased libido and rhinitis¹².

An intensive investigation of SrE has begun since the eighties. For this purpose, most commonly used product has been Permixon, n-hexane lipidosterolic extract of French producer Pierre Fabre Medicament. Of all herbal remedies, Permixon has been subjected to greater scientific scrutiny and is associated with more clinical trials and pharmacologic analyses than any other preparation of SrE⁷.

Today, a large number of preparations containing SrE, whether plain or in combination with other plant extracts and minerals is on the market. The composition of these preparations, depending on the manufacturer, is very different in terms of percentage of free fatty acids (from 40.7% to 80.7%) and other ingredients¹³.

Also, the lack of products' standardization is an additional problem. Analyzing six different preparations, Feifer AH et al¹⁴ concluded that the amount of active ingredients ranging from -97% to +140% compared to the one that was specified on the package.

Foregoing means that the results obtained in studies with Permixon could not be applied to the products of other manufacturers. In this regard and recommendations of the 5th International Consultation on BPH that each product, although derived from the same plant, should be evaluated individually and rigorously through both clinical and pharmacological trials¹⁵.

German brand Prostatamol-uno^R (Berlin-Chemie AG, Berlin, Germany) is the most commonly used herbal product for BPH in Montenegro. In the form of soft capsules containing 320 mg of the active substance, it can be purchased in state and private pharmacies. Our recent investigation found that its safety and tolerability during three-month treatment of patients with lower urinary tract symptoms (LUTS) due to BPH, was comparable to standard

pharmacotherapy¹⁶. Some earlier data also suggest that Prostatamol-uno^R is efficient and well tolerated by most users and is not associated with serious adverse effects¹⁷⁻¹⁸.

Bearing in mind the above recommendations, the purpose of this study was to enlarge the current cognition about tolerability and toxicity profile of SrE, in particular of Prostatamol-uno^R, in two dosage regimes in experimental animals.

Material and Methods

Study Design

Twenty-four sexually mature male Wistar laboratory rats, approximately weighing 250-300 g, were randomly distributed into 4 groups of 6 animals. The first control group (O) received only water (1 ml/kg BW p.o.) and second control group (OO) received olive oil (1 ml/kg BW p.o.) every day for 30 days. The third and fourth groups of rats (SR5 and SR10) were treated with SrE dissolved in olive oil. Preparations were prepared daily. Animals received the equal volume of solvent (olive oil) per kilogram of BW in which was dissolved the determined dose of SrE. Both control and investigated substances were administered every morning by feeding needle. Animals' bodyweight was measured weekly, so the applied dose was adapted to the new measured weight.

Substances and Doses

Following substances were used: extract of *Serenoa repens* (Prostatamol-uno^R, soft capsules 30 × 320 mg, Berlin-Chemie AG, Menarini Group, Berlin, Germany); olive oil (bottle 50 ml, Pharmacy "Biljana", Novi Sad, Serbia); urethane solution 25%; sulphosalicylic acid 4%; Ellman's reagent (0.2 mmol/dm³ DTNB* [*5.5'-dithiobis-(2-nitrobenzoic acid)] in 0.1 mol/dm³ K₃PO₄, pH=8); Biuret reagent (0.15% CuSO₄·5H₂O; 0.6% K, Na-tartrate, 0.1% KJ dissolved in 0.85 mmol/dm³ NaOH).

In determining the pharmacological dose of SrE in rats there was used Clark's formula, by which dose for rats were calculated in relation to the human dose:

$$X = \frac{D/kg \times \sqrt[3]{BW (man)}}{\sqrt[3]{BW (animal)}}$$

X = dose for animal; BW = bodyweight; D/kg = human dose

In order to investigate the tolerability and toxicological profile of SrE, obtained value (30 mg/kg BW/day) was multiplied with five (150 mg/kg BW/day) and ten (300 mg/kg BW/day), that means five and ten times higher dose than human recommended daily dose.

Experimental Procedures

After 30 days of treatment, the animals were measured, then anesthetized with 25% urethane solution (2.5 ml/kg intraperitoneally). Blood was collected to prepare serum for biochemical analyses. The liver was measured; a portion of 1 mg was removed for the preparation of homogenate, which was used for the determination of total protein and glutathione content in homogeneous suspension of hepatocytes. Left kidney, prostate and left testis were also separated and measured.

Tolerability and toxicity of SrE were estimated on the basis of daily monitoring of behavior and bodyweight gain (BWG) at weekly intervals and at the end of treatment. Additional parameters were liver weight/body weight (LW/BW) ratio, kidney weight/body weight (KW/BW) ratio, prostate weight/body weight (PW/BW) ratio and testis weight/body weight ratio (TW/BW). The value of urea, creatinine, electrolytes, transaminases (ALT and AST) and lactate dehydrogenase (LDH) was also measured using standard methodology.

For determination of glutathione content in liver homogenate and hepatocyte suspension, we used a method that was applied by Kapetenovic IM et al¹⁹ (modified Elman's method).

Total protein content was measured by a biuret method, modified by Gornall and Bardwall.²⁰

Animals

Rats were bred in the vivarium at the Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Novi Sad, Serbia. Animals were kept in standard plexiglass cages (six per cage) at standard laboratory conditions (light period of 12h/day, constant room temperature $21 \pm 1^\circ\text{C}$ and humidity $55\% \pm 1.5\%$). They were fed by standard laboratory rat feed, produced by the Veterinary Institute in Zemun, Serbia, with free access to food and water. Rats were allowed to acclimatize for two weeks before the application of preparations. Animal care and all experimental procedures were carried out in compliance with the Animal Care

Committee regulations of Medical School in Novi Sad, Serbia, and adhere to the recommendations from the Declaration of Helsinki.

Statistical Analysis

All the data were expressed as mean \pm SD of the mean and were analyzed by analysis of variance (ANOVA) for multiple comparisons, followed by Tukey's test where appropriate. The accepted level of significance was $p < 0.05$.

Results

By continual monitoring of experimental animals, there wasn't noticed any change in their behavior that could be attributed to the possible toxicological effect of applied preparations. No animal has died during treatment and food and water use was similar in all groups. There wasn't observed any change in rats' behavior during weekly measurements and cleaning of the cage.

However, from the fifth day to the end of one-month treatment, almost all rats from SR10 (300 mg/kg BW/day) refused application of the extract by feeding needle and expressed anxiety and visual aggressiveness during treatment.

Bodyweight gain (BWG) in rats that were treated with SrE in two dose regimes compared to control groups, in intervals of seven days, is shown on Figure 1.

BWG was significantly unchanged in SR5 (150 mg/kg BW/day) and SR10 (300 mg/kg BW/day) compared to both controls in all intervals of measurement and at the end of thirty-day treatment ($p > 0.05$). Also, there is no statistically significant difference in BWG between control groups ($p > 0.05$).

The influence of SrE in two doses on the liver weight/body weight (LW/BW), kidney weight/body weight (KW/BW), prostate weight/body weight (PW/BW) and testis weight/body weight (TW/BW) ratio in experimental animals is shown in Table I.

One-month treatment of experimental animals with SrE in a higher dose of 300 mg/kgBW daily significantly increased the LW/BW ratio compared with the control group O ($p < 0.01$), but not with OO ($p > 0.05$).

The PW/BW ratio was different between control groups of animals. As shown in Table I, the PW/BW ratio is significantly higher in OO compared to O ($p < 0.05$), and values of SR5 and

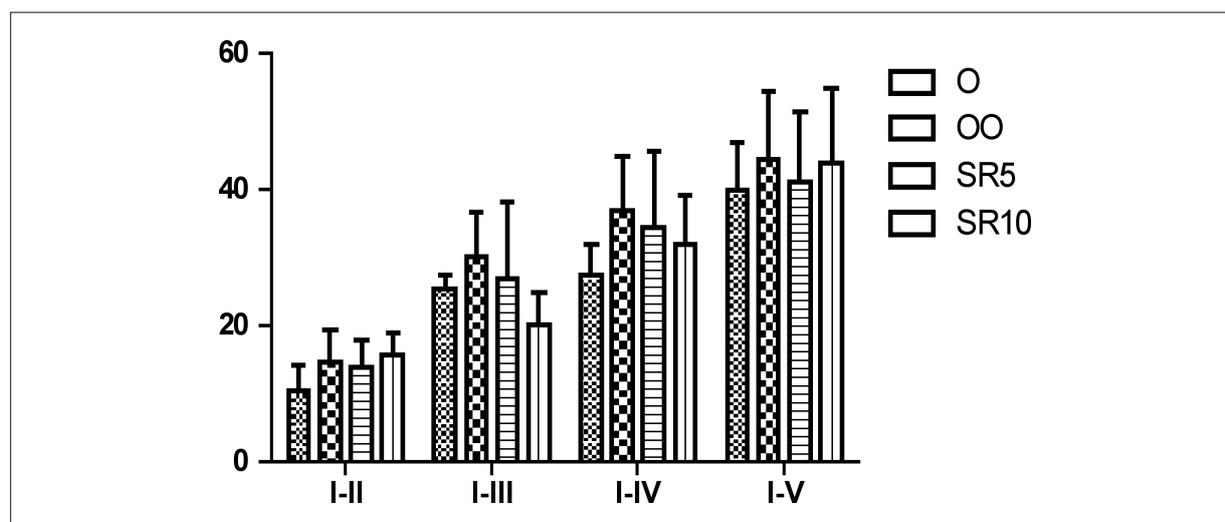


Figure 1. Bodyweight gain (BWG) in rats chronically treated with SrE (SR5 and SR10) compared to control substances (O and OO), in intervals of seven days, expressed in percentages (%) (Mean \pm SD) ($p > 0.05$). O: first control group (n=6), water (1 ml/kg BW/day p.o.); OO: second control group (n=6), olive oil (1 ml/kg BW/day p.o.); SR5: first experimental group (n=6), extract of *Serenoa repens* (150 mg/kg BW/day p.o.) dissolved in olive oil; SR10: second experimental group (n=6), extract of *Serenoa repens* (300 mg/kg BW/day p.o.) dissolved in olive oil.

SR10 are between obtained results, but without statistically significant difference ($p > 0.05$).

One-month treatment with both SrE didn't cause any difference in KW/BW and TW/BW ratio.

SrE in two doses also caused some changes in the values of biochemical parameters that were analyzed. The results are shown in Table II.

Five times higher dose of SrE than human significantly increased ($p < 0.05$) the values of serum creatinine and potassium compared to the control group O. SrE in tenfold higher daily dose high significantly increased ($p < 0.01$) the creatinine compared to the first control group of animals (O), but significantly ($p < 0.05$) compared to the OO. The same dose high significantly ($p < 0.01$) increased the serum potassium values in relation to both control groups.

The effect of SrE (150 and 300 mg/kg BW/day) on AST, ALT, LDH, total protein content in the liver (in mg of proteins per g of the liver) and glutathione content in liver homogenates and suspensions of hepatocytes, is shown in Table III.

At first, it must be noted here that one-month treatment with olive oil high significantly reduced ($p < 0.01$) the content of total proteins in the liver compared to the control O.

Chronic treatment with SrE in a lower dose significantly decreased ($p < 0.05$) ALT values in relation to the first control group, but AST and LDH values remained statistically unchanged compared to both controls. SrE in dose of 300 mg/kg BW/day increased the values of transaminases and LDH, but the difference didn't achieve statistical significance ($p > 0.05$).

Table I. Body weight (BW) at the end of treatment, liver weight/body weight (LW/BW), kidney weight/body weight (KW/BW), prostate weight/body weight (PW/BW) and testis weight/body weight (TW/BW) ratio in rats who were treated with SrE (150 and 300 mg/kg BW/day p.o.) compared with control groups (O and OO) (Means \pm SD, n=6 rats).

Group	BW (g \pm SD)	LW/BW ($\times 10^{-3}$) \pm SD	KW/BW ($\times 10^{-3}$) \pm SD	PW/BW ($\times 10^{-3}$) \pm SD	TW/BW ($\times 10^{-3}$) \pm SD
O	414.33 \pm 6.85	31.87 \pm 0.19	3.27 \pm 0.05	1.37 \pm 0.12	3.60 \pm 0.22
OO	415.83 \pm 34.17	33.55 \pm 2.34	3.27 \pm 0.17	1.78 \pm 0.27**	3.42 \pm 0.53
SR5	417.05 \pm 37.89	31.17 \pm 2.98	3.55 \pm 0.33	1.53 \pm 0.17	3.23 \pm 0.49
SR10	432.80 \pm 33.25	34.38 \pm 0.98*	3.13 \pm 0.16	1.58 \pm 0.24	3.50 \pm 0.32

* $p < 0.01$ vs. first control group (O); ** $p < 0.05$ vs. first control group (O).

Table II. The values of serum urea, creatinine, potassium, sodium and chloride in Wistar rats who were treated with SrE (150 and 300 mg/kg BW/day) for 30 days and control groups of animals (O and OO) (Means \pm SD, n=6 rats).

Group	Urea (mmol/l)	Creatinine (μ mol/l)	Potassium (mmol/l)	Sodium (mmol/l)	Chlorides (mmol/l)
O	9.50 \pm 0.45	55.67 \pm 4.50	4.10 \pm 0.67	153.00 \pm 2.83	96.00 \pm 0.82
OO	9.18 \pm 0.93	55.50 \pm 7.61	4.50 \pm 0.70	155.50 \pm 1.38	101.00 \pm 1.15
SR5	9.00 \pm 0.91	63.00 \pm 5.73*	5.12 \pm 0.37*	155.20 \pm 0.98	104.00 \pm 1.79 [§]
SR10	10.85 \pm 0.67	69.50 \pm 6.54 ^{§, #}	5.98 \pm 0.33 ^{§, ‡}	153.75 \pm 1.64	102.75 \pm 1.30 [§]

* $p < 0.05$ vs. first control group (O); [§] $p < 0.01$ vs. first control group (O); [#] $p < 0.05$ vs. second control group (OO); [‡] $p < 0.01$ vs. second control group (OO).

Lower dose of 150 mg/kg BW/day increased both the content of total proteins and glutathione in the liver in comparison to control groups, but statistically significant difference was observed only in total protein content compared to second control group OO ($p < 0.01$).

Applying the statistical analysis to the results in Table III, there hasn't been found statistically significant difference in the values of glutathione in liver homogenates between groups of animals which were treated with SrE (SR5, SR10) and control groups (O and OO).

Discussion

Thirty-day treatment with SrE in five (150 mg/kgBW) and ten (300 mg/kgBW) times higher daily doses than human, experimental animals survived well and none died during treatment. If it is known that applied dose of 300 mg/kgBW is approximately sixty times higher in comparison to human daily dose of SrE and did not exhibit any lethal effect in experimental animals during one-month treatment, it most likely confirms a favorable toxicological profile of SrE, in particular of

Prostamol-uno^R. In favor of previous assumption, both doses of SrE didn't significantly alter BWG in SR5 and SR10 compared to both controls in all intervals of measurement and at the end of treatment ($p > 0.05$). Although, possible protective effect of olive oil as a solvent, as the relatively small number of treated animals (n=6), can be limiting factors in making a final conclusion.

Continuously observing the behavior of experimental animals, it was noticed the existence of anxiety and expressed aggressiveness during per oral application of SrE in group of animals who received the ten times higher daily dose in relation to the human. It could be the consequence of many possible influences of SrE in applied dose (unpleasant taste and/or smell, possible gastrointestinal disturbances, some psychotropic effect of high dose of SrE) or influence on some another level, that we can't specify on the basis of obtained results.

The liver weight/body weight (LW/BW) ratio was higher in SR10 compared to both control groups and also with SR5, but achieved statistically significant difference ($p < 0.01$) only in comparison with control that received water (O). This could indicate the potential hepatotoxicity of SrE in applied dose of 300 mg/kg BW/day. However, it must be noted here that LW/BW ratio was ap-

Table III. AST, ALT, LDH, total protein and glutathione content in liver homogenates and suspension of hepatocytes, measured after one-month treatment with SrE (150 and 300 mg/kg BW/day), compared with control groups (O and OO) (Means \pm SD, n = 6 rats).

Group	AST (U/l)	ALT (U/l)	LDH (U/l)	Total liver proteins (X \pm SD)(mg/g)	Glutathione (X \pm SD) x10 ⁻⁶ (mol/mg)
O	196.33 \pm 9.57	62.00 \pm 9.20	2745.00 \pm 304.62	371.00 \pm 11.43	0.0131 \pm 0.0009
OO	207.17 \pm 51.65	54.17 \pm 16.36	3568.17 \pm 1129.25	346.50 \pm 9.69*	0.0136 \pm 0.0021
SR5	197.20 \pm 58.49	50.60 \pm 5.78 [§]	3084.40 \pm 772.32	402.50 \pm 33.02 [#]	0.0142 \pm 0.0016
SR10	279.75 \pm 91.00	72.00 \pm 13.04	3664.50 \pm 1485.01	362.25 \pm 10.35	0.0132 \pm 0.0013

* $p < 0.01$ vs. first control group (O); [§] $p < 0.05$ vs. first control group (O); [#] $p < 0.01$ vs. second control group (OO).

proximately the same in the group of animals that received five times higher dose of SrE (150 mg/kg BW/day) as in group O, while olive oil in a dose of 1 ml/kg BW/day also caused a rise of LW/BW ratio to some degree in relation to group O. Since olive oil was used as the solvent for both doses of SrE, it can not be ruled out that the increase of LW/BW ratio in group SR10 has been partially caused by the contribution of olive oil. In these terms, a final position on possible hepatotoxicity of SrE in a dose of 300 mg/kg BW/day can not be made based on the obtained results.

Five times higher dose of SrE didn't influence animals' behavior, BWG or LW/BW ratio significantly ($p > 0.05$), but its potentially toxic effects can not be excluded because olive oil as a solvent could act as hepatoprotective factor, that was shown in some studies.²¹⁻²³ According to previously stated, for the adoption of a more precise conclusion about the possible hepatotoxicity of SrE, it would be necessary to eliminate olive oil as a solvent and, if it is possible, give animals the pure extract.

One-month treatment with olive oil (1 ml/kg BW/day) significantly increased the prostate weight/body weight (PW/BW) ratio in Wistar rats compared to animals who received only water (O) ($p < 0.05$), but SrE showed the tendency to decrease those difference. Some studies found that increased consumption of both butter and margarine was positively associated with BPH risk, but no overall association was found with respect to consumption of olive oil²⁴. Without pathological examination of rats' prostate and some additional analyses, it can't be possible to determine precisely at which level olive oil influenced the relative weight of rats' prostate. Also, additional studies are needed to investigate the reduction of effects of olive oil on rats' prostate by SrE to some extent. If we know that both proliferation of prostate tissues and anti-androgenic effect of drugs (5-alpha reductase inhibitors and androgen receptor antagonists) need more time than one month, it will be interesting to explore obtained findings with more details.

Significantly higher values of serum creatinine, potassium and chloride in experimental animals which received SrE in both dosage regimes compared with controls, could be the consequence of nephrotoxicity and/or skeletal muscle toxicity of SrE in applied doses. Similar results hasn't been published till know, although the existing research mainly was done with small doses compared to ours.

Unchanged values of urea, transaminases, lactate dehydrogenase and glutathione content in the liver ($p > 0.05$) probably speak in favor of the absence of oxidative damage and/or reduction of the functional capacity of the liver under the influence of applied SrE. Our results are consistent with the findings of Singh et al¹¹ who came to the same conclusion by measuring SrE effects on several enzymes and formation of malondialdehyde (MDA), but with too much smaller doses in comparison with ours (9.14 and 22.86 mg/kg BW/day).

An interesting result is that the lower dose of SrE significantly increased ($p < 0.01$) the content of total liver protein compared with the control that received olive oil, while the content of protein does not differ in group SR10 compared to both controls ($p > 0.05$). Neither here it can not be eliminated the effect of olive oil. High significantly reduction of total protein content in the liver as the result of one-month treatment of rats with olive oil in a dose of 1 ml/kg BW/day, could be explained as the consequence of the increased hepatic fat accumulation and fatty infiltration of the liver²²⁻²³. Richter et al²⁵ in a similar experimental model have found the existence of moderate, non-degenerative fatty infiltration of the liver which started in periportal spaces. These changes were attributed to the effects of monounsaturated oleic acid. In this regard, possible conclusion could be that the dose of SrE of 150 mg/kgBW/daily actually acted as a protective factor against fatty infiltration of the liver caused by the olive oil.

Conclusions

The findings of this investigation have expanded the current state of knowledge about the tolerability and toxicity of SrE, in particular of German brand Prostamol-uno^R. If we know that a human dose of SrE is 320 mg daily, that is about 5 mg/kgBW, it can be concluded that investigated preparation is safe for human use most likely.

Olive oil as the solvent has been shown as the limiting factor in the precise interpretation of obtained results, because it demonstrated specific effects and probably influenced the tolerability and toxicity of SrE to some extent. Also, in order to obtain more exact conclusions, it would be desirable to examine the tolerability

and toxicity of SrE and in another animal model over a longer period of time by applying additional experimental procedures that were identified in this study.

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