

NEW EXPERIMENTAL DESIGN: DEHYDRATION INDUCED UREMIA AND OXIDATIVE STRESS ON MALE ALBINO RATS, INNOVATIVE APPROACH TO RESEARCHERS FOR FURTHER STUDY ON KIDNEY DISEASE.

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Summary

Uremia means excess nitrogenous waste products in the blood and their toxic effects. Dehydration or water deprivation of body that decreases urine excretion allows urea and other protein waste products to accumulate and oxidative stress develops. The aims of this present study are to investigate uremia & oxidative stress are caused by dehydration and this study is the introductory experiment for further research of "Protection of dehydration induced uremia and oxidative stress by some selective medicinal plant extract" and a new methods introduction to dehydration. In this context, this experimental studies set up dehydration induce uremia and oxidative stress on albino rats in five groups-one control-normal food and water provided, and four dehydrating groups based on duration dependent of water providing and limited water providing. After 45 days of experiment we have observed body weight, super-oxide dismutase (SOD) and catalase activity lower and blood urea, blood creatinine and Malondialdehyde (MDA) in dehydrating groups are higher than control group. We have concluded that those rats received little amount of water than other dehydrating animals and dehydration period was long than others, these rats had been achieved more dehydration by significantly lower its body weight, catalase & SOD enzyme in plasma & higher in blood urea level, blood creatinine level & lipidperoxidation (MDA activity) than other three dehydrating groups. So, severe uremia and oxidative stress occurred in dehydrating animals.

Key Words: Dehydration, Uremia, Oxidative stress, Plant extract, Medicinal Plants.

Introduction

Uremia means excess nitrogenous waste products in the blood and their toxic effects.¹ Dehydration or water deprivation of body is the risk factors at this point urine production declines & finally no urine output (anuria).¹ Without urinary excretion of waste products, dangerous levels of urea accumulate in the blood.^{1,2} Prolonged dehydration resulting renal disorder or kidney impairment where dialysis or kidney transplant may be required.^{3,4} A cause of increase production of urea & decrease eliminating of urea due to water deprivation or dehydration.^{4,5} Recent studies have shown that oxidative stress is highly present in patients with renal disease.⁶ Wynckel A et. al., proposed that acute renal failure (ARF) may occur in patients who are taking angiotensin converting enzyme (ACE) inhibitors in situations associated with decreased glomerular filtration pressure, such as dehydration caused by acute diarrhea or diuretic therapy.⁷ Tsuchiya R et. al., experimentally induced urethra-obstruction results the blood urea nitrogen (BUN) and serum creatinine values increased at constant rates.⁸ There is good evidence indicating that uraemia in general is associated with enhanced oxidative stress^{9,10}, and treatment of uraemic patients with haemodialysis or peritoneal dialysis has been suggested to particularly contribute to oxidative stress and reduced antioxidant levels in uremic patients^{11,12}. Uremia is a potentially fatal condition that demands immediate treatment.⁶ Treatment options for uremia include kidney transplant, dialysis, and other treatments typically associated with kidney failure.⁸ Uremia and increasing nitrogenous end product and oxidative stress is correlated.⁸ The aims of this present study are to investigate uremia & oxidative stress are caused by dehydration and this study is the introductory experiment for further research of “ Protection of dehydration induced Uremia and Oxidative stress by some selective medicinal plants extract”.¹²

Materials and Methods

Selection of Animals and Care

The study was conducted on thirty healthy, adult, male albino rats of Wister strain (Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata-54) having a body weight of 100 ± 15 g. They were acclimatized to laboratory condition for 2 weeks prior to experimentation. Animals were housed one per cage in a temperature-controlled room ($22 \pm 2^\circ\text{C}$) with 12-12 h dark-light cycle (8.00-20.00 h light: 20.00-8.00 h dark) at a humidity of 50 ± 10 %. They were provided with standard food (pellet diet) and water *ad libitum*. The principle of laboratory animal care National Institute of Health (NIH, 1985) USA guideline was followed through out the duration of experiment¹³ & Our University Ethics Committee approved the experimental protocol.

Grouping of animals & experimental procedure:

Dehydration Protocol: Animals were randomly placed in 1 rats /cage with free access with dry food (pellet diet-Supplied from Ghosh animal, animal foods and animal cages Supplier, Kolkata) and adequate water and daily measured water intake /rat and dehydration was achieved by withdrawing the drinking water bottle for the dehydration period of experimentation followed by Lucien N et. al.,¹⁴ and further modification was assumed by a new method to introduce to dehydration.

The rats were divided into five equal groups as follows-

Group-I or control Group (Gr.) - Six animals were subjected to control Gr.. They were housed at room temperature ($25 \pm 3^\circ\text{C}$) & feed dry food (palate diets) & 15 ml water to each rat once. for 45 days.

Group-II or dehydration Gr.- Six animals were randomly placed in 1 rats /cage with free access with dry food. Dehydration was achieved by providing the drinking 8 ml water/ rat once after interval of every 24 hrs for 45 days starting from experiment.

Group-III or dehydration Gr.- Six animals were randomly placed in 1 rats /cage with free access with dry food. Dehydration was achieved by providing the drinking 4 ml water/ rat once after interval of every 24 hrs for 45 days starting from experiment.

Group-IV or dehydration Gr.- Six animals were randomly placed in 1 rats /cage with free access with dry food. Dehydration was achieved by withdrawing the drinking water bottle for every 48 hrs and provides 8 ml water/rat once after interval of every 48 hrs for 45 days starting from experiment.

Group-V or dehydration Gr.- Six animals were randomly placed in 1 rats /cage with free access with dry food. Dehydration was achieved by withdrawing the drinking water bottle for 48 hrs and provides 4 ml water after interval of every 48 hrs for 45 days starting from experiment.

Body Weight (BW): BW was measured prior to experimentation and during experiment in every week and final BW was taken before sacrificed the rat but anaesthetized rat are place on a pan of weight machine and measured weight.

Antioxidant Enzymes:

Biochemical assay of catalase (CAT) activity

CAT activity was measured biochemically.¹⁵ For the evaluation of CAT activity in kidneys were homogenized separately in 0.05 M Tris Hydrochloric acid (HCl) buffer (Merck, India) solution (pH-7.0) at the tissue concentration of 50 mg/ml. These homogenate were centrifuged separately at 10,000 g at 4°C for 10 min. In spectrophotometric cuvette, 0.5 ml of hydrogen peroxide (H_2O_2) and 2.5 ml of distilled water were mixed and reading of absorbance was noted at 240 nm. Tissue supernatant was added at volume of 40 μ l separately the subsequent six reading were noted at 30 sec. Interval.

Biochemical assay of superoxide dismutase (SOD)

Kidneys were homogenized in ice-cold 100mM Tris-cocodylate buffer (LOBA Chem, India) to give a tissue concentration of 50 mg/ml centrifuged at 10,000g for 20min at 4°C. The SOD activity of these supernatant were estimated by measuring the percentage of inhibition of the pyragallol (HIMEDIA, India) autoxidation by SOD.¹⁶ The buffer was 50mM Tris (pH-8.2) containing, 50 mM cocodylic acid(pH-8.2), 1 mM ethylene diamine tetra acetic acid (EDTA-HIMEDIA, India) and 10mM hydrochloric acid (Hcl). In a spectrophotometric cuvette, 2ml of buffer, 100 μ l of 2 mM pyragallol and 10 μ l of of supernatant were poured and the absorbance was noted in spectrophotometer at 420 nm for 3 mi. One unit of SOD was defined as the enzyme activity that inhibited the autoxidation of pyragallol by 50 percent.

Estimation of Lipid Peroxidation from the levels of Malondialdehyde (MDA).

The kidneys were homogenized separately at the tissue concentration of 50 mg/ml in 0.1 M of ice-cold phosphate buffer (pH-7.4) and the homogenates were centrifuged at 10,000 g at 4°C for 5 min individually, supernatant were used for the estimation of MDA. For the measurement of MDA 0.5 ml homogenate and plasma were mixed separately with 0.5 ml normal saline (0.9 g% NaCl) and 2 ml of TBA-TCA mixture (0.392 g of TBA in 75 ml of 0.25 N HCl with 15 g of TCA, the volume of the mixture was made up to 100 ml by 95% ethanol) and boiled at 100°C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000 g for 10 min. The whole supernatant and plasma was transferred in spectrophotometer cuvette and read at 535 nm. Calibration was performed by using the acid hydrolysis of 1,1,3,3 tetra methoxy propane (Merck, India) are a standard. The MDA in sample was calculated by using the extinction coefficient of 1.56x 105 M/cm and expressed in the unit of nM/mg of tissue or nM/ml of plasma.¹⁷

Blood Uremia Profile:

Biochemical estimation Blood Urea.

The collected blood was centrifuged and plasma fraction was separated. Urea level of plasma measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyser (Merck, Japan) by standard protocol for phtotometric determination of urea according to the Urease GLDH method (kinetic UV test). First 10 μ l of urea standard (50 mg/ 100ml) was mixed with 1000 μ l monoreagent (Composed of Tris pH7.8 -120mmol/l, 2-Oxoglutarate-7mmol/l,ADP-0.6mmol/l,urease-6ku/l,Glutamate dehydrogenase-1ku/l and NADH-0.25 mmol/l) to incubate for approx. 60 sec. at 25°C and read absorbance at 37°C for standardization then 10 μ l sample is used above procedure and automatic calculated reading has taken.¹⁸

Biochemical estimation Blood Creatinine

The collected blood was centrifuged and plasma fraction was separated. Creatinine level of plasma measured by commercially available standard Blood Urea Kit (Merck, Japan) by Semiautoanalyser (Merck, Japan) by standard protocol for phtotometric determination of creatinine based on Jaffe kinetic method without deproteinization. First 100 μ l of creatinine standard (1 mg/ 100ml) was mixed with 1000 μ l monoreagent (Buffer:NaOH-313mmol/l + 12.5 mmol/l and Picric acid-8.73mmol/l) to incubate for approx. 0-5 min at 25°C and read absorbance at 37°C for standardization then 100 μ l sample is used above procedure and calculated reading has taken.¹⁹

Toxicity study:

Biochemical estimation of Glutamate Oxaloacetate Transaminase (GOT) and biochemical estimation of Glutamate Pyruvate Transaminase (GPT)

For the assessment of toxicity in plasma and kidney we measured GOT and GPT according to the method of Goel.²⁰

Statistical Analysis:

Analysis of variance (ANOVA) followed by a multiple two-tail ‘t’ test with Bonferroni modification was used for statistical analysis of the collected data. Difference were considered significant when $p < 0.05$.

Results

Fig 1 shows that water intake by different groups of rat and achieved dehydration. Body weight (Fig.2), SOD (Fig: 6) and catalase (Fig: 5) are significantly decreased in Group II, Group III, Group IV, Group V respectively, than Group I (control). Blood urea (Fig: 3), blood creatinine (Fig: 4) and Lipid peroxidation (Fig: 7) were increased significantly in dehydrating animals than control. Table.1 enlightened toxicity status in different groups. Group V was achieved more toxicity than others.

Fig-1: Achieved to dehydration by providing little amount of drinking water/day to male albino rats. Data are expressed as Mean \pm SE (n=6).

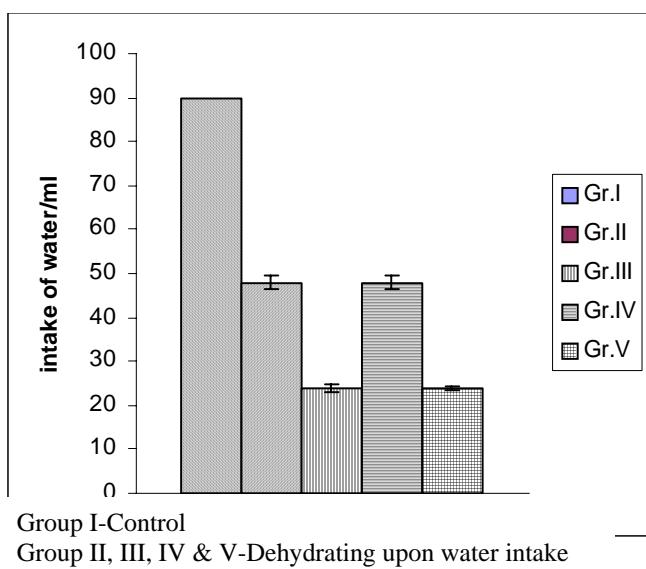


Fig-2: Effect of dehydration by withdrawing drinking water on Body Weight. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. Bars for a specific data differ from each other significantly ($p < 0.05$).

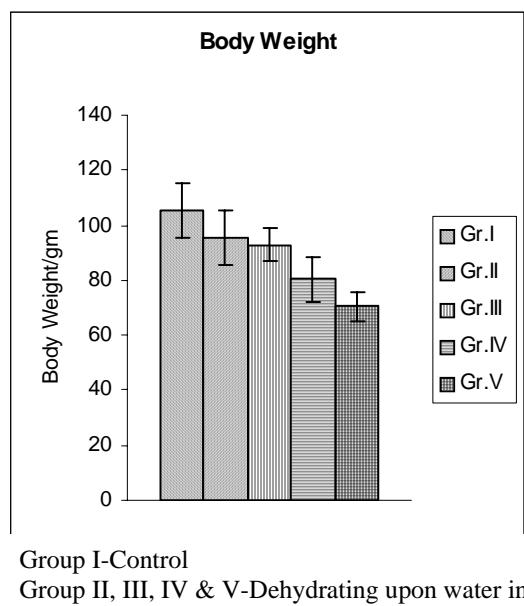
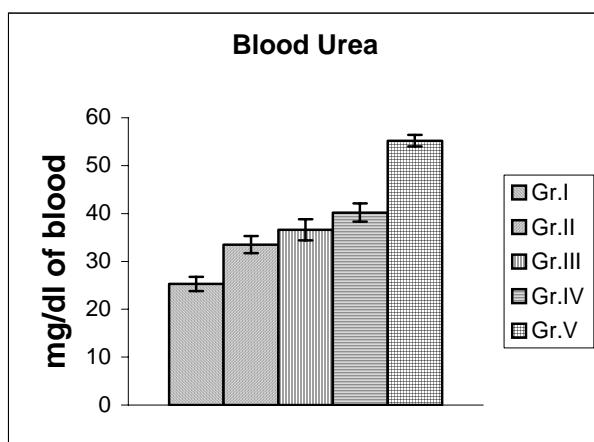


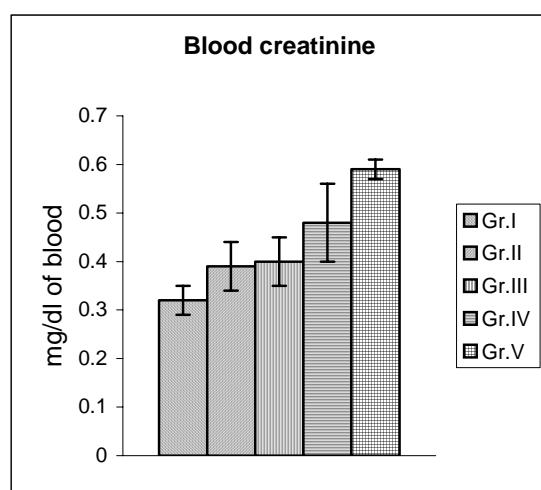
Fig-3: Effect of dehydration by withdrawing drinking water on Blood Urea. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. Bars for a specific data differ



Group I-Control

Group II, III, IV & V-Dehydrating upon water intake

Fig-4: Effect of dehydration by withdrawing drinking water on Blood Creatinine. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. for a specific data differ from each other significantly ($p < 0.05$).



Group I-Control

Group II, III, IV & V-Dehydrating upon water intake

Fig-5: Effect of dehydration by withdrawing drinking water on Catalase in kidney. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. for a specific data differ from each other significantly ($p < 0.05$).

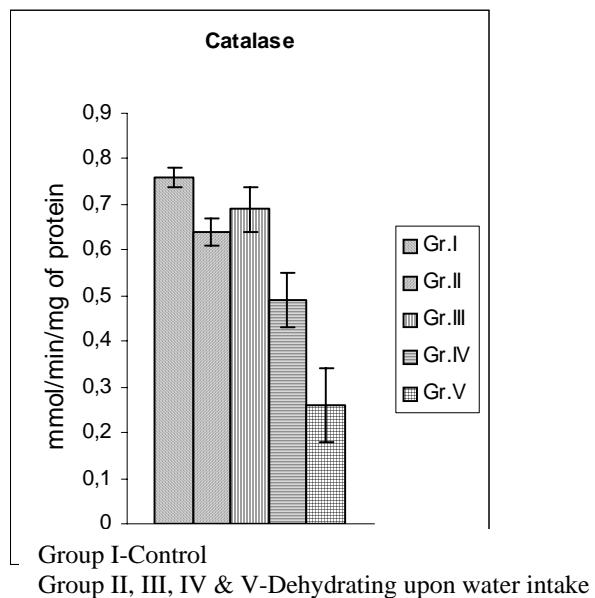


Fig-6: Effect of dehydration by withdrawing drinking water on SOD in kidney. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. Bars for a specific data differ from each

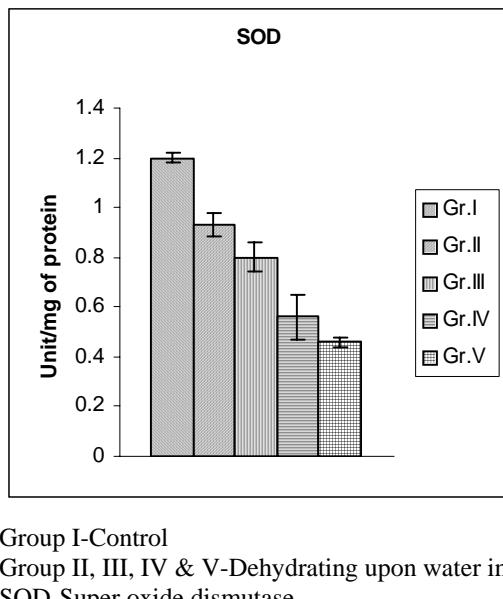
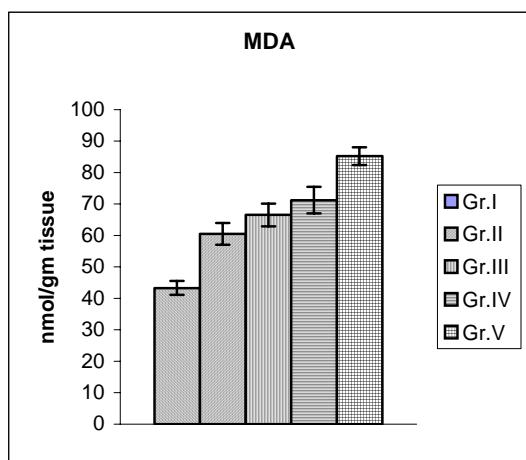


Fig-7: Effect of dehydration by withdrawing drinking water on MDA in kidney. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two tail t-test. Bars for a specific data differ from each other significantly ($p < 0.05$).



Group I-Control

Group II, III, IV & V-Dehydrating upon water intake

MDA-Melon Di aldehyde

Table.1. Effect of dehydration by withdrawing drinking water on plasma and kidney GOT and GPT activities in dehydration induced oxidative stress and uremia in rat. Data are expressed as Mean \pm SE (n=6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d and e) a specific vertical column did differ from each other significantly . ($P<0.05$).

Group	Plasma		Kidney	
	GOT (Unit/ Lit of plasma)	GPT (Unit/ Lit of plasma)	GOT (Unit/ mg of tissue)	GPT (Unit/ mg of tissue)
I	15.4 \pm 0.5 ^a	23.4 \pm 0.2 ^a	113.3 \pm 2.3 ^a	124.4 \pm 1.3 ^a
II	25.8 \pm 0.4 ^b	33.6 \pm 0.2 ^b	133.6 \pm 1.2 ^b	133.6 \pm 1.2 ^a
III	26.9 \pm 0.8 ^c	38.1 \pm 0.1 ^c	142.9 \pm 3.1 ^c	137.1 \pm 1.5 ^a
IV	36.1 \pm 0.7 ^d	42.2 \pm 0.3 ^d	153.2 \pm 2.5 ^d	144.2 \pm 1.2 ^a
V	45.1 \pm 0.6 ^e	47.2 \pm 0.3 ^e	162.2 \pm 2.5 ^e	157.2 \pm 1.2 ^a

Group I-Control

Group II, III, IV & V-Dehydrating upon water intake

GOT-Glutamate Oxaloacetate Transaminase

GPT-Glutamate Pyruvate Transaminase

Discussion

Dehydration is the risk factors at this point urine production declines & finally no urine output (anuria). Here, dehydration is only used for causing oxidative stress, elevation of blood urea and creatinine levels.³. Without urinary excretion of waste products, dangerous levels of urea accumulate in the blood.³ Decreased blood volume occurs with deficient fluid intake cause reduced blood flow in kidney means decreased Glomerular Filtration Rate (GFR) this may leads to ARF.⁴ So, Prolonged blood volume deficiency ultimately produces renal damage⁴. Prolonged increase urea & creatinine & decrease GFR causes Chronic Renal Failure (CRF) & ultimately kidney gets damage.⁴. When GFR decrease 90% than normal GFR then End Stage Rena Disease (ESRD) may occur. So, main cause of increase production of urea & decrease eliminating of urea due to, urinary outflow reduction & Water deprivation or dehydration.^{5,19} Fig: 1 represents five groups of rat drunk water in different amount, showing Gr.V have intake water less. Dehydration reduces body weight much in Gr. V than other groups significantly (Fig: 2). Dehydrating induced oxidative stress in blood has been established here by noting the low activities of SOD in kidney (Fig: 6) and CAT in kidney (Fig: 5) these are the important antioxidant enzymes.²⁰ The decrease in antioxidant enzyme activities due to dehydration might be due to their use against the free radicals destruction and their inhibition by free radicals species.²¹ It is well established that SOD activity is inhibited by hydrogen peroxide that reduced Cu²⁺ to Cu⁺ in SOD.²² The reduction of hydrogen peroxide is catalyzed by Catalase that protects the tissues from highly reactive hydroxyl radicals.²³ Increased the levels of products of free radicals like MDA (Fig : 7) in blood in dehydrating group again indicate the low level of antioxidant enzyme activity that increase the lipid peroxidation. Another possibility for such elevation in MDA may be due to ischemia-reperfusion phenomenon^{24,25} or due to high rate of catecholamine secretion that generate free radicals either through auto oxidation or through metal ion or superoxide-catalyzed oxidation.^{26,27}. Recent studies have shown that oxidative stress is highly present in patients with renal disease.^{8,28} It is known that LDL from uremic patients presents an elevated susceptibility to oxidation. Uremic oxidative stress is characterized from a biochemical point of view as a state of reactive aldehyde and oxidized thiol group accumulation, together with depletion of reduced thiol groups, which are particularly important as part of antioxidant defense.²⁷ As a consequence of diminished renal catabolism and function, uremic oxidant mediators accumulate urea (Fig: 3) and creatinine (Fig: 4) in blood.^{29,30} There is toxicity occur (Table.1). So, we concluded that in this study it has been revealed that Gr. V dehydrating animals had been achieved dehydration by significantly lower its body weight, catalase & SOD Enzyme in serum & higher in blood urea level, Blood creatinine level & lipidperoxidation (MDA activity) than other four groups. So, this present study shows that dehydration induced method (for GR. V animals) are appropriate for the further study on uremia and oxidative stress.

Acknowledgements

We thanks to Dr. Uday Chand Pal, Principal, Raja N. L. Khan Women's College , Midnapore-721102, West Bengal, India for using department for this project, also thanks to Prof Dipak Sarkar, Chairman and Dr. Nirmalya Roy, M.O, Dialysis Unit, Vidyasagar Institute of Health, (a 100 beded Private Hospital) Centre for advancement of Higheir Education & Research Rangamati, Paschim Medinipur, West Bengal, Pin-721101, India.

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