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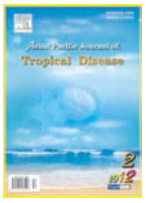

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Effect of embelin on lithium-induced nephrogenic diabetes insipidus in albino rats

Ashish K Sahu[1,2], MK Gautam[2], Pradeep T Deshmukh[1*], Lokendra S Kushwah[1], Narendra Silawat[1], Zafar Akbar[1], MS Muthu[2]

[1] *Department of Pharmacology and Toxicology, BR Nahata College of Pharmacy, Mandsaur-458001, India*
[2] *Department of Pharmacology, Faculty of Medicine, Institute of medical sciences, Banaras Hindu University, Varanasi-221005, India*

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ABSTRACT

Objective: To evaluate the nephroprotective and anti-polyuric role of embelin on lithium induced nephrogenic diabetes insipidus (NDI) in albino rats. **Methods:** NDI induced by lithium chloride (4 meq/kg/day, i.p. for 6 days) which leads to huge amount of urine excretion. After induction of NDI, embelin (50 and 100mg/kg) was administered orally, once daily for 21 day in rats and N-acetyl cysteine (10mg/kg, twice daily, i.p.) was used as a standard drug for treatment of NDI. The body weight, urine protein, urine creatinine, plasma creatinine, blood urea nitrogen were assessed at 0, 7, 14 and 21 day. At the end of the study glutathione (GSH) content in kidney was assessed and histopathology of kidney was performed. **Results:** Embelin 50 and 100 mg/kg showed increase in the body weight and decrease in plasma and urine creatinine, blood urea nitrogen levels, and urine protein level. Embelin acts as a potent antioxidant; it increases the level of glutathione in kidney. Histopathological examination of the kidney indicated that embelin 50 and 100 mg/kg were reduced the vascular degeneration of tubules as well as slight degeneration and dilatation of renal tubules, however N-actyl cysteine (NAC) treated rats showed normal glomeruli and renal tubule with slight degeneration. **Conclusions:** Embelin seemed to be effective in NDI by its predominant effect on promoting antioxidant status and decrease the urine excretion may be due to the blocking of sodium channels.

1. Introduction

Diabetes insipidus is a syndrome in which huge amount of urine output, low urine-specific gravity, high plasma osmolality and high plasma sodium. It is produce when the kidney is not able to control plasma osmolality due to a defect in the action of arginine vasopressin (AVP)[1]. Nephrogenic diabetes insipidus (NDI) is a type of diabetes insipidus, manifested by a lack of response of the collecting duct to circulating anti-diuretic hormone (ADH) and cause frequent and excessive urination (Polyuria). Prolonged polyuria of any cause can result in partial NDI as a consequence of dissolution of the intrarenal solute gradient that facilitates renal water resorption. NDI results when the kidneys are unable to respond to ADH. Polyuria is a frequent complication in long-term use of lithium therapy. The kidney ability to respond to ADH can be impaired by drugs like lithium and by chronic disorders including polycystic kidney disease, sickle cell disease, kidney failure, partial blockage of the ureters, and inherited genetic disorders[2]. Polyuria is a frequent complication in long-term use of lithium therapy. Lithium is a frequent therapeutic agent used to treat patients with various manic depressive illness. However, this medication has a narrow therapeutic index and, therefore, frequent side effects. It has been associated with several forms of renal injury, which is impaired urinary concentrating ability. It is estimated to be present in at least 50% individuals on chronic lithium therapy. Initially, the decreased urinary concentrating ability is largely reversible after cessation of lithium. However, with continued treatment, this defect translates into irreversible polyuria and polydipsia in up to 20% of population, which is resistant to the actions of arginine vasopressin (AVP). This functional lesion is associated with a chronic focal interstitial fibrosis predominantly in the medullary region of the kidney, which may be progressive, leading to renal failure[3, 4].

Embelin (3-undecyl 2,5-dihydroxy, 1,4-benzoquinone) is an naturally occurring alkyl substituted hydroxy

*Corresponding author: Dr. Pradeep T Deshmukh, Department of Pharmacology and Toxicology, BR Nahata College of Pharmacy, Mandsaur-458001, India
Tel: +91-9369614200
Fax: -91-0542-2367568
E-mail: sahuaship@gmail.com

benzoquinone obtained from dried fruits of *Embelia ribes* (Myrsinaceae), is reported to be an effective analgesic and anti-fertility agent. Embelin is reported to posses anti-bacterial, wound healing, free radical scavenging and antioxidant activity, anti-fertility, anti-implantation, anti-tumour, anti-inflammatory and analgesic and anti-ulcer property[5, 6]. It is also reported to impair the inflammatory signaling though inhibition of nuclear factor kappa B (NF-kappa B) activity and beneficiary effect on acetic acid-induced colitis model[7].

2. Materials and methods

2.1. Drugs and chemicals

Lithium chloride and embelin purchased from Loba chemie, Mumbai and Natural Remedies, Benguluru, India, respectively. All other chemical and reagents used were of analytical grade.

2.2. Animals

Male albino rats (150-200 g) of wistar strain were procured from animal house, BRNCP, Mandsaur and maintained under laboratory condition of 12 h light/12 h dark cycle, 25±3 ℃ and 35%-60% humidity. The rats were maintained at standard chow diet and water ad libitium. Animals described as fasted had been deprived of food for 16 hr but has been allowed to free access to water. 'Principles of laboratory animal care' (NIH publication no. 82-23, revised 1985) guidelines were followed. Approval from the Institutional Animal Ethical Committee was taken prior to the experimental (121/MPh/09/IAEC/BRNCP/08-09/Mandsaur).

2.3. Induction of NDI and treatment protocol

Lithium chloride (4 meq/kg/day or 27.76 mg/kg/day, once a day) dissolved in normal saline and injected intraperitonialy for 6 days[6]. Animals with profound polyuria were selected for study. Daily urine output was assessed for confirmation of NDI. The drug treatment was started after 6th day of lithium chloride administration. Normal control rats were received normal saline (i.p.), Lithium treated control rats were received normal saline (i.p.), other NDI animals treated with embelin 50 and 100 mg/kg dose in 1% tween 80 vehicle, orally[6]. Standard control rats were received N-acetyl cysteine (10mg/kg i.p. twice a day)[8].

2.4. Sample collection

Blood was collected by retro-orbital method from the eye. The withdrawn blood was centrifuged with 5000 r/min for 10 min by using centrifuge and plasma was collected and used for the determination of creatinine and blood urea nitrogen. Urine was collected with the help of metabolic cages in graduated tubes and volume was measured. Biochemical parameters like urine protein and urine creatinine were assessed. After 14 days of the treatment the animals were euthanized by over dose of ether anaesthesia and the kidney was collected for the determination of GSH content and histopathology.

2.5. Effect on body weight after induction of NDI

The above parameters were measured on the 0, 7, 14 and 21 day of the experiment. Each rat was individually weighed using standard rat weighing machine and their respective weights were noted down.

2.6. Biochemical parameters

Plasma creatinine and blood urea nitrogen (Transasia Biomedicals); urine protein and urine creatinine were measured using a commercial kit (Span Diagnostics).

2.7. Estimation of glutathione level in kidney

Kidney was isolated and mixed with saline (2 ml), further it was homogenized in 5 mL of cold KCl (1.15%) and precipitated with trichloroacetic acid (0.5 mL). From this, 0.5 mL of supernatant was taken and in this 2 ml phosphate buffer (pH 8.9) was added. Subsequently, 0.1 ml of DTNB (0.04%) solution was added and absorbance was taken against blank prepared at 412 nm[9].

2.8. Statistical analysis

Statistical comparison was performed using either unpaired't' test or one way analysis of variance (ANOVA) and for multiple comparisons versus control group was done by Dunnett's test.

3. Results

3.1. Effect of embelin on body weight after induction of NDI

Little or no change was observed on body weight between the lithium-treated and embelin/N-acetyl cysteine treated animals from 0 day to 21 day of study treatments.

3.2. Effect of embelin on plasma creatinine and blood urea nitrogen after induction of NDI

The effect of embelin on plasma creatinine and blood urea nitrogen in lithium chloride induced NDI is presented in Table 1 and 2 respectively. The result showed that the lithium chloride increase the plasma creatinine and blood urea nitrogen in lithium control animals while, treatment with embelin 50 and 100 mg/kg was significantly decrease (P<0.001) plasma creatinine and blood urea nitrogen on 14 day compared to lithium control group. N-acetyl cysteine also decrease the level (P<0.001) on 14 day (Table 1 and 2).

3.3. Effect of embelin on urine volume, urine protein and urine creatinine after induction of NDI

Urine volume was increased after lithium treatment in all groups of the animal compared to normal control group and after treatment with embelin/N-acetyl cysteine urine volume was significantly decreased (P<0.001) after 14 days treatment (Figure 1). Urine protein and urine creatinine level significantly increase in lithium treated rats. After treatment

Table 1.
Effect of embelin on plasma creatinine (mg/dL) after induction of NDI in rats.

Groups	Before treatment		After treatment	
	0 day	7 day	14 day	21 day
Normal control	0.4478 ± 0.065	0.4523 ± 0.063	0.4523 ± 0.063	0.4523 ± 0.063
Lithium control	0.4250 ± 0.034	1.1170 ± 0.013*	1.1230 ± 0.015*	1.1460 ± 0.010*
Lithium + Embelin 50 mg/kg	0.4767 ± 0.021	1.1420 ± 0.018	0.7170 ± 0.006 c	0.7100 ± 0.006 c
Lithium + Embelin 100 mg/kg	0.4833 ± 0.051	1.1700 ± 0.007	0.7110 ± 0.0006 c	0.7100 ± 0.005 c
Lithium + N-acetyl cysteine 10 mg/kg	0.4317 ± 0.015	1.1530 ± 0.015	0.7040 ± 0.001 c	0.7000 ± 0.001 c

Results are mean ± SEM (n=6). * P<0.001 compared to respective normal control group and c P<0.001 compared to respective lithium control group.

Table 2.
Effect of embelin on blood urea nitrogen (mg/dl) after induction of NDI in rats.

Groups	Before treatment		After treatment	
	0 day	7 day	14 day	21 day
Normal control	22.23 ± 0.675	22.22 ± 0.673	22.21 ± 0.672	22.20 ± 0.665
Lithium control	22.36 ± 0.824	87.99 ± 0.332*	88.78 ± 0.173*	88.78 ± 0.170*
Lithium + Embelin 50 mg/kg	22.74 ± 0.961	87.32 ± 0.470	63.96 ± 0.010c	63.91 ± 0.003c
Lithium + Embelin 100 mg/kg	23.04 ± 1.050	87.64 ± 0.483	63.42 ± 0.134c	63.38 ± 0.130c
Lithium + N-acetyl cysteine 10 mg/kg	22.54 ± 0.830	86.87 ± 0.360	61.41 ± 0.300c	61.35 ± 0.300c

Results are mean ± SEM (n=6). * P<0.001 compared to respective normal control group and c P<0.001 compared to respective lithium control group

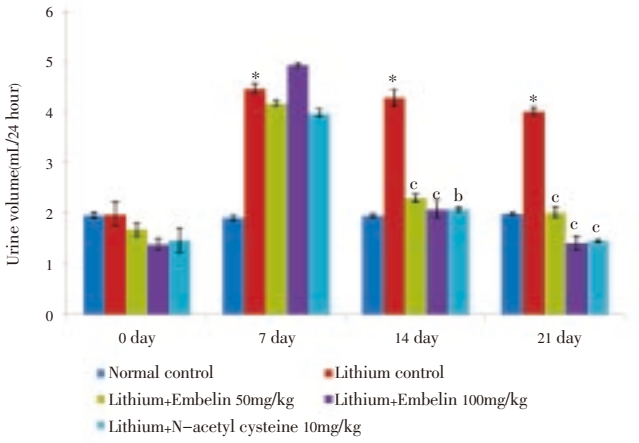

Figure 1. Effect of embelin on urine volume (ml/24 hour). Results are mean ± SEM (n=6), *P<0.001 compared to respective normal control group and bP<0.01, cP<0.001 compared to respective lithium control group.

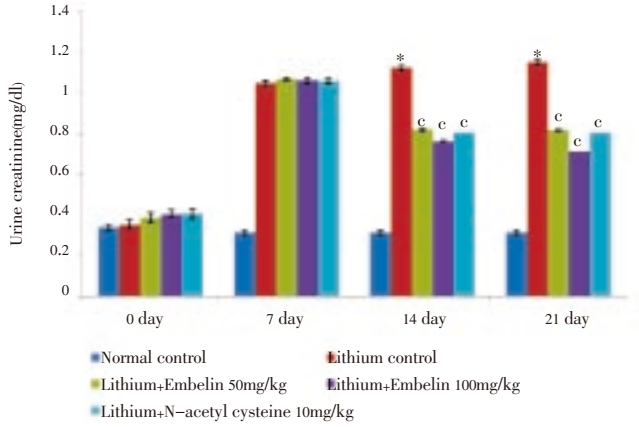

Figure 2. Effect of embelin on urine creatinine (mg/dl). Results are mean ± SEM (n=6), *P<0.001 compared to respective normal control group and cP<0.001 compared to respective lithium control group.

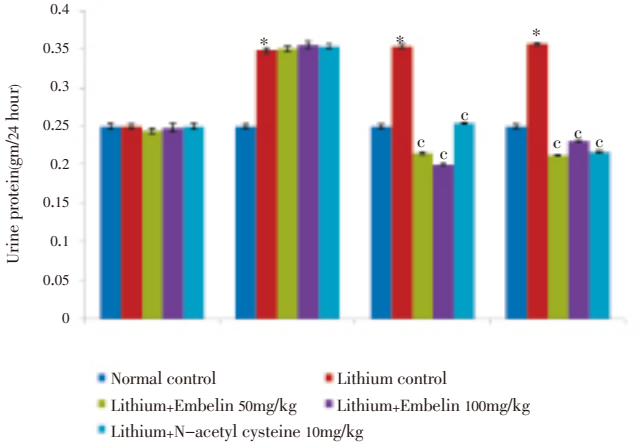

Figure 3. Effect of embelin on urine protein (gm/24 hour). Results are mean ± SEM (n=6), *P<0.001 compared to respective normal control group and cP<0.001 compared to respective lithium control group.

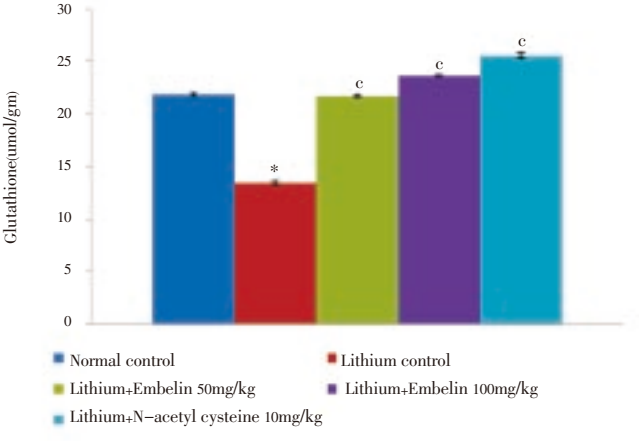

Figure 4. Effect of embelin on glutathione level (μ mol/gm). Results are mean ± SEM (n=6), *P<0.001 compared to respective normal control group and cP<0.001 compared to respective lithium control group.

with embelin/N-acetyl cysteine, the level of urine protein and urine creatinine was significantly decreased (P<0.001)

on 7 and 14 day (Figure 2 and 3).

3.4. Effect of embelin on kidney glutathione after induction of NDI

Kidney glutathione level was decrease in lithium induced NDI rats while, treatment with embelin 50 and 100 mg/kg glutathione level was increases significantly ($P<0.01$) in kidney tissue (Figure 4).

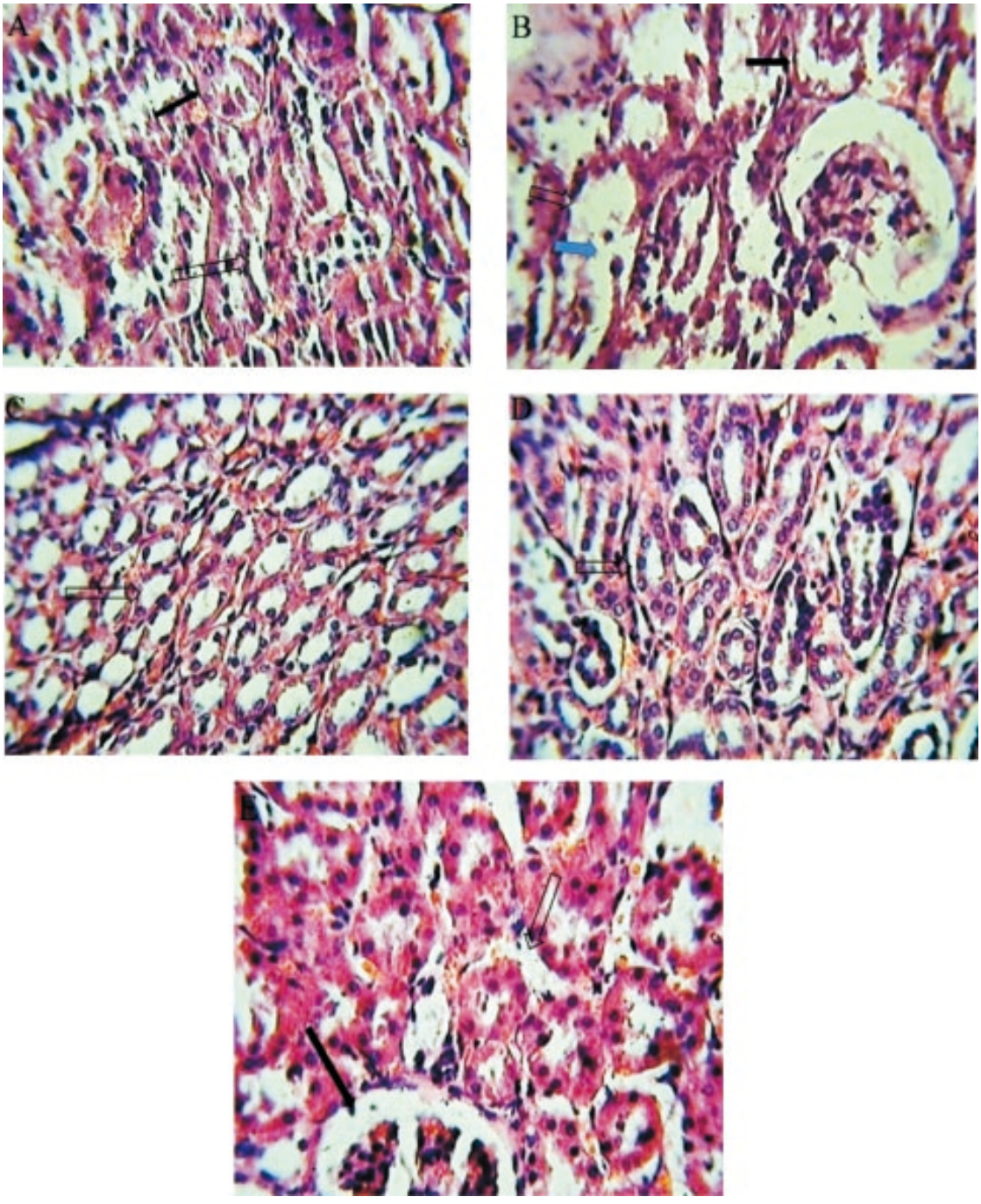

Figure 5. Photomicrographs of rat kidney (10x magnifications)
A: Histopathology of normal rat kidney showing normal renal tubule (showed by hollow arrow) and glomeruli (showed by solid black arrow); B: Histopathology of lithium control group showing renal tubular damage (showed by hollow arrow) characterized by dilatation of renal tubules and focal flattening of renal tubular epithelial cells (showed by solid blue arrow); C: Histopathology of embelin 50 mg/kg treated rats showing vascular degeneration of tubules (showed by hollow arrow) is reduced; D: Histopathology of embelin 100 mg/kg treated rats showing slight degeneration of renal tubules (showed by hollow arrow) and slight dilatation of renal tubules; E: Histopathology of N-acetyl cysteine treated rats showing normal glomeruli (showed by solid black arrow) and renal tubule (showed by hollow arrow) with slight degeneration.

4. Discussion

Prolonged use of lithium therapy may deteriorate the renal function even after discontinuing lithium treatment and could induce renal failure in a slowly progressive manner and its rate of progression is related to the both duration and cumulative dose of the drug. NDI is the most common side effect of lithium therapy, possibly resulting from down regulation of aquaporin-2 expression in the collecting

duct. With long-term use, chronic tubulointerstitial nephritis and tubular microcysts are identified[10]. Lithium accumulates within the collecting tubule cells, after entering the cells through sodium channels in the luminal membrane, lithium then interferes with ability of ADH. The predominant form of renal disease associated with lithium therapy is tubulointerstitial nephropathy; electron microscope examinations have demonstrated severe lesions in the mitochondria and endoplasmic reticulum in renal tissue of patients with lithium nephrotoxicity, implicating ischaemia as one of the pathogenetic mechanism. Under such circumstances, any other co-morbid conditions such as diabetes or a decrease in renal blood flow may result in a further reduction in tissue oxygenation and augment lithium- mediated toxicity[11, 12].

In our present study treatment with lithium caused polyurea relative to the normal control. Embelin 50 and 100 mg/kg and N-acetyl cysteine showed no change in body weight of the rats in comparison to disease control group treated with lithium chloride. Administration of lithium chloride showed a significant rise in plasma creatinine, blood urea nitrogen, urine protein, urine creatinine and urine volume with respect to the vehicle control, which clearly indicates intrinsic acute renal failure[8]. From our study embelin at a dose of 50 and 100 mg/kg rendered significant protection against lithium induced nephropathy which was evident by the decreased plasma creatinine, blood urea nitrogen levels, and urine protein. Also embelin 50 and 100 mg/kg showed an inhibitory effect on polyuria induced by lithium. This inhibitory action on polyuria may be due to the sodium channel blocking action of embelin.

Cellular antioxidants are protective against the free radicals which are deleterious for the tissue milieu. This antioxidant system was deranged after treatment with Lithium chloride. Oxidative stress and subsequently cellular damage due to lithium could decrease the glutathione level[13]. Renal medullary cells are exposed to the stress of long-term hyperosmolality and respond to this stress by accumulating compatible organic osmolytes. Renal vasoconstriction is may be due to increased oxidative stress as a result increased local production of superoxide hence decrease in nitric oxide bioavailability[8,14]. Embelin in a dose of 100 mg/kg acts as potent antioxidant or free radical scavenger which induces the glutathione level. The histopathological findings of kidney shows that rats treated with lithium chloride caused diffused tubular necrosis and several dilated tubuli with intraluminal tubular cells fragments. Histopathology renal study of rat showed that embelin in different dosages significantly protected the glomerular and tubular injury in comparison to negative control group. Embelin 50 mg/kg and 100 mg/kg in lithium treated rats reduces the vascular degeneration of tubules as well as slight degeneration and dilatation of renal tubules; however N-acetyl cysteine treated rats showed normal glomeruli and renal tubule with slight degeneration.

Embelin seemed to be effective in NDI by its predominant effect on promoting antioxidant status and decrease the urine excretion may be due to the blocking of sodium channels. Further research is needed in order to reveal molecular mechanism and mode of action of embelin in NDI.

Conflict of interest statement

We declare that we have no conflict of interest.

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