Experimental Studies of *Achyranthes aspera* (L) Preventing Nephrotoxicity Induced by Lead in Albino Rats

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The present study was designed to evaluate the nephroprotective role of methanolic extract of *Achyranthes aspera* (*A. aspera*) an important herb in the Indian system of medicine against lead acetate-induced nephrotoxicity in rats. Toxicity was induced in male albino rats (Wistar strain) by administering lead acetate (0.2%) in drinking water for 6 weeks, followed by extract of *A. aspera* (200 mg/kg body weight). Changes in kidney weights encountered upon lead administration improved after extract with *A. aspera*. Lead damage to the urine was evident from increase in the activity of γ-glutamyltranspeptidase (γ-GT), Cathespin D, alkaline phosphatase (ALP), acid phosphatase (ACP), β-glucuronidase lactate dehydrogenase (LDH) and N-acetyl-β-D-glucosaminidase (NAG) in urine along with some urinary constituents (urea, uric acid, creatinine, protein and phosphorous). The effects of lead were also studied in kidney (γ-GT, β-glucuronidase, NAG, Cathespin D and LDH) and showed a decline upon extract administration. Increased activities of urinary enzymes were accompanied by increase in the urinary constituents. Treatment with methanolic extract of *A. aspera* after lead induction completely ameliorated the lead-induced renal damage.

Key words——*Achyranthes aspera*, lead, nephrotoxicity, urinary enzyme

INTRODUCTION

The common environmental toxic metal lead, a potent neurotoxin has been known to produce lethal effects nearly 3000 years.[1] Worldwide, it is estimated that peoples lead exposure is 300–500 times greater than background or natural levels. Acute lead poisoning occurs at high levels of exposure, causing symptoms of blindness, brain damage, kidney disease, convulsions and cancer.[2] The effects of pervasive exposure to lower levels of lead are easily miscredited; lead poisoning has thus been called an "aping disease" because its symptoms are frequently similar to those of other known ailments.[3] The evolution of lead nephropathy is usually silent. The central event appears to be the progressive destruction of tubular cells by lead and their replacement with fibrosis.[4] Clinical manifestations of impairment, such as elevations in blood urea nitrogen (BUN) or serum creatinine, do not ordinarily become evident until 50–75% of the nephrons have been destroyed.[5] Renal damage can be difficult to assess clinically. The kidney has excess functional capacity and in most cases a significant proportion of the kidney must be ablated before clinical changes in kidney function are observed.[6]

Currently available biomarkers, such as BUN or serum creatinine, are not very sensitive because although both represent direct measures of renal function, increases in the serum concentration of these markers are generally observed only after significant renal damage has occured.[7] Consequently, there has been an ongoing interest in developing new, early biomarkers of renal damage.[8] The renal enzyme N-acetyl-β-D-glucosaminidase (NAG) and the brush border enzyme γ-glutamyltranspeptidase (γ-GT) have also been used to assess renal toxicity.[9, 10] When tubules are damaged, NAG and

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Gamma-glutamyl transpeptidase (GGT) are excreted into the lumen of the tubule and can be detected in the urine. However, a limiting factor in the use of these enzymes, particularly NAG is the considerable intra/inter-individual variation in urinary enzyme activity.[11]

Medicinal value of plants has been widely investigated for centuries. Many herbs were considered conventional medicines. *Achyranthes aspera* (*A. aspera*) Linn.[12] (Family Amaranthaceae), commonly known as Rough chaff tree in English, is an annual herb that grows throughout India.[13] *A. aspera* Linn. is used in the Indian system of medicine as a remedy for several diseases.[14] *A. aspera* leaves have been assessed for cancer chemopreventive activity,[15] the non alkaloid fractions are valuable antitumour promoters in carcinogenesis. In rats *A. aspera* extract induced changes in thyroid hormone concentration.[16] *A. aspera* contain immunostimulatory compounds in root extract.[17]

MATERIALS AND METHODS

Animals —— Male albino rats (Wistar strain) procured from Tamilnadu University for Veterinary and Animal Sciences, Chennai, India, weighing 120 ± 20 g (10–12 weeks old) were used for the study. Animals were utilized as per the guidelines from the Institutional Animal Ethics Committee (No: 07/043/05). The rats were fed with a standard rat pellet diet and had free access to water ad libitum. The rats were housed under conditions of controlled temperature (26 ± 2°C) with 12 hr light and 12 hr dark exposure.

Collection of Plant Material —— The indigenous medicinal plant is widely distributed throughout Asia, South America and Africa. The whole plant of *A. aspera* was collected in November 2004. The plant specimen was authenticated by Dr. S. Jayaraman, Plant Anatomy Research Center, Chennai, and Tamilnadu, India. A voucher specimen has been deposited at the herbarium unit of the Department of Pharmacology and Environmental Toxicology, University of Madras, Taramani, Chennai.

Preparation of Plant Extract —— The whole plant was shade dried and coarsely powdered. The powder was then extracted with methanol using soxhlet extractor. The extracts were dried under pressure using flash evaporator. The yield of total extract was 11.47% w/v.

Experimental Design —— The rats were divided into four groups with 6 animals in each group. Group I rats received isotonic saline (0.5 ml/day *i.p.*) for 6 week only (control). Group II rats received lead (lead acetate) (0.2% w/v) in drinking water for 6 weeks. Group III rats received lead (lead acetate −0.2% w/v) for 6 weeks + *A. aspera* 200 mg/kg body weight for 6 weeks simultaneously. Group IV rats received *A. aspera* plant extract alone for 6 weeks.

Collection of Urine Sample —— On the last day of the experimental period the rats were housed in metabolic cages. The urine was collected on ice and was free from fecal contamination. Urine samples were centrifuged and aliquots separated. One portion was acidified with concentrated hydrochloric acid and used for the analysis of urea, uricacid and creatinine. The remaining portion was dialyzed at 4°C against distilled water for 3 hr and later was used for the determination of various enzymes and proteins.

Preparation of Tissue Samples —— At the end of experimental period, animals were subjected to mild ether anesthesia; blood was collected from retro orbital plexus and the serum was separated by centrifugation at 3000 rpm at 4°C for 15 min. Animals were sacrificed by cervical decapitation and the kidney was excised, washed in ice cold saline and blotted to dryness. A 1% homogenate of the kidney tissue was prepared in Tris–Hcl buffer (0.1 M; pH 7.4) and centrifuged at 1000 rpm at 4°C for 10 min to remove the cell debris. The clear supernatant was used for further biochemical assays. Urea was estimated by the method of Natelson *et al.* (1951),[18] Uric acid was estimated by the method of Caraway (1963),[19] creatinine by the method of Slot (1965).[20] The activity of γ-GT was estimated by the method of Rosalki and Rau (1972),[21] alkaline phosphatase (ALP) and acid phophatase (ACP) (Orthophosphoric-monoester phosphohydrolase) were estimated by the method of Bergmeyer (1963)[22] as described by Balasubramanian *et al.* (1983),[23] lactate dehydrogenase (LDH) was assayed by the method of King (1965a),[24] cathepsin-D activity was estimated by the method of Sapolsky *et al.* (1973);[25] NAG was assayed by the method of Marhun (1976).[26]

Statistical Analysis —— The data obtained were subjected to one-way analysis of variance (ANOVA) and Tukey's multiple comparison test was performed using SPSS statistical package (Version 7.5). Values are expressed as mean ± scanning

electron microscope (SEM), Values $p < 0.05$ were considered significant.

RESULTS

Table 1 illustrates the effect of *A. aspera* on mean body weight and kidney weight of the control and experimental animals. Body weight was decreased slightly in Group II animals treated with lead acetate compared with Group I control animals ($p < 0.05$), which was slightly increased in Group III, animals treated with *A. aspera* ($p < 0.05$) when compared with Group II. On the other hand, the kidney weight was slightly increased in Group II toxicity-induced animals ($p < 0.05$), whereas in *A. aspera*-treated animals the kidney weight was significantly decreased ($p < 0.05$) when compared with Group II. There was no significant change in Group IV *A. aspera*-treated animals when compared with control animals.

Table 2 represents BUN level in serum of all the experimental animals. It was observed that increased levels ($p < 0.05$) of BUN, uric acid and serum creatinine were observed in the lead-acetate intoxicated animals (Group II) when compared with control animals (Group I). Following treatment with *A. aspera* levels of BUN, uricacid and serum creatinine were significantly ($p < 0.05$) decreased in Group III animals when compared with Group II animals. No significant changes were observed in *A. aspera* alone treated animals (Group IV) when compared with control animals.

The level of protein in serum was significantly ($p < 0.05$) decreased in Group II animals. On the other hand following treatment with *A. aspera* the protein level was increased significantly ($p < 0.05$) when compared with Group II animals. No remarkable change was observed in Group IV drug control animals.

Table 3 Shows the levels of urinary constituents such as urea, uric acid, creatinine and protein in control and experimental animals. Due to renal dysfunction caused by lead acetate in Group II ani-

Table 1. Body and Kidney Weight of Control and Experimental Animals

Particular	Group I (Control)	Group II (Pb)	Group III (Pb + *A. aspera*)	Group IV (*A. aspera*)
Body weight (gm)	186 ± 13.56	153 ± 14.78^{a*}	174 ± 15.37^{a*,b*}	182 ± 16.74^{a,NS}
Kidneyweight (gm)	0.94 ± 0.097	1.53 ± 0.14^{a*}	1.27 ± 0.18^{a*,b*}	1.01 ± 0.12^{a,NS}

Each value represents mean ± SEM of 6 individual animals. Significance at $^*p < 0.05$. NS-Not significant. a-compared with Group I and b-compared with Group II.

Table 2. Levels of Uinary Constituents of Control and Experimental Animals

Particulars (mg/dl)	Group I (Control)	Group II (Pb)	Group III (Pb + *A. aspera*)	Group IV (*A. aspera*)
Blood-Urea	23.41 ± 7.43	44.49 ± 6.73^{a*}	35.03 ± 4.73^{a*,b*}	24.18 ± 2.30^{a,NS}
Protein	8.18 ± 0.83	6.45 ± 0.56^{a*}	7.39 ± 0.91^{a*,b*}	8.45 ± 0.71^{a,NS}
Uric acid	1.78 ± 0.34	2.37 ± 0.43^{a*}	1.67 ± 0.32^{a*,b*}	1.82 ± 0.65^{a,NS}
Serum creatinine	0.37 ± 0.04	2.06 ± 0.61^{a*}	0.99 ± 0.08^{a*,b*}	0.33 ± 0.04^{a,NS}

Each value represents mean ± SEM of 6 individual animals. Significance at $^*p < 0.05$. NS-Not significant. a-compared with Group I and b-compared with Group II.

Table 3. Evels of Urinary Constituents of Control and Experimental Animals

Particulars (mg/dl)	Group I (Control)	Group II (Pb)	Group III (Pb + *A. aspera*)	Group IV (*A. aspera*)
Urea	23.27 ± 2.01	47.56 ± 4.63^{a*}	37.41 ± 3.4^{a*,b*}	22.79 ± 2.14^{a,NS}
Uric acid	4.26 ± 0.40	6.30 ± 0.58^{a*}	5.12 ± 0.54^{a*,b*}	4.25 ± 0.39^{a,NS}
Creatinine	9.65 ± 0.92	14.67 ± 1.38^{a*}	11.47 ± 1.2^{a*,b*}	9.62 ± 0.97^{a,NS}
Protein	8.36 ± 0.81	15.27 ± 1.45^{a*}	9.76 ± 0.89^{a*,b*}	8.32 ± 0.79^{a,NS}

Each value represents mean ± SEM of 6 individual animals. Significance at $^*p < 0.05$. NS-Not significant. a-compared with Group I and b-compared with Group II.

Table 4. Activities of Marker Enzymes in Urine of Control and Experimental Animals

Particulars (mg/dl)	Group I (Control)	Group II (Pb)	Group III (Pb + *A. aspera*)	Group IV (*A. aspera*)
γ-glutamyltranspeptidase	3.24 ± 0.31	4.98 ± 0.37^{a*}	3.84 ± 0.32^{a*,b*}	3.14 ± 0.31^{a,NS}
Cathespin D	0.02 ± 0.01	0.98 ± 0.09^{a*}	0.41 ± 0.04^{a*,b*}	0.03 ± 0.01^{a,NS}
ALP	0.04 ± 0.02	1.68 ± 0.14^{a*}	0.44 ± 0.11^{a*,b*}	0.05 ± 0.03^{a,NS}
ACP	0.03 ± 0.04	1.27 ± 0.13^{a*}	0.46 ± 0.07^{a**b*}	0.07 ± 0.04^{a,NS}
β-glucuronidase	0.34 ± 0.02	0.94 ± 0.08^{a*}	0.51 ± 0.04^{a*,b*}	0.32 ± 0.03^{a,NS}
LDH	0.21 ± 0.01	0.42 ± 0.03^{a*}	0.34 ± 0.03^{a*,b*}	0.24 ± 0.03^{a,NS}
N-acetyl-β-D-glucosamidase	0.52 ± 0.03	1.26 ± 0.14^{a*}	0.61 ± 0.5^{a*,b*}	0.51 ± 0.04^{a,NS}

Each value represents mean ± SEM of 6 individual animals. Significance: $^{*}p < 0.05$, NS-Not significant. Units—ALP, ACP and β-D-Glucuronidase-µmol of p-nitrophenol liberated/mg protein/min, γ-Glutamyl transpeptidase-(IU/l), LDH: µmoles of pyruvate liberated/mg protein/min, Cathespin-nmoles tyrosine formed/h/mg protein, N-acetyl-β-D-Glucosaminidase: µmoles of phenol liberated/mg protein/min a-compared with Group I and b-compared with Group II.

Table 5. Levels of Marker Enzymes in Isolated Brush-border Membrane in Urine of Control and Experimental Animals

Particulars (mg/dl)	Group I (Control)	Group II (Pb)	Group III (Pb + *A. aspera*)	Group IV (*A. aspera*)
γ-glutamyltranspeptidase	1.77 ± 0.15	0.94 ± 0.08^{a*}	1.36 ± 0.14^{a*,b*}	1.64 ± 0.14^{a,NS}
β-glucuronidase	1.69 ± 0.13	1.14 ± 0.16^{a*}	1.42 ± 0.16^{a*,b*}	1.58 ± 0.17^{a,NS}
N-acetyl-β-D-glucosaminidase	1.76 ± 0.14	1.04 ± 0.14^{a*}	1.37 ± 0.15^{a*,b*}	1.72 ± 0.16^{a,NS}
Cathespin D	2.24 ± 0.23	1.41 ± 0.16^{a*}	1.91 ± 0.18^{a*,b*}	2.12 ± 0.25^{a,NS}
LDH	1.62 ± 0.12	1.31 ± 0.14^{a*}	1.54 ± 0.16a^{a*,b*}	1.63 ± 0.17^{a,NS}

Each value represents mean ± SEM of six individual animals. Significance: $^{*}p < 0.05$, NS-Not significant. Units—γ-Glutamyl transpeptidase-(IU/l), LDH: µmol of pyruvate liberated/mg protein/min, Cathespin-nmoles tyrosine formed/h/mg protein, N-acetyl-β-D-Glucosaminidase: µmol of phenol liberated/mg protein/min a-compared with Group I and b-compared with Group II.

mals, all the abovementioned constituents were significantly increased ($p < 0.05$) in urine, when compared with Group I control animals. Whereas, in Group III animals all the abovementioned constituents were significantly ($p < 0.05$) reverted to near normal when compared with Group II animals. No significant changes were observed in group IV *A. aspera* alone treated animals when compared with Group I control animals.

The lysosomal marker and brush border enzymes such as ALP, ACP, γ-GT, Cathepsin-D, LDH, NAG and β-D-glucuronidase in the urine of control and experimental animals are shown in Tables 4 and 5. Significantly increased activities ($p < 0.001$) of these marker enzymes were noted in urine due to tubular cell damage in the kidney of lead-acetate induced Group II animals when compared with Group I control animals. On the other hand the activities of ALP, ACP and NAG were reverted towards normal with ($p < 0.05$) significance; Cathapsin-D, β-glucuronidase and LDH activities were moderately ($p < 0.05$) reverted and the γ-GT activity was slightly ($p < 0.05$) reverted towards normal in *A. aspera*-treated Group III animals when compared with Group II animals. No significant changes in marker enzyme activities were observed in Group IV *A. aspera* alone treated animals when compared with Group I control animals.

Figure 1 shows the histopathological findings in kidney viewed under light microscope in control and experimental animals. Group I control animals showed normal architecture. Group II lead treated animals showed tubular damage as seen from increased number of hyper necrotic, irregular nuclei in the cells. Group III lead plus *A. aspera* treated animals showed reduced tubular damage as evident from reduced number of hyper necrotic irregular cells Group IV *A. aspera* alone treated animals showed normal kidney architecture.

DISCUSSION

The present study investigated the therapeutic potential of *A. aspera* in reducing lead-induced renal tubular defects. Increase in kidney weights as observed in the present study reflected hypertrophy of the total kidney mass, which is in accordance with a previous report.[27] Decreases of body weight of lead induced animals are also consistent with pre-

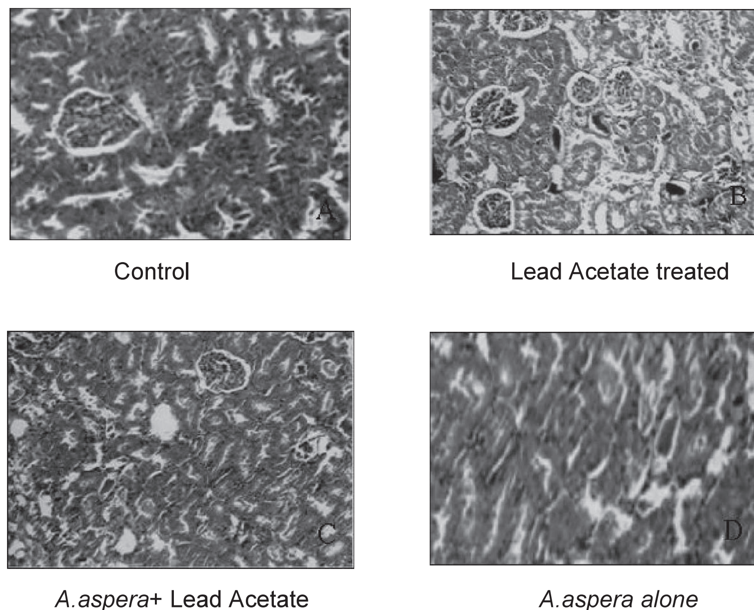

Fig. 1. Histopathological Studies of Kidney Viewed under Light Microscope in Control and Experimental Animals

Group I: Control animals showing normal kidney architecture (H & E × 40). Group II: Pb treated animals showing tubular damage as seen from increased number of hyper necrotic, irregular nuclei in the cells (H & E × 40). Group III: Pb + *A. aspera* animals showing reduced tubular damage as evident from reduced number of hyper necrotic irregular cells (H & E × 40). Group IV: *A. aspera* alone treated animals showing normal kidney architecture as that of control animals (H & E × 40).

vious report.[28)] Rapid progression of the toxic syndrome substantiates the increased levels of the lead in renal tissue. Lane (1968)[29)] has reported that occupationally exposed workers exhibit blood lead (BPb) between 40 and 120 g/dl and urinary lead values that consistently exceed 100 g/g creatinine. Positive correlations have been found between indicators of lead toxicity and urinary lead.[30)] From the study of Sanchez-Fructuoso *et al.* (2002),[31)] it is conspicuous that exposure of 0.5% lead acetate over a 90-day period resulted in increased levels of BPb, urine lead and tissue lead levels in rats. *A. aspera* might have protected the kidney by improving the thiol status.[32)] This might be the reason for the constructive role of *A. aspera* in the recovery of body weight and kidney weight, particularly in reducing the oxidative stress in blood and kidney tissue.

Urinary excretion of nitrogenous waste products (urea and uric acid) and creatinine was increased significantly in lead poisoned rats coupled with phosphaturia. Elevation of urinary urea might be a consequence of impaired solute transport in the proximal tubules. Rejection of solutes in the proximal tubule would retard water reabsorption and ultimately enhance fluid delivery to the distal nephron. This would increase the driving force for urea reabsorption in the collecting duct.[33)] Lin *et al.* (2001)[34)] have suggested that the inhibition of urate excretion can be markedly improved by chelation therapies.

Protienuria is a sensitive indicator for renal damage.[35)] Lead intoxicated rats develop heavy protienuria, indicating extensive renal damage, which may be due to direct local toxic effect of lead on the kidney. Combination of several protein measurements may provide a better sensitivity for detection of the site of action of the toxic material.[36)] Proteinuria most often reflects loss of the normal glomerular impermeability to filtration of plasma proteins and or diminished capacity of the proximal tubules to reabsorb and catabolize proteins. Tubular injury could be attributed to the increased oxidative stress imposed by lead, a condition where increased peroxidation of membrane lipids and a fall in antioxidant status are observed. This is in agreement with reports stating the effectiveness of combined treatment in reversing parameters indicative of oxidative stress in renal tissue and blood.[37)] Hence the pronounced efficacy of *A. aspera* study could be attributed to its antioxidant property. *A. aspera* might have protected the kidney by improving the thiol status.[32)] This might be the reason for the beneficial role of *A. aspera* in the recovery of altered biochemical variables, particularly in reducing oxidative stress in blood and kidney tissue.

Modulatory effect of *A. aspera* level on phosphatase enzymes was studied in urine and kidney tissues. Activity of ALP and ACP levels were increased significantly. In contrast, these enzymes were decreased in kidney of lead intoxicated rats.[38] These membrane-bound enzymes exhibit high activity in the brush-border membrane and their alteration is likely to affect the membrane permeability and produce derangements in the transport of metabolites. Our findings suggest that the change in urinary enzyme activities, which leaked from inside of brush border or lysosome, indicated the degree or localization of tubular damage, because renal tubules were selectively injured by lead acetate.

The investigation of LDH, a regulator of many biochemical functions in the body and fluids and distributed over most parts of the nephron has proved most sensitive in a variety of experimental nephropathies. Maruhn *et al.* (1983)[39] and Vyskocil (1995)[40] have proposed that increased excretion of LDH in urine occurs after exposure to lead acetate along with a profound increase in kidney weight. Urinary β-Glucuronidase derives from the lysosomes of tubular cells and from the epithelial cells of urinary tract.[35] Lead-induced production of Reactive Oxygen Species (ROS)[41] could lead to leakage of lysosomal enzymes in the urine indicating lysosomal membrane damage.[42] Determination of γ-GT has been shown useful in the diagnosis of proximal tubular damage. The increased excretion of γ-GT reflects extensive damage to the proximal tubular epithelium and shedding of the brush-border membrane in the urine. Early change in the kidney after chemical insult results in alteration of the brush-border enzymes. It appears logical, therefore, to look for an index of this part of the nephron, which would permit early recognition of alterations to brush-border by quantifying brush-border enzyme activities.[43] ALP, a membrane bound enzyme, has high activity in the brush-border membrane and its alteration is likely to affect the membrane permeability and produce derangements in the transport of metabolites. Decrease in ALP activity in renal tissue was correlated to the increase in activity in the urine[44] which supports the present finding. Biosynthesis of Reduced Glutathione (GSH) is balanced by γ-GT an enzyme located on the luminal side of brush-border of the straight proximal tubule.[45] Loss of brush-border leads to a fall in γ-GT activity and hence the active turnover of GSH could be affected leading to a fall in GSH status. This has been consistent with the previous reports, wherein a fall in GSH levels in renal tissues of rats exposed to lead was observed.[46] Decrease in brush-border enzyme activities could be based on decreased biosynthesis or due to accelerated membrane degradation by lead. A significant change in the activity of γ-GT with an increase in ALP activity in the brush-border fractions of lead poisoned rats was observed by Teichert-Kuliszewska and Nicholls (1985).[47] The renal integrity assessment of *A. aspera* showed marked improvement with respect to the enzyme.

Examination of paraffin sections stained with hematoxylin-eosin failed to reveal consistent differences in the histology of the kidney tissue of *A. aspera* alone treated animals when compared with that of control animals. Lead- acetate administered animals showed (focal) tubular necrosis, whereas in animals treated with lead acetate and *A. aspera* these changes were reverted as evidenced in Fig. 1.

In conclusion, we have shown that some signs of lead nephrotoxicity are mitigated by co-administration of *A. aspera*. If this interaction is confirmed in further experiments using more elaborate indices of nephrotoxicity, it may be of some human clinical relevance. It would also be of interest to try, in future work, a metabolite of Achyranthine and betaine which has stronger renal and urinary antioxidant activity than *A. aspera.*

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