

## Research Article

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## Therapeutic effect of eel serum in an experimental rat model of chronic kidney failure

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### Abstract:

**Background:** Chronic kidney failure causes deficits in glomerular filtration. Although it has been reported that eel serum stops this deficit, the specific mechanisms are unknown.

**Objective:** To determine whether eel serum improves creatinine clearance in a rat model of chronic kidney failure.

**Methods:** 40 rats were divided into 4 groups. Three groups received eel serum (at  $1 \times 10^{-1}$  %,  $1 \times 10^{-4}$  % and  $1 \times 10^{-7}$  %, in a volume of 0.5 ml) and one received the vehicle (control), all for a period of 2.5 months.

**Setting:** Laboratory of University of Colima's Faculty of Medicine.

**Primary Outcome Measures:** Significant change in creatinine clearance.

**Results:** With two of the doses of eel serum ( $1 \times 10^{-1}$  % and  $1 \times 10^{-7}$  %) there was no significant change in creatinine clearance. However, with  $1 \times 10^{-4}$  % of eel serum the creatinine clearance in rats decreased only 0.2%, whereas an 18% decrease in this parameter was found in the control group.

**Conclusion:** With a dose of  $1 \times 10^{-4}$  % of eel serum, there was a significant detention in the normal continuous drop in creatinine clearance in a rat model of chronic kidney failure.

### INTRODUCTION:

Kidney failure occurs as a result of the progressive and irreversible destruction of nephrons, leading to structural and functional hypertrophy of remaining nephrons. Among chronic degenerative disorders, this pathology deserves special attention due to the alarming increase in its incidence, the decrease in the quality of life for patients, and the high costs of treatment for health institutions.

The current increase in incidence is due almost entirely to diabetic nephropathy and to a much lesser extent arterial hypertension (1,2). Today treatment of this pathology is still very limited, in the best of cases only managing to detain the process. Consequently, there is an increase in patients who progress to the latter stages of the disease, known as the predialytic (fourth) and dialytic (fifth) phases (3,4).

The aim of the present study was to test the possible effectiveness of eel serum, a homeopathic medicine, to

clear urine of creatinine in an experimental rat model of chronic diabetic kidney failure (5,6).

### METHODS

A study was carried out using an experimental model of chronic kidney failure with 3- to 4-month-old male Wistar rats weighing 200-300 g. The rats had no other apparent pathologies before or during the experiment. Rats were excluded if they did not develop kidney failure.

### Animals

Male Wistar rats were obtained from the animal house of the Superior School of Medicine, National Polytechnic Institute, Mexico City, Mexico. In the laboratory of University of Colima's Faculty of Medicine, they were maintained under conditions established by the Official Mexican Regulation for the handling and care of experimental animals NOM-062-ZOO-1999. The study was approved by the Ethics Committee of the institution.

### Induction of diabetes

Diabetes was introduced to the rats with streptozotocin, a derivative of *Streptomyces acromogenes* and an antibiotic classified as a nitrosourea. Streptozotocin is preferable to aloxane for introducing diabetes in animals due to its greater selectivity for beta cells in pancreatic isolates. It has reversible toxic effects in the kidney and liver that are dependent on the dose. Its most important toxic effect is proximal tubular damage, without causing kidney failure (7). In a few cases there is hematological toxicity that results in anemia, leukopenia or thrombocytopenia. It tends to accumulate and can cause death. Streptozotocin was administered intraperitoneally, at 30 mg/kg of body weight, to induce hyperglycemia (8). After 48 hours the level of glucose began to rise. At 30 days the levels of creatinine and urea started to increase.

### Treatment with eel serum

At one and a half months post-streptozotocin administration, creatinine clearance in urine began to decrease. At approximately 2.5 months, when creatinine clearance reached 20% of the normal parameter, treatment with eel serum began. Rats were divided into four groups (n=10), 3 of which received eel serum at different concentrations ( $1 \times 10^{-1}$ %,  $1 \times 10^{-7}$ % and  $1 \times 10^{-4}$ % in a volume of 0.5 ml) and the fourth (control) received the vehicle (in the same volume), homeopathic (76%) alcohol (9). Treatment lasted 2 and one half months for all groups. Eel serum was administered daily at the same time of the morning subcutaneous administration.

### Laboratory tests

Animals consumed only water for at least 8 hours before the serum glucose test. The level of this substance was determined by the Trinder method with a reactive that restrains oxidation of glucose, and with chromophore 4-aminophenazone in a buffer solution. A value of glucose of 55-110 mg/dl (3.05-6.11 mmol/l) was considered normal (10,11).

Urea was hydrolyzed in the presence of urease, in ammoniac and carbon dioxide. The ammoniac produced by this reaction combines with  $\alpha$ -cetoglutarate and NADH in the presence of glutamate dehydrogenase to produce glutamate and NAD. Normal serum levels are 20-50 mg/dl (3.3-8.3 mmol/l) (10,11). Plasmatic creatinine levels were determined by reacting plasma with an alkaline medium that contained proteins with picrate to form a reddish-orange compound. Normal levels are 0.6-1.3 mg/dl (10,11).

The following formula was used to calculate the clearance of creatinine in urine at 24 h:  $Ucr \times V / Pcr$

Where, Ucr is the creatinine concentration in urine, V is the volume of urine formed per minute, and Pcr is the concentration of creatinine in plasma (10,11).

### Statistical analysis

All values are expressed as the mean  $\pm$  standard error (SE). The paired and independent Student's *t*-test was employed to analyze the significance of differences in case of similar variance and in case of different variance, the Wilcoxon and U of Mann-Whitney tests were utilized alternately. A p-value  $< 0.05$  was considered significant.

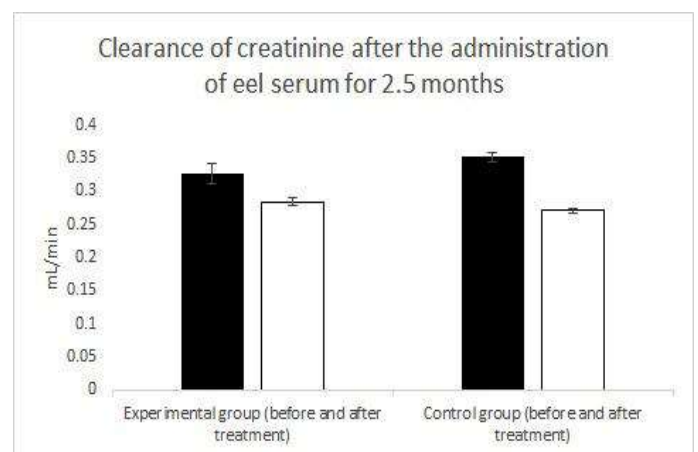
## RESULTS

### Treatment with $1 \times 10^{-1}$ % of eel serum

At approximately 2 and one half months after administration of streptozotocin, rats showed a 20% decrease in the clearance of creatinine in urine at 24 h. At this point kidney failure was established and eel serum was administered. With the concentration of  $1 \times 10^{-1}$  % of eel serum, no final significant difference was found between the experimental and control groups in creatinine clearance (Table 1, Fig. 1). On the other hand, when an intragroup comparison was done, in both groups there were significant difference, indicating that decrement in creatinine clearance values was continuing in both groups.

**Table 1: Values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-1}$  %**

Groups	Before (ml/min)	After (ml/min)	P-value
Experimental n= 10	0.327 $\pm$ 0.015	0.284 $\pm$ 0.006	0.003
Control n= 10	0.352 $\pm$ 0.007	0.271 $\pm$ 0.004	0.001
P-value	0.090	0.209	



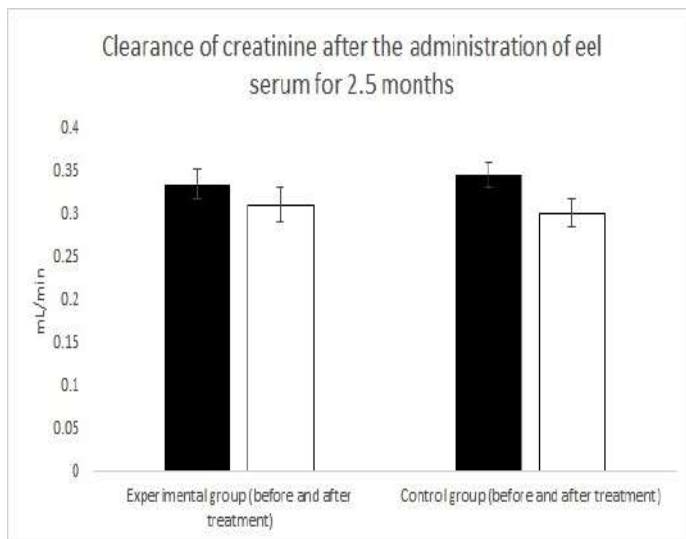
**Figure 1. Average values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-1}$  %. With this dose there is no change in the measured parameter. This was the highest dose tested.**

**Treatment with  $1 \times 10^{-7}$  % of eel serum**

With the concentration of  $1 \times 10^{-7}$  % of eel serum, no final significant difference was found between the experimental and control groups in creatinine clearance (Table 2, Fig. 2). On the other hand, in the intragroup comparison in experimental group, no significant difference was observed indicating the possible detention of kidney damage, meanwhile in the intragroup comparison in control group, the decrement in creatinine clearance values persisted, and the difference was on borderline of significance.

**Table 2: Values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-7}$  %**

Groups	Before (ml/min)	After (ml/min)	P-value
Experimental n= 10	0.335 ± 0.018	0.311 ± 0.020	0.229
Control n= 10	0.346 ± 0.015	0.301 ± 0.016	0.059
<b>P-value</b>	0.333	0.392	



**Figure 2. Average values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-7}$  %. With this dose there is a tendency indicating the possible detention of kidney damage. This was the lowest dose tested.**

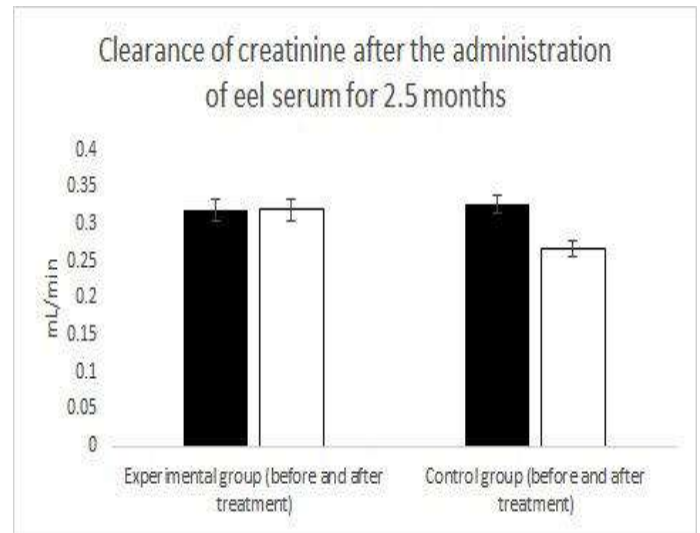
**Treatment with  $1 \times 10^{-4}$  % of eel serum**

With the concentration of  $1 \times 10^{-4}$  % of eel serum, a significant difference was found between the experimental and control groups in creatinine clearance at the end of the study (Table 3, Fig. 3;  $P = 0.017$ ). No significant difference existed in this treated group between the initial and final values ( $0.319 \pm 0.015$  vs.  $0.319 \pm 0.014$ ), once again possibly reflecting the detention of kidney damage. However a significant difference did indeed exist between the initial and final

values of this parameter in the control group ( $0.327 \pm 0.012$  vs.  $0.266 \pm 0.011$ ;  $P = 0.0008$ ).

**Table 3: Values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %**

Groups	Before (ml/min)	After (ml/min)	P-value
Experimental n=10	0.319 ± 0.015	0.319 ± 0.014	<b>0.289</b>
Control n=10	0.327 ± 0.012	0.266 ± 0.011	<b>0.0008</b>
<b>P-value</b>	<b>0.388</b>	<b>0.017</b>	



**Figure 3. Average values of creatinine clearance before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %. With this dose the effect is clear, there is a significant difference between groups in the measured parameter. This was the middle dose tested.**

**Values for glucose, urea, creatinine and animal weight**

For the experimental group receiving  $1 \times 10^{-4}$  % of eel serum, measurements were made of other parameters, because in this group a significant difference was observed between the values of creatinine clearance before and after treatment. Animals showed a significant weight loss during the 2.5 months of treatment with eel serum, but there was no significant difference between treated and control animals regarding this parameter (Table 4). There was a significant rise in glucose levels during the treatment period, but there was no significant difference between treated and control animals regarding this parameter (Table 5). The significant increase in serum urea and creatinine levels in the control group at the end of the study (compared to initial values) was not found in the treated animals, and therefore there was a significant difference between treated and control animals with respect to these two parameters at the end of 2.5 months of administration of eel serum (Tables 6 and 7).

**Table 4: Average weight of rats before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %**

Groups	Before (grams)	After (grams)	P-value
Experimental	218.4 ± 4.60	189.6 ± 3.89	0.042
Control	219.8 ± 6.16	190.8 ± 2.92	0.026
P-value	0.432	0.287	

**Table 5: Average level of serum glucose before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %**

Groups	Before (mg/dl)	After (mg/dl)	P-value
Experimental	204.3 ± 4.67	253.1 ± 3.26	0.000
Control	200.8 ± 3.23	254.3 ± 3.61	0.000
P-value	0.283	0.447	

**Table 6: Average level of serum urea before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %**

Groups	Before (mg/dl)	After (mg/dl)	P-value
Experimental	63.4 ± 1.12	69.142 ± 2.0	0.213
Control	69.1 ± 1.81	88.2 ± 1.78	0.002
P-value	0.077	0.000	

**Table 7: Average level of serum creatinine before and after 2.5 months of eel serum treatment at a dose of  $1 \times 10^{-4}$  %**

Groups	Before (mg/dl)	After (mg/dl)	P-value
Experimental	0.63 ± 0.03	0.66 ± 0.03	0.307
Control	0.61 ± 0.02	0.83 ± 0.04	0.000
P-value	0.388	0.017	

## DISCUSSION

To our knowledge this is the first report of the mechanism by which this substance acts on the organism. The present results show that compared to the control group eel serum at a concentration of  $1 \times 10^{-4}$  % can detain the drop in creatinine clearance in a rat model of chronic kidney failure ( $P = 0.017$ ). No significant difference existed in the group treated with this concentration of eel serum between the initial and final values of creatinine clearance ( $0.319 \pm 0.015$  vs.  $0.319 \pm 0.014$ ), possibly reflecting the detention of kidney damage. However a significant difference did indeed exist between the initial and final values of this parameter in the control group ( $0.327 \pm 0.012$  vs.  $0.266 \pm 0.011$ ;  $P = 0.0008$ ). The decrease in this parameter was constant during the 2.5 months of the treatment period in the control group. Considering that the mortality of kidney failure has been unaffected by any treatment to date (12,13), this finding represents an important breakthrough. Indeed, the morbidity and mortality of kidney failure has increased in Mexico in the last decade, to the point of becoming a public health problem.

Diabetes was induced in the current contribution by administering streptozotocin. At one and a half months of treatment with this antibiotic, the clearance of creatinine in urine began to decrease, and when it reached 20% of the normal parameter the animals were in a condition defined as the second stage of chronic kidney failure (14). The treatment with eel serum began, using three different concentrations ( $1 \times 10^{-1}$ ,  $1 \times 10^{-4}$  and  $1 \times 10^{-7}$  %). In the groups treated with  $1 \times 10^{-1}$  % and  $1 \times 10^{-7}$  % of eel serum, the drop in the clearance ability of creatinine was slightly less than in the control group. However, this difference was not significant. With regard to the effects achieved with lower doses of serum eel, there are evidences obtained with the use of other drugs, which with low concentrations produce a determined effect and at higher concentrations, the final effect is less or inclusive absent (15). In the present report, the 3 different doses tested were applied at different rats. Be interesting to extend the dose-response curve in future research.

The parameters other than creatinine clearance were only recorded for the experimental group treated with  $1 \times 10^{-4}$  % of eel serum. A weight loss and an increase in serum glucose levels were found in the treated and control groups, with no significant difference between these two groups at the beginning or end of the study. This indicates that eel serum has no effect on diabetes mellitus itself.

On the other hand, a significant difference did indeed exist at the end of the study between the levels of serum urea and creatinine in the treated and control groups. That is, there was a significant rise in these values found in the control group, but not in the group treated with  $1 \times 10^{-4}$  % of eel serum. Urea and creatinine are important substances regarding the capacity of the organism to detain the pathological process of kidney failure. The effects obtained in this experimental group are comparable to those obtained when patients are treated with alphas-keto-analogues of aminoacids or with losartan (16).

It has been reported that when blockers of mineralocorticoid receptors are administered, together with inhibitors of the enzyme that converts angiotensin and/or with blockers of angiotensin, proteinuria is diminished 15-54%, with the majority of patients showing a decrease of 30-40%. Blockers of mineralocorticoid receptors are associated with a significant reduction in blood pressure and in the rate of glomerular filtration. However, these studies have important limitations, such as data without assessment

of variance, and experimental design that neglected to include a placebo group (17).

Preclinical data from a study in a rat model of hypertensive nephropathy suggest that aliskerin, a direct inhibitor of renin, has effects similar to those of blockers of mineralocorticoid receptors in respect to kidney protection. Similarly, in a multicentered, multinational, controlled, double-blind study with 599 patients suffering from type II albuminuria diabetes, there is a preliminary report that albuminuria was reduced by 20% after 6 months of treatment with aliskerin (300 mg, once per day) plus losartan (100 mg) (18).

As is well-known, for the endothelial cell surface in the kidney to act as an effective filtration barrier, it must conserve its negative charge and express a series of specific proteins (19, 20, 21), which are involved in pedicelular processes interdigitated and horseshoe diaphragm. Together these electrical and mechanical mechanisms impede the passage of low molecular weight, negatively charged proteins. However, this filtering function is altered by the development of diabetic nephropathy, which is characterized by an alteration in the structure of the mesangium and the basal membrane leading to proteinuria. This condition causes a reduction in the synthesis of proteoglycans, which in turn results in a loss of the negative charge of the glomerular basal membrane, thus permitting the passage of low-molecular weight, negatively charged proteins such as albumin (22, 23).

To conserve the negative charge on endothelial cell surfaces in the kidney (in the superficial and deep glomerulus), the presence of lectins is required. Perhaps the signals from these lectins also reduce intraglomerular hypertension. Therefore, the fact that treatment with  $10^{-4}$  % of eel serum in the present study avoided an increase in serum creatinine and urea may owe itself to the high lectin content of eel serum (24), which could have restored a negative charge to the surfaces of endothelial cells of the glomerulus and mesangium in the kidney.

A limitation of the present study was the fact that we did not carry out a histopathological study as evidence of diabetic nephropathy. Currently there is a worldwide epidemic of chronic kidney failure associated with diabetic nephropathy, and this problem has not yet reached its peak. Although, as Brosius and Palm point out (25, 26), there is currently no ideal model for diabetic nephropathy, certain parameters are considered key indicators. They include: (i) a decrease in glomerular filtration greater than 50 %, (ii) a 100-fold or greater

increase in the rate of excretion of urinary albumin, and (iii) a histopathological study. What can be discarded in the present study is nephropathy caused by streptozotocin, as this would have manifested itself in the first 3-4 weeks after the administration of this compound (27, 28).

In spite of these limitations, the present study clearly demonstrates the effectiveness of eel serum in detaining the normally continuous decline in creatinine clearance during the development of chronic kidney failure. The current results provide the basis for further research into the best dose and combination of treatments. Moreover, it serves as an initial exploration of the mechanism by which eel serum acts, including the possibility that the high lectin content of eel serum restores a negative charge to the endothelial cell surface in the glomerulus and mesangium.

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