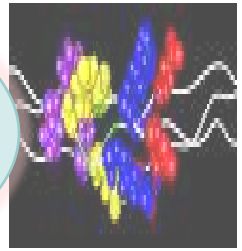
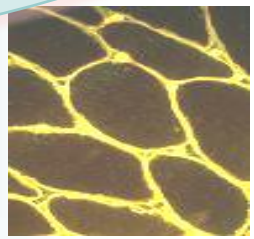
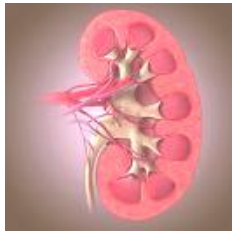
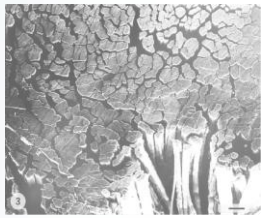
The effect of sodium fluoride administration on hydroxyproline concentration in rat kidneys

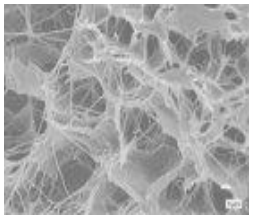
Eman Ali Al-Omireeni











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  b) **Nephrons**
  c) **Hydroxyproline**
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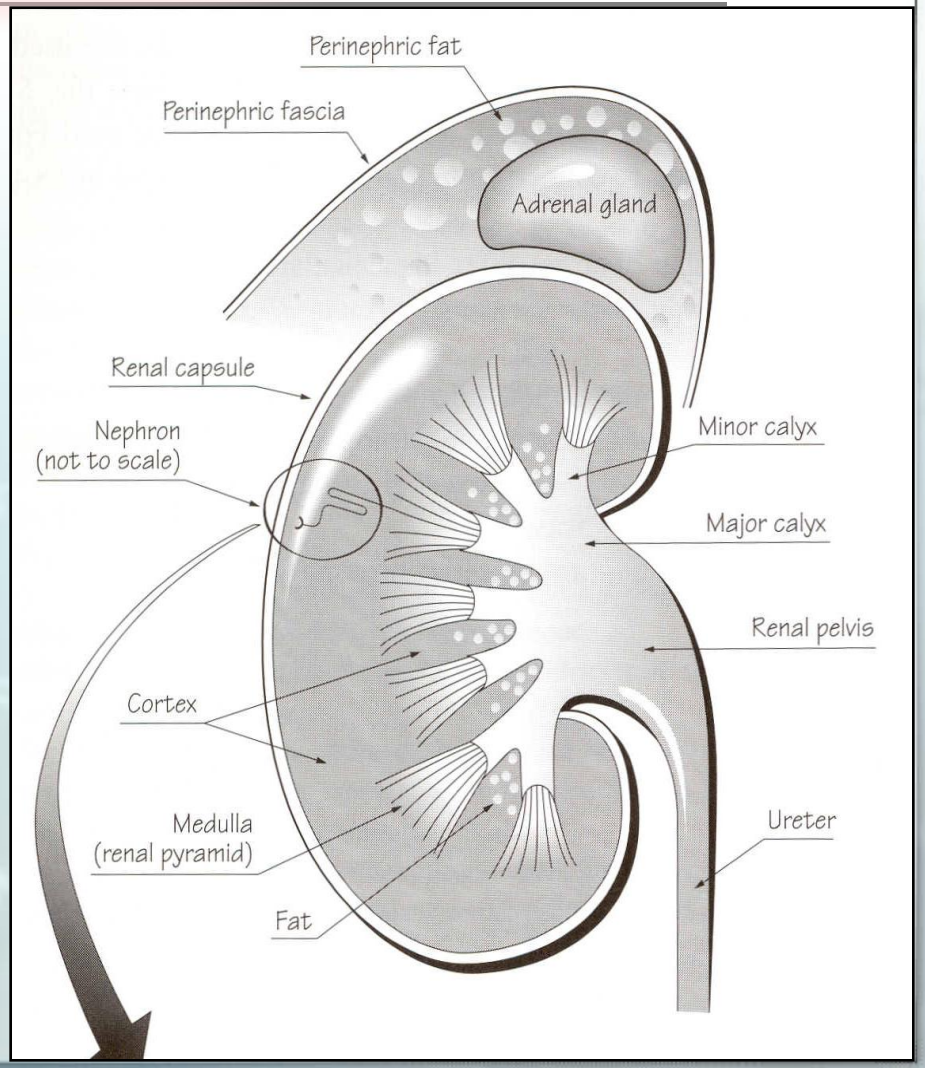
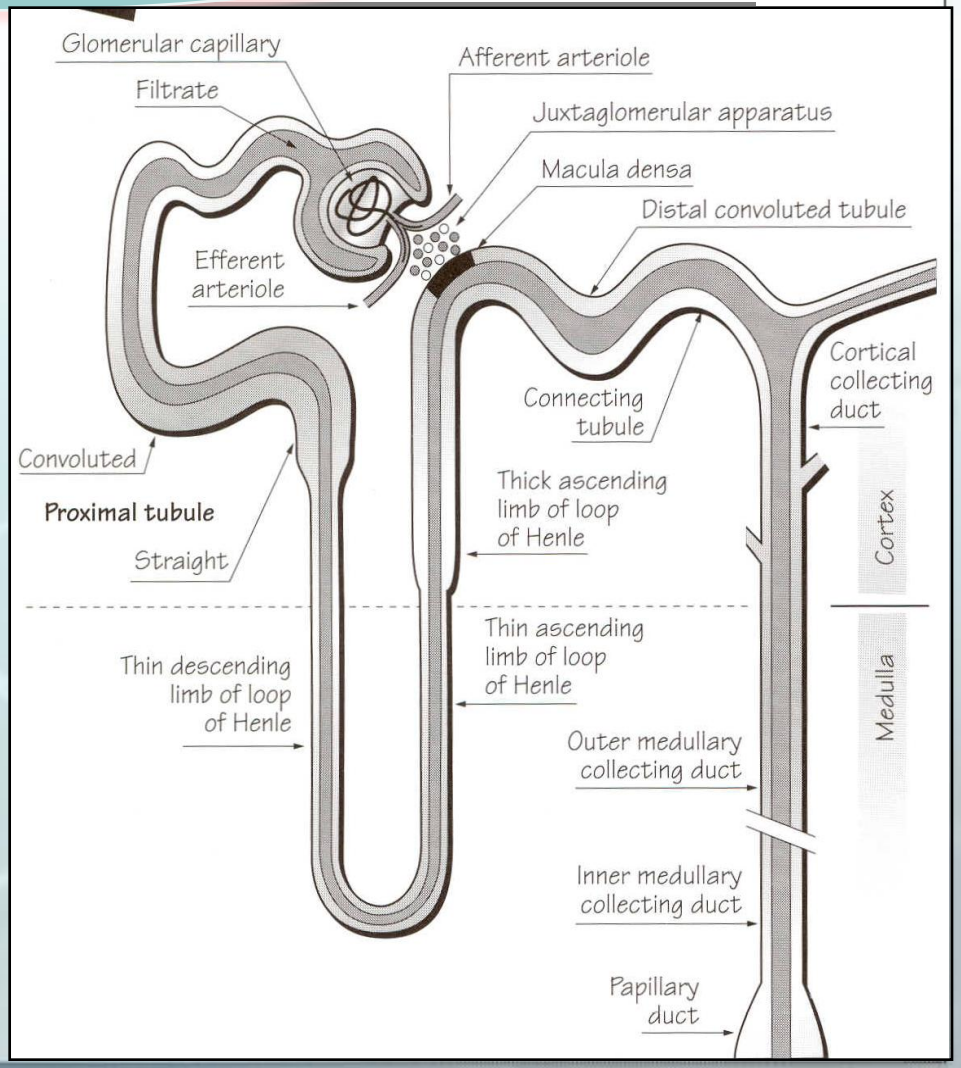
Kidneys

- Kidneys are paired, bean-shaped organs situated in a retroperitoneal position on the posterior aspect of the abdominal cavity, on either side of the vertebral column.
- The kidney is covered by a fibrous capsule. The outer zone of the kidney is the renal cortex which contains the glomeruli and the inner zone is the medulla.



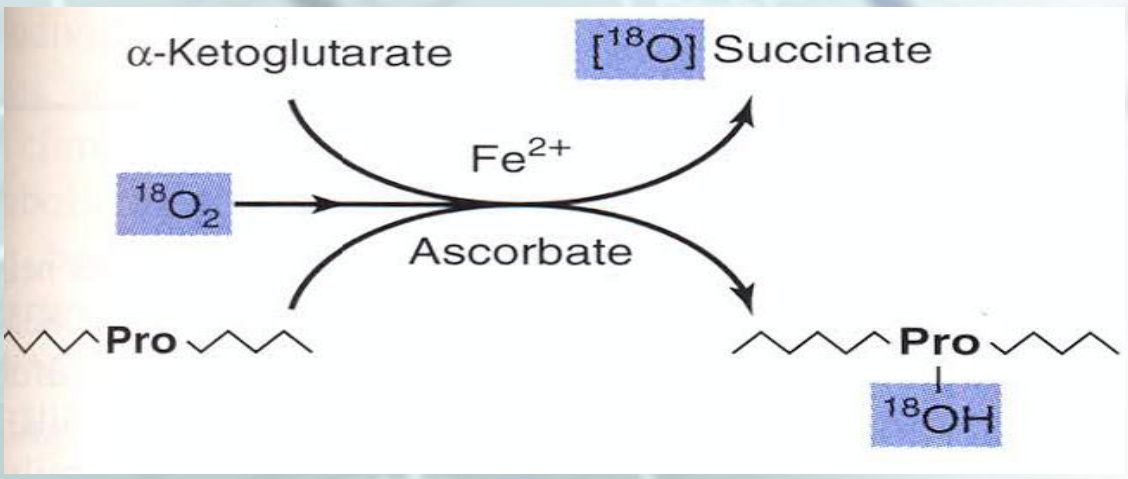
Nephron

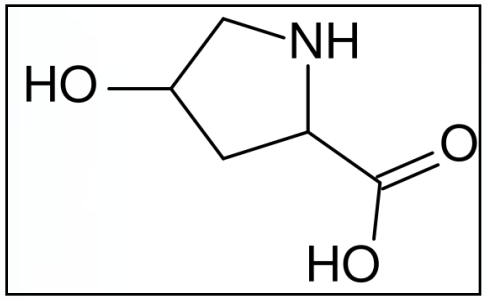
- The nephron is the structural and functional unit of the kidney.
- Each kidney contains 30,000 to 35,000 nephrons in the rats and about 1 million in humans .
- Each nephron is made up of a renal corpuscle (glomerulus) and a complex tubular portion, which drains into a unifying tubular system called the collecting duct system.



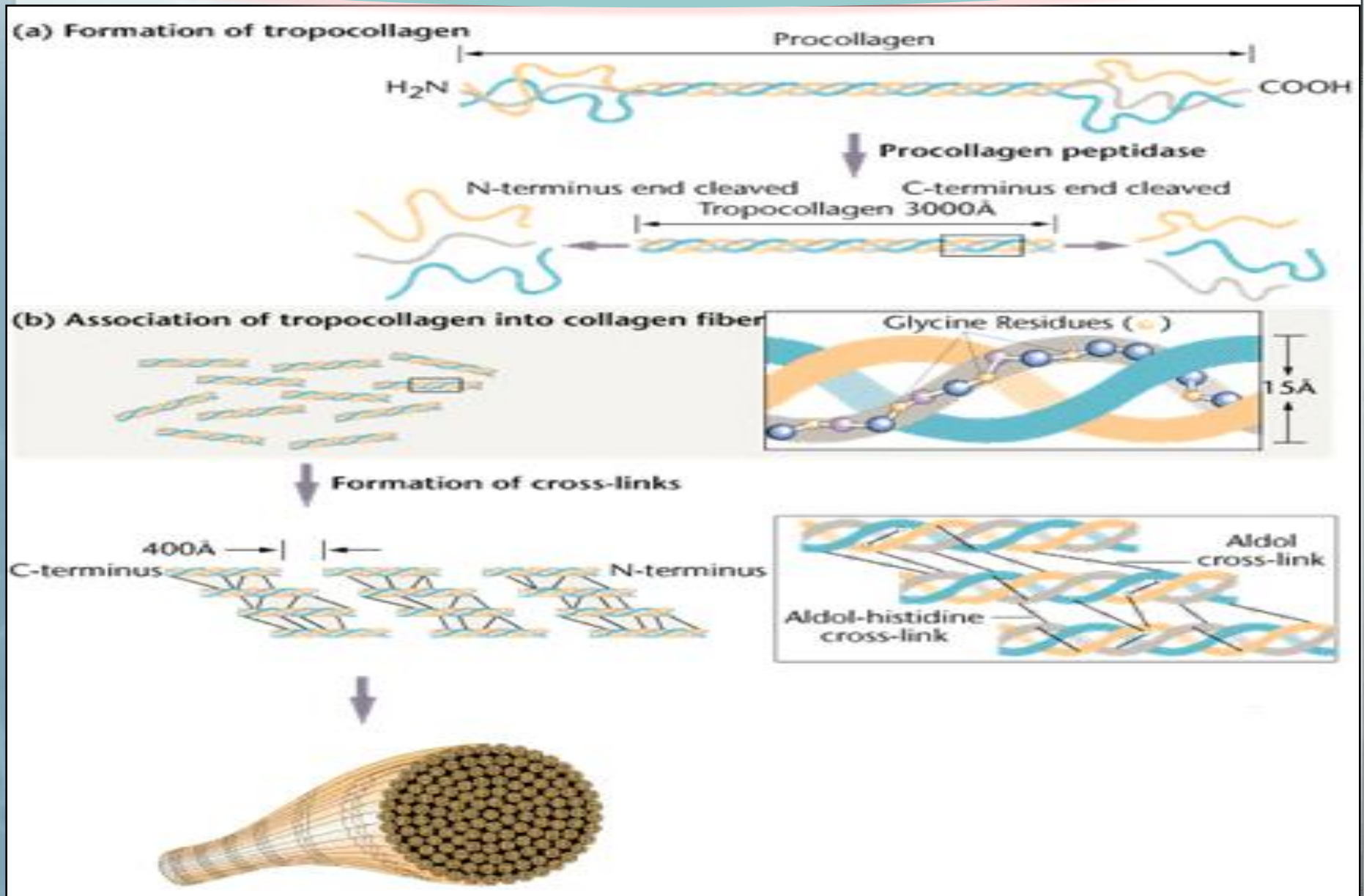
Hydroxyproline (Hyp)

- Hydroxyproline is a modified amino acid that is derived from proline by post-translation hydroxylation occuring within the peptide chain in biosynthesis of **collagen**.
- The occurrence of this amino acid is thought to be confined exclusively to **collagen**, where it is present in the Y position of the Gly-X-Y repeating tripeptide .





Collagen fibers

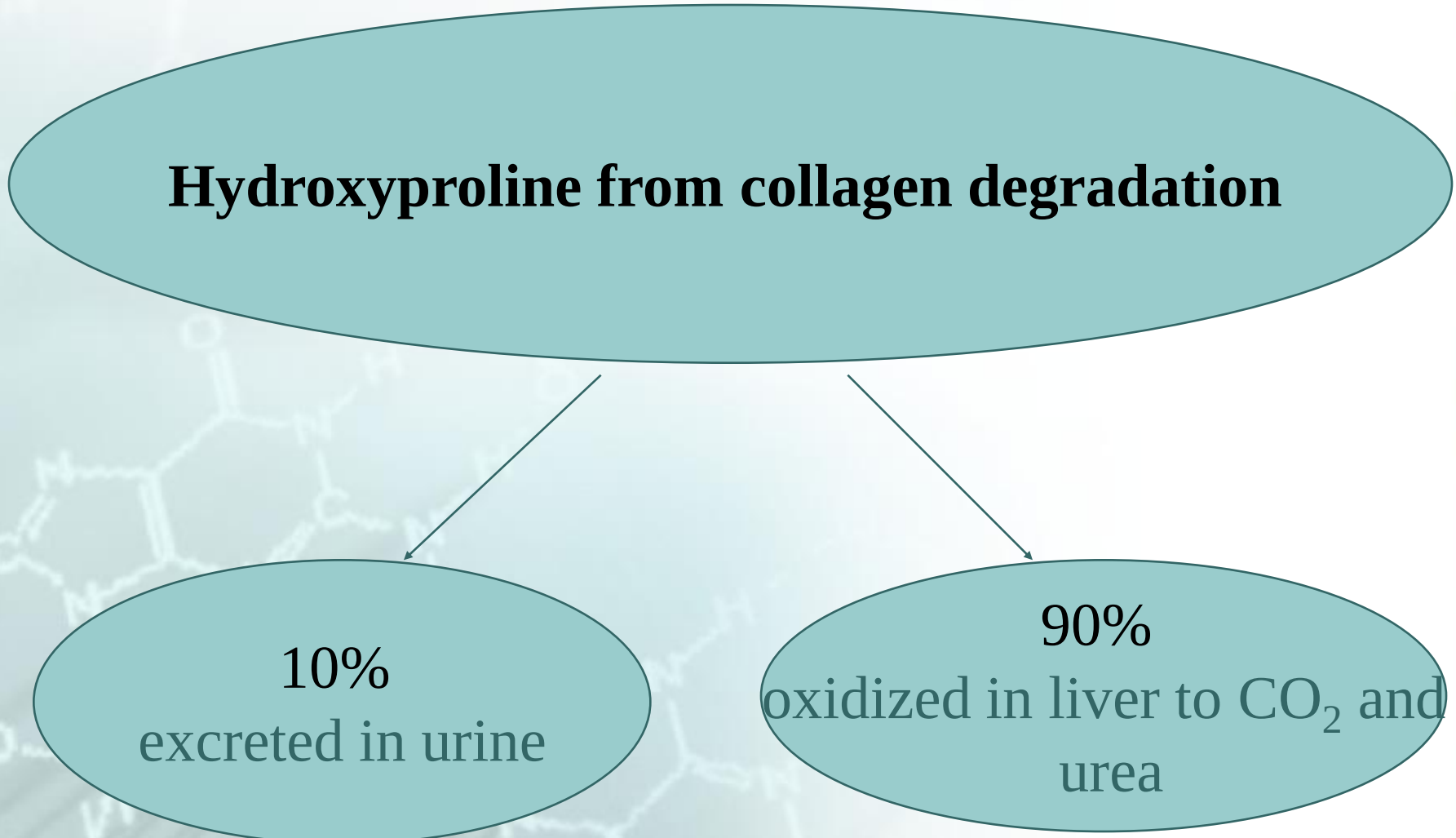


Forms of Hydroxyproline in the Tissues

1. Free Hydroxyproline.
2. Peptide- bound Hydroxyproline.
3. Protein- bound Hydroxyproline.
4. Soluble collagen hydroxyproline and insoluble collagen hydroxyproline

Breakdown or remodeling of collagen occurs normally in tissues in response to growth or injury.
However, during rapid growth and in disease states, the extent of collagen degradation is extensive.

Collagen content in tissue is determined by measuring its hydroxyproline content


Hydroxyproline from collagen degradation
10%
excreted in urine
90%
oxidized in liver to CO_2 and urea

Fluoride

- Fluoride is taken mainly in drinking water beside various nutrition products and beverages like tea, drugs, fluoride containing salt
- Fluoride has both beneficial and detrimental effects on human health, with a narrow range between the intakes associated with its beneficial and adverse health effects.

Aim

- To study the effect of sodium fluoride administration on hydroxyproline/collagen concentration in rat kidneys

- To study the reported protective effect of magnesium chloride on sodium fluoride induced changes in rat kidney hydroxyproline/collagen.

Materials and Methods

Protocol

Injection of sodium fluoride (intraperitoneally)

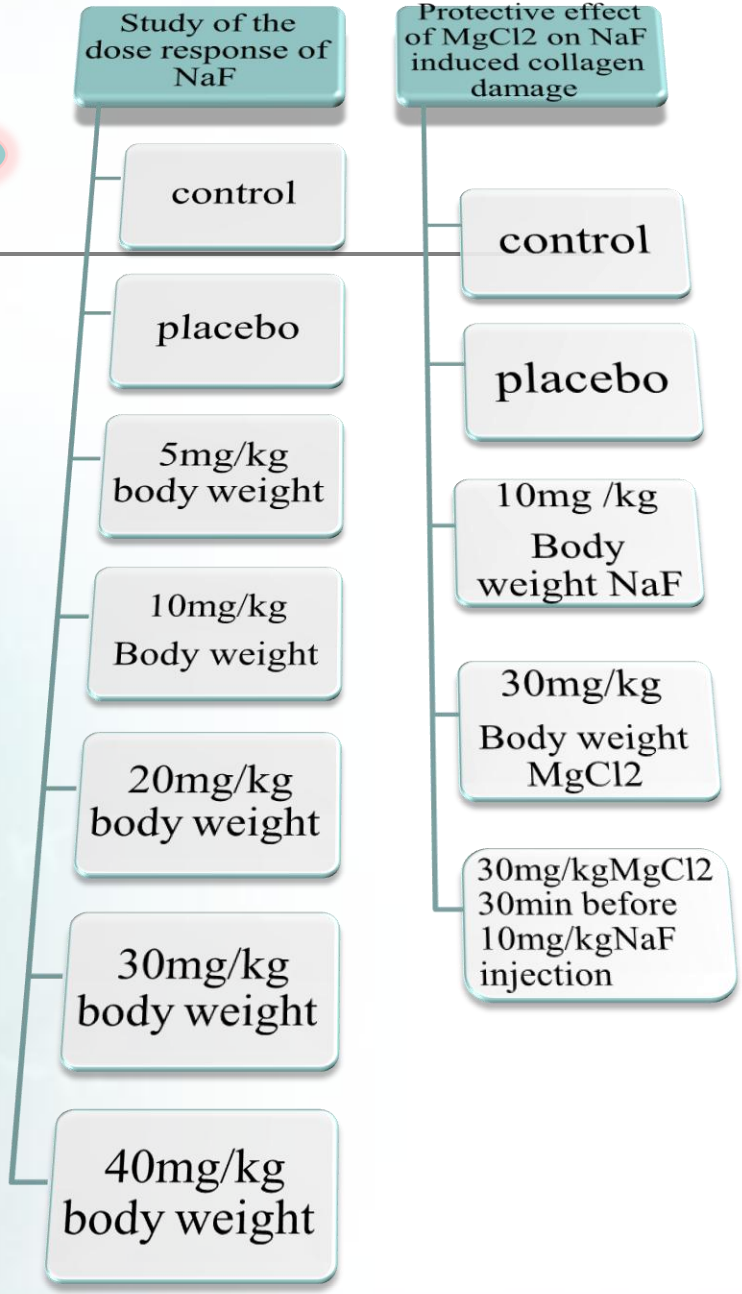
Kidneys removed and homogenized

Extraction of different hydroxyproline fractions

Determination of hydroxyproline/collagen concentration

Injection

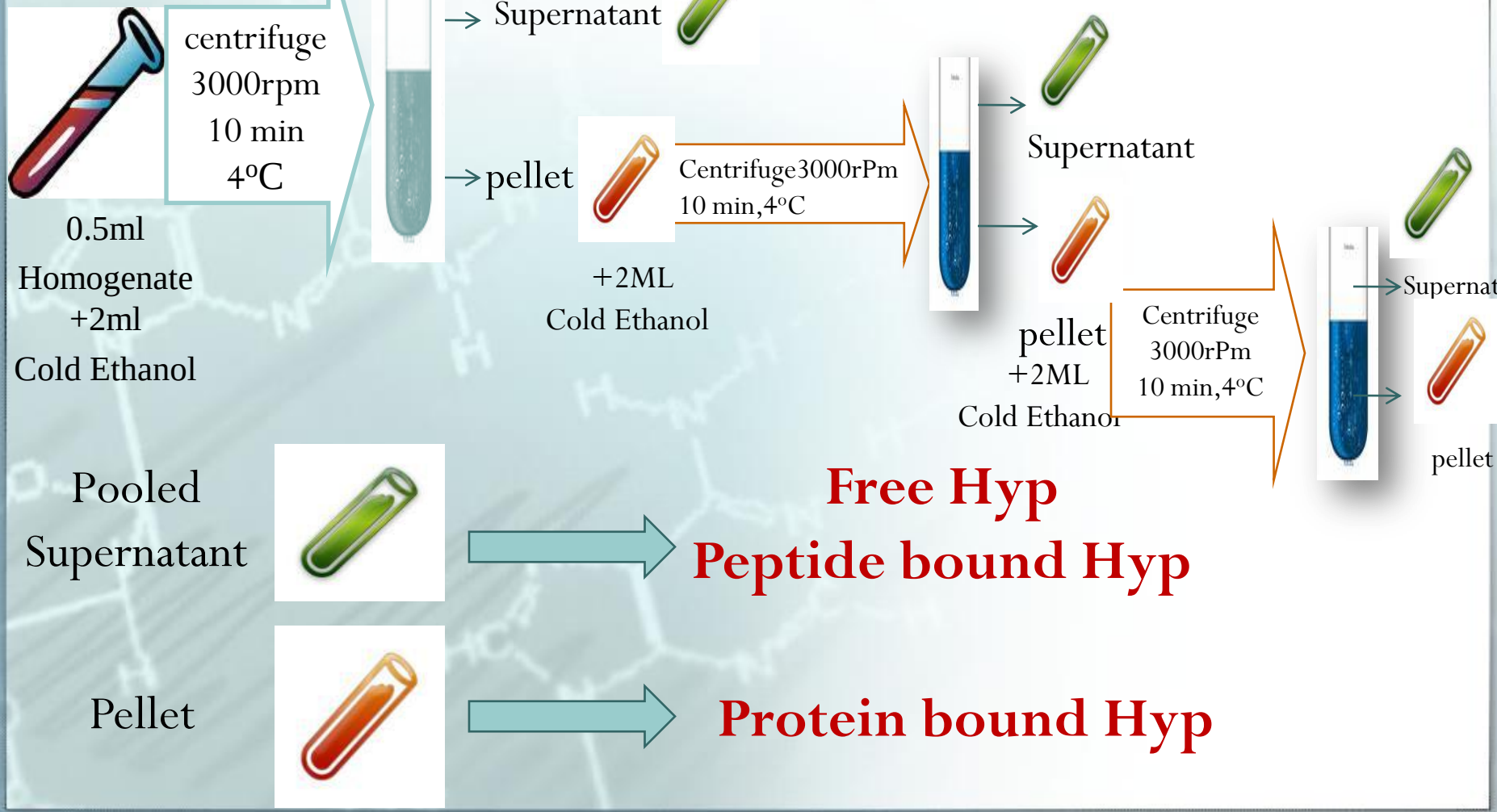
- All the groups consisted of
- 6 –8 rats of the same age and weight.
- The rats are injected with $NaF/MgCl_2$through intraperitoneal route.



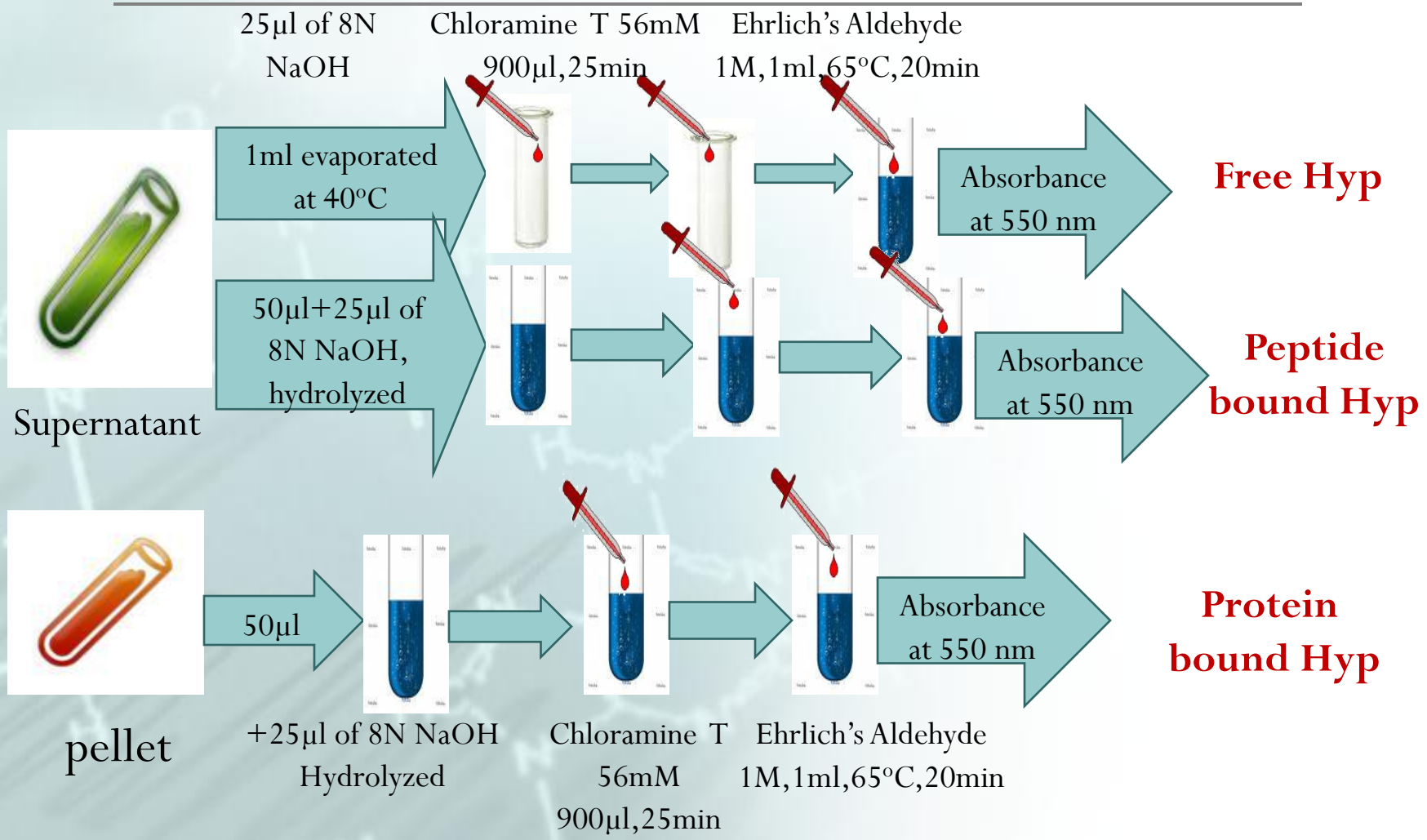
Kidneys removed and Homogenized

- All the rats were sacrificed 24 hours after the treatment.
- Kidneys were taken from rats, washed in buffered saline, frozen immediately in liquid nitrogen and stored at -80°C until processed.
- Tissues were homogenized in normal saline (0.85% NaCl). The homogenate was used to determine the free, peptide and protein-bound Hyp.
- Tissues were homogenized in 0.45% NaCl to determine the soluble and insoluble collagen Hyp.

Extraction of Free, Peptide-bound and Protein-bound Hyp



Determination of Free, Peptide-bound and Protein-bound Hyp



Extraction of soluble in soluble collagen Hyp

centrifuge
13000rpm
60 min
4°C

3ml
Homogenate

→ Supernatant

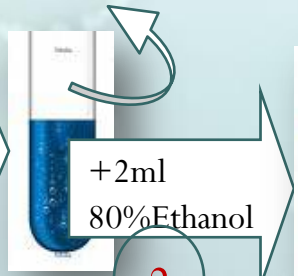
Soluble collagen Hyp

→ pellet

Insoluble collagen Hyp

+4ml 100% ethanol
centrifuge3000rpm
10 min,4°C

1ml
Supernatant



+2ml
80%Ethanol

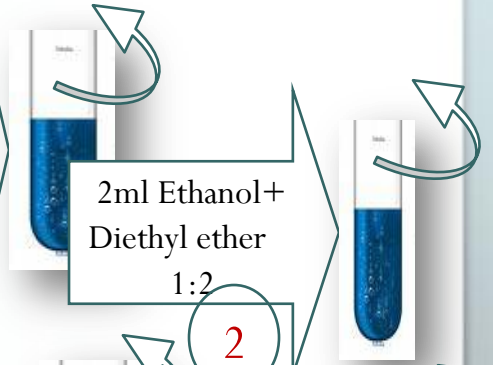
2

+2ml 100%
Ethanol

2

+2ml
Diethyl ether

2



2ml Ethanol+
Diethyl ether
1:2

2

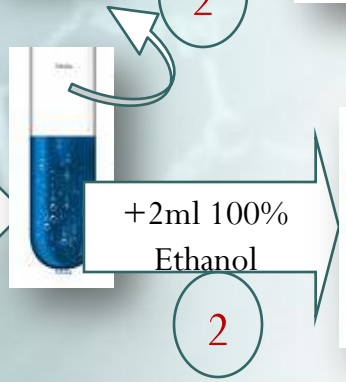
+2ml NaCl
centrifuge3000rpm
10 min,4°C

pellet

2

2ml DDH2O

2



+2ml 100%
Ethanol

2

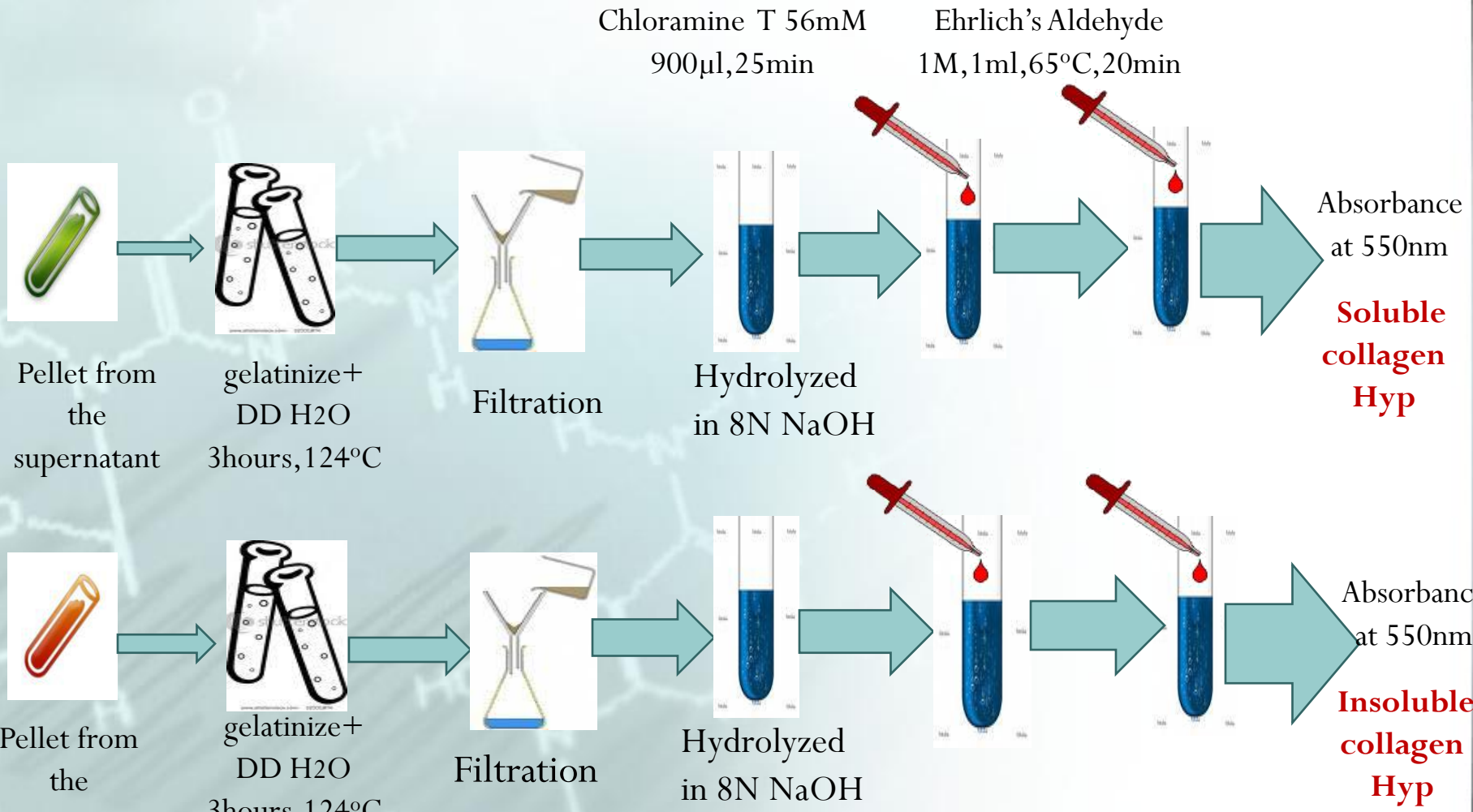
+2ml
Diethyl ether

2

2ml Ethanol+
Diethyl ether
1:2

2

Determination of soluble in soluble collagen Hyp



Results

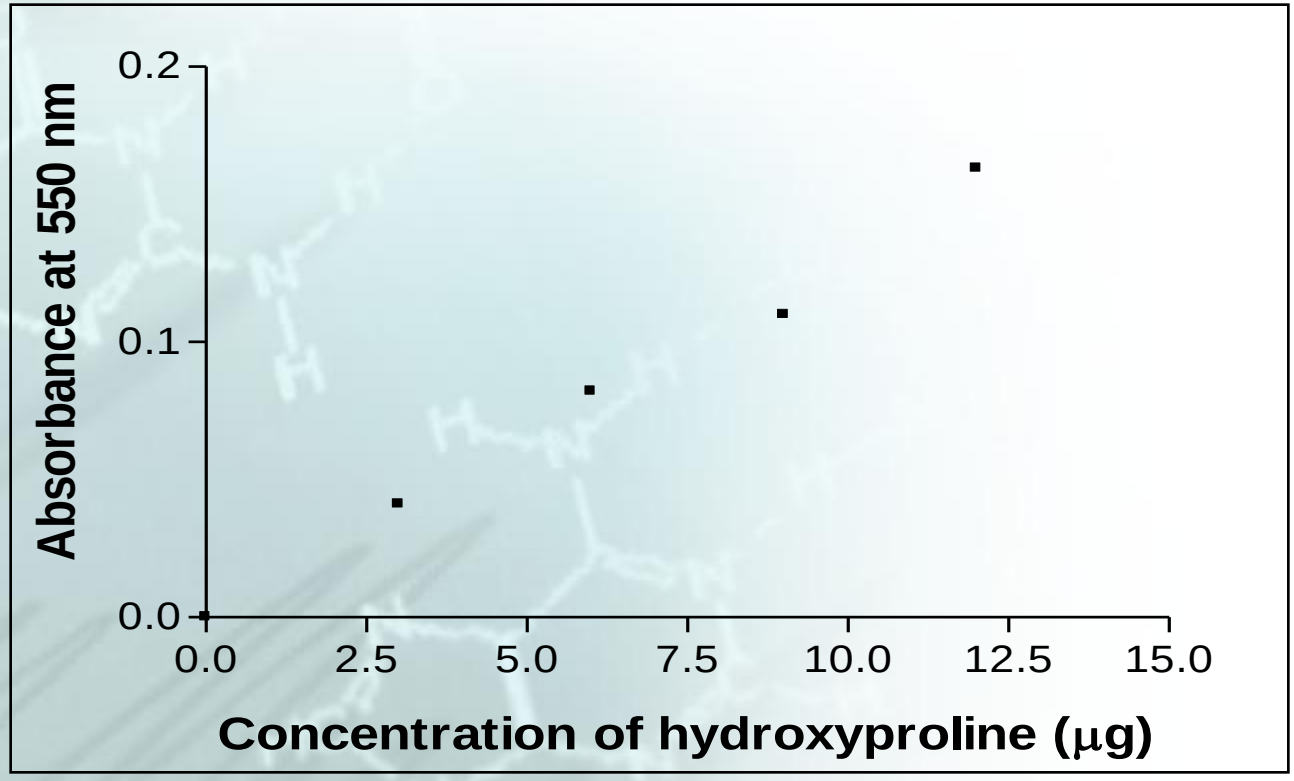
Standard graph

Effect of different doses of NaF on renal hydroxyproline/ col1agen in rats

Effect of$MgCl_2$ on NaF induced changes in renal hydroxyproline/ collagen in rats

Standard graph

Standard graph of hydroxyproline



Effect of different doses of NaF on kidney hydroxyproline/collagen in rats*.

* Al-Omireeni EA, Siddiqi NJ, Alhomida AS. Effect of different doses of sodium fluoride on various hydroxyproline fractions in rat kidneys. Kidney Research Journal, http://scialert.net/abstract/?doi=krj.0000.13409.13409. ISSN 1819-3374, (2009).

Effect of different doses of NaF on survival in rats

Serial number	Dose of NaF mg/kg body weight	Number of rats injected with NaF	Number of rats Survived	Percentage survival
1	Control	12	12	100
2	Placebo	12	12	100
3	5	12	12	100
4	10	6	6	100
5	20	6	6	100
6	30	15	10	67
7	40	15	4	27

Effect of NaF treatment on body weight, kidney weight and kidney protein in rats

Serial number	Experimental Groups (NaF treated)	Body Weight (grams)	Kidney Weight (grams)	Kidney protein (mg/gm fresh tissue)
1	Control	228.3 ± 3.20	1.20 ± 0.16	167.3 ± 37.96
2	Placebo	235.2 ± 7.55 ns	1.15 ± 0.12 ns	154.4 ± 43.56 ns
3	5 mg/kg	231.8 ± 15.35 ns	1.14 ± 0.14 ns	74.33 ± 8.98 ***
4	10 mg/kg	183.8 ± 16.92 ***	1.09 ± 0.17 ns	113.7 ± 22.64 *
5	20 mg/kg	250.0 ± 0.00 **	1.04 ± 0.04 ns	159.9 ± 15.61 *
6	30 mg/kg	270.0 ± 0.00 ***	1.06 ± 0.1 ns	59.33 ± 6.64 ***

Effect of NaF treatment on organosomatic index in rat kidney

Serial number	Dose of NaF (mg/kg body weight)	Organ somatic index
1	Control	0.58 ± 0.08
2	Placebo	0.61 ± 0.05^{ns}
3	5	0.50 ± 0.07^{ns}
4	10	0.60 ± 0.07^{ns}
5	20	$0.41 \pm 0.02^{***}$
6	30	$0.40 \pm 0.04^{***}$

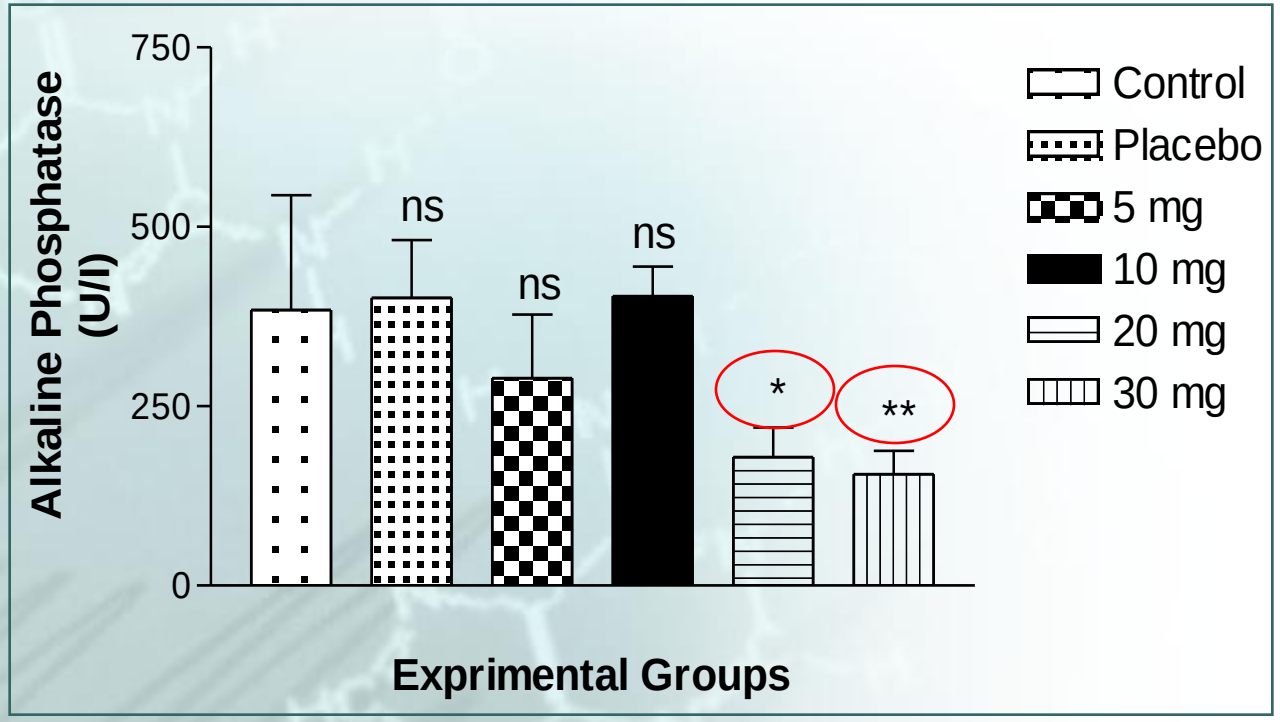
Effect of NaF treatment on serum biochemical parameters in rats

Biochemical Parameters	Control	Placebo	5 mg/kg body weight	10 mg/kg body weight	20 mg/kg body weight	30 mg/kg body weight
Urea (mg/dl)	33.5 ± 3.12	34.50 ± 3.32^{ns}	40.75 ± 4.92^{ns}	62.0 ± 9.31***	57.0 ± 8.83***	30.50 ± 4.44*
BUN (mg/dl)	17.75 ± 3.30	17.25 ± 2.22 ^{ns}	19.75 ± 2.36 ^{ns}	17.25 ± 1.5*	30.00 ± 6.05**	23.75 ± 6.19 ^{ns}
Creatinine (mg/dl)	0.37 ± 0.07	0.40 ± 0.06 ^{ns}	0.55 ± 0.05*	0.36 ± 0.06 ^{ns}	0.51 ± 0.03 ^{ns}	0.47 ± 0.09 ^{ns}
Uric acid (mg/dl)	1.33 ± 0.21	1.28 ± 0.52 ^{ns}	1.40 ± 0.27 ^{ns}	1.63 ± 0.15 ^{ns}	0.70 ± 0.14 ^{ns}	1.50 ± 0.52 ^{ns}
Albumin (g/dl)	3.56 ± 0.45	3.71 ± 0.33 ^{ns}	3.54 ± 4.50 ^{ns}	3.95 ± 0.17 ^{ns}	3.10 ± 0.34 ^{ns}	2.66 ± 0.21 ^{ns}
Protein (g/dl)	6.20 ± 0.45	6.44 ± 0.43 ^{ns}	7.35 ± 0.38 ^{ns}	7.63 ± 0.60*	5.55 ± 0.77 ^{ns}	5.02 ± 0.59*

Effect of NaF treatment on serum electrolytes concentration in rats

Serum Electrolytes	Control	Placebo	5 mg/kg body weight	10 mg/kg body weight	20 mg/kg body weight	30 mg/kg body weight
Sodium (mEq/I)	143.8 ± 4.35	144.5 ± 6.46 ns	148.8 ± 6.5 ns	142.8 ± 3.1 ns	139.5 ± 2.52 ns	136.0 ± 4.24
Potassium (mEq/I)	5.58 ± 0.96	6.50 ± 3.48 ns	6.20 ± 0.51 ns	11.75 ± 0.87***	4.63 ± 0.17 ns	4.68 ± 0.50 ns
Calcium (mg/dl)	12.34 ± 0.66	12.82 ± 0.23 ns	12.75 ± 1.20 ns	11.99 ± 0.25 ns	9.49 ± 0.13***	8.55 ± 0.19**
Magnesim (mg/dl)	2.45 ± 0.04	2.54 ± 0.32 ns	3.21 ± 0.47*	2.44 ± 0.22 ns	2.44 ± 0.12 ns	2.34 ± 0.45 ns
Phosphorous (mg/dl)	10.24 ± 1.64	10.24 ± 2.23 ns	12.42 ± 2.43 ns	10.80 ± 0.86 ns	6.42 ± 0.61*	6.79 ± 0.94**

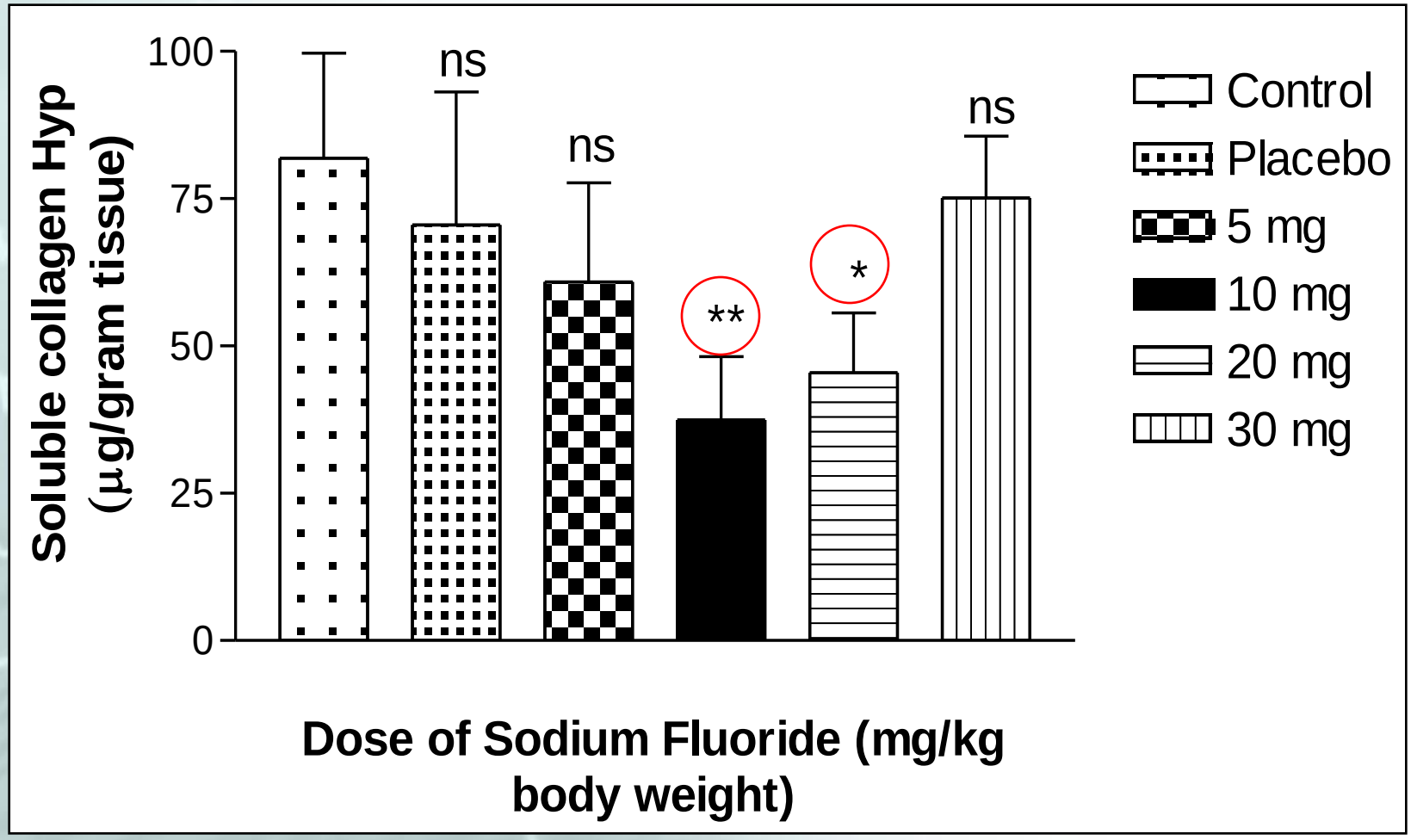
Effect of NaF treatment on serum alkaline phosphatase in rats



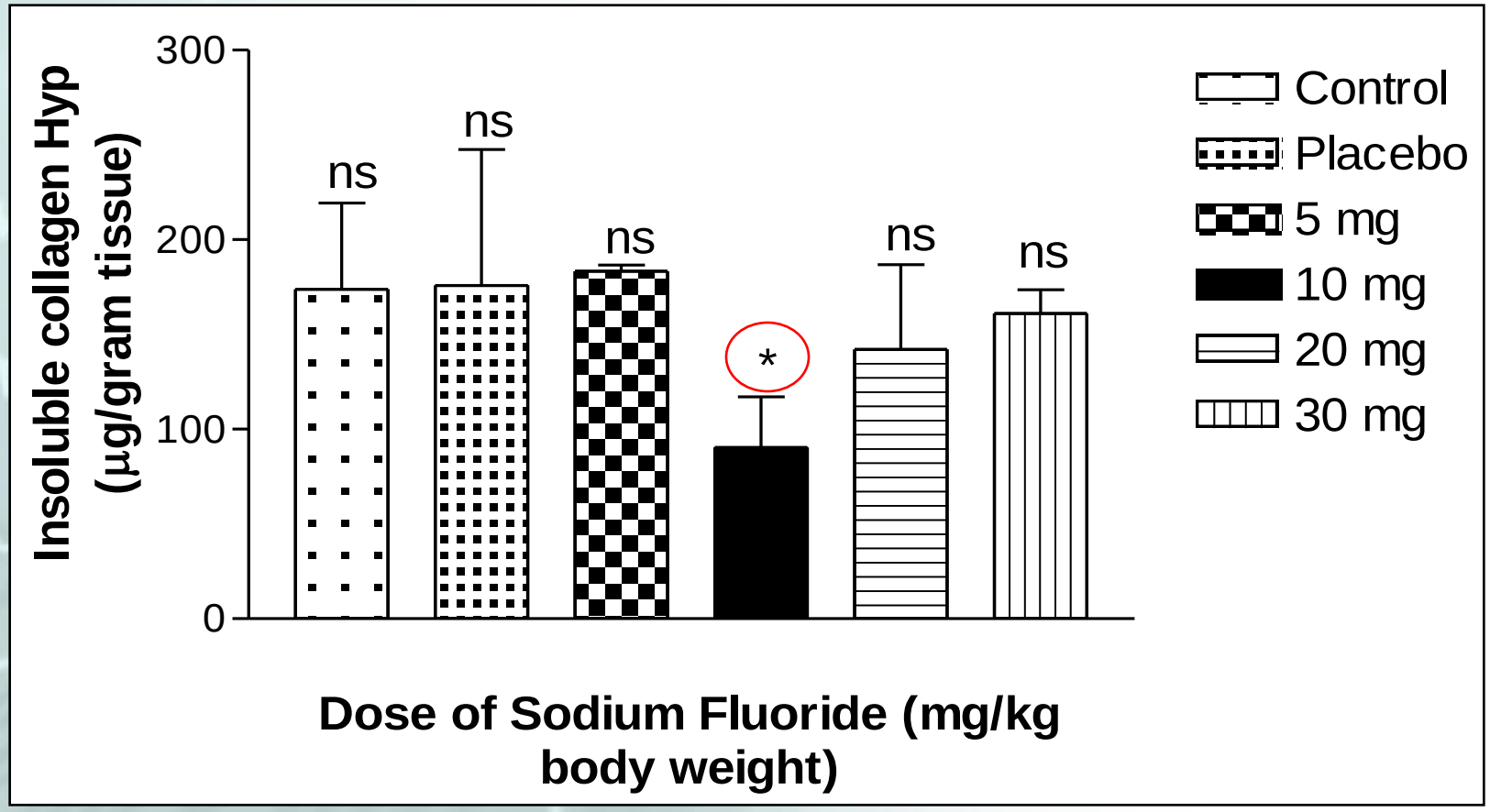
Effect of NaF treatment on different hydroxyproline fractions in rat kidneys

Experimental Groups (NaF treated)	Free Hyp (µg/gm fresh tissue)	Peptide-bound Hyp (mg/gm fresh tissue)	Protein-bound Hyp (mg/gm fresh tissue)	Total Hyp (mg/gm fresh tissue)
Control	329.3 ± 54.65	11.12 ± 0.92	1.048 ± 0.15	12.32 ± 2.76
Placebo	276.8 ± 24.66^{ns}	5.92 ± 3.2*	1.22 ± 0.37 ^{ns}	6.86 ± 0.59**
5mg/kg body weight	283.5 ± 50.7 ^{ns}	8.91 ± 2.9 ^{ns}	2.76 ±0.40***	11.76± 3.07 ^{ns}
10 mg/kg body weight	125.3±15.94***	1.61± 0.9***	1.26 ± 0.23 ^{ns}	2.93 ±0.75***
20 mg/kg body weight	245.5 ± 40.5*	1.89 ± 0.41**	1.49 ± 0.21 ^{ns}	2.98 ±0.67***
30 mg/kg body weight	333.2 ± 49.5 ^{ns}	1.30 ± 0.25**	1.308 ± 0.29 ^{ns}	2.62 ±0.49***

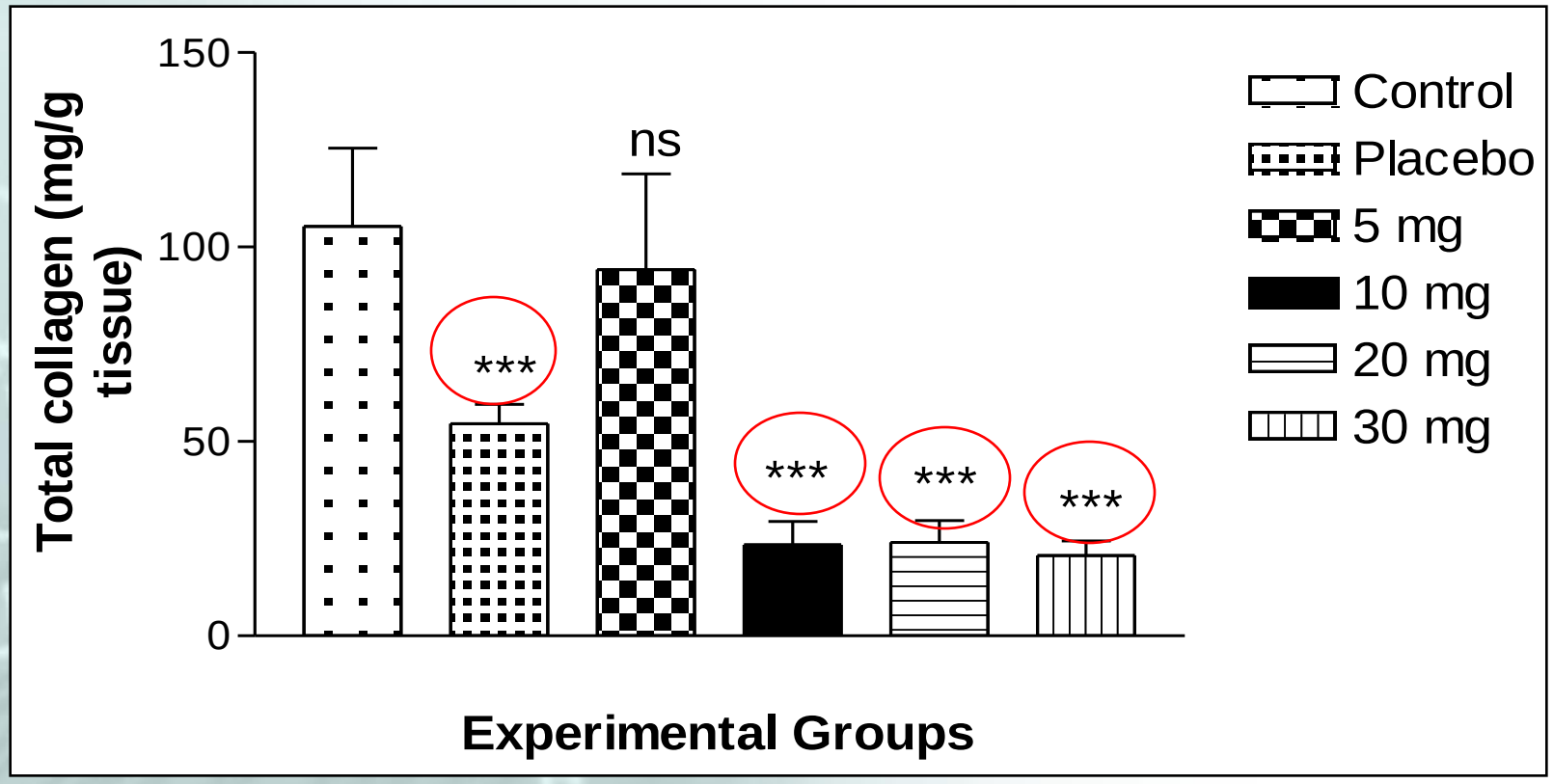
Effect of NaF on soluble collagen hydroxyproline in rat kidneys



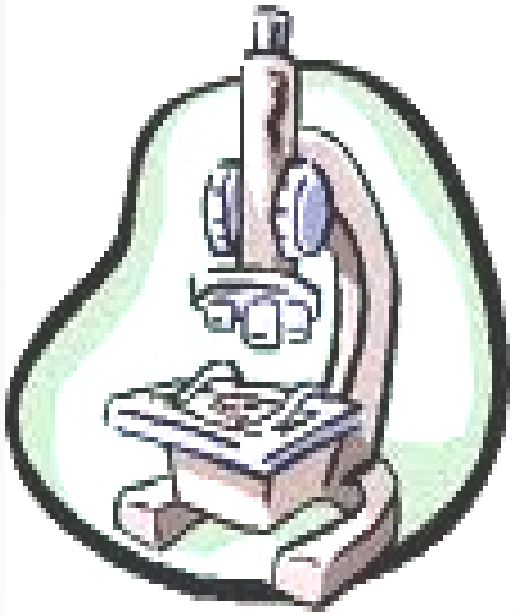
Effect of NaF on insoluble collagen hydroxyproline in rat kidneys



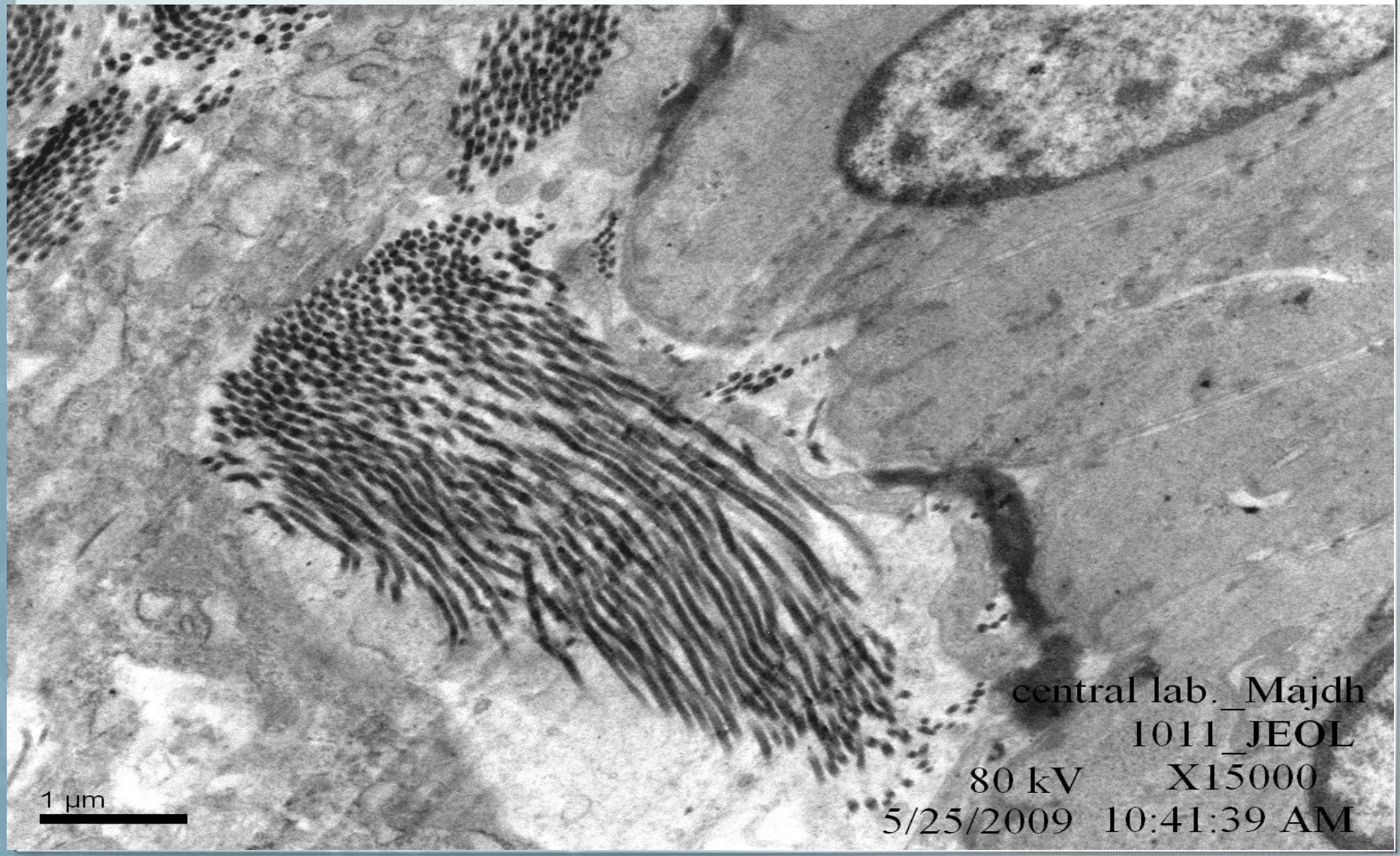
Effect of NaF on total collagen hydroxyproline in rat kidneys



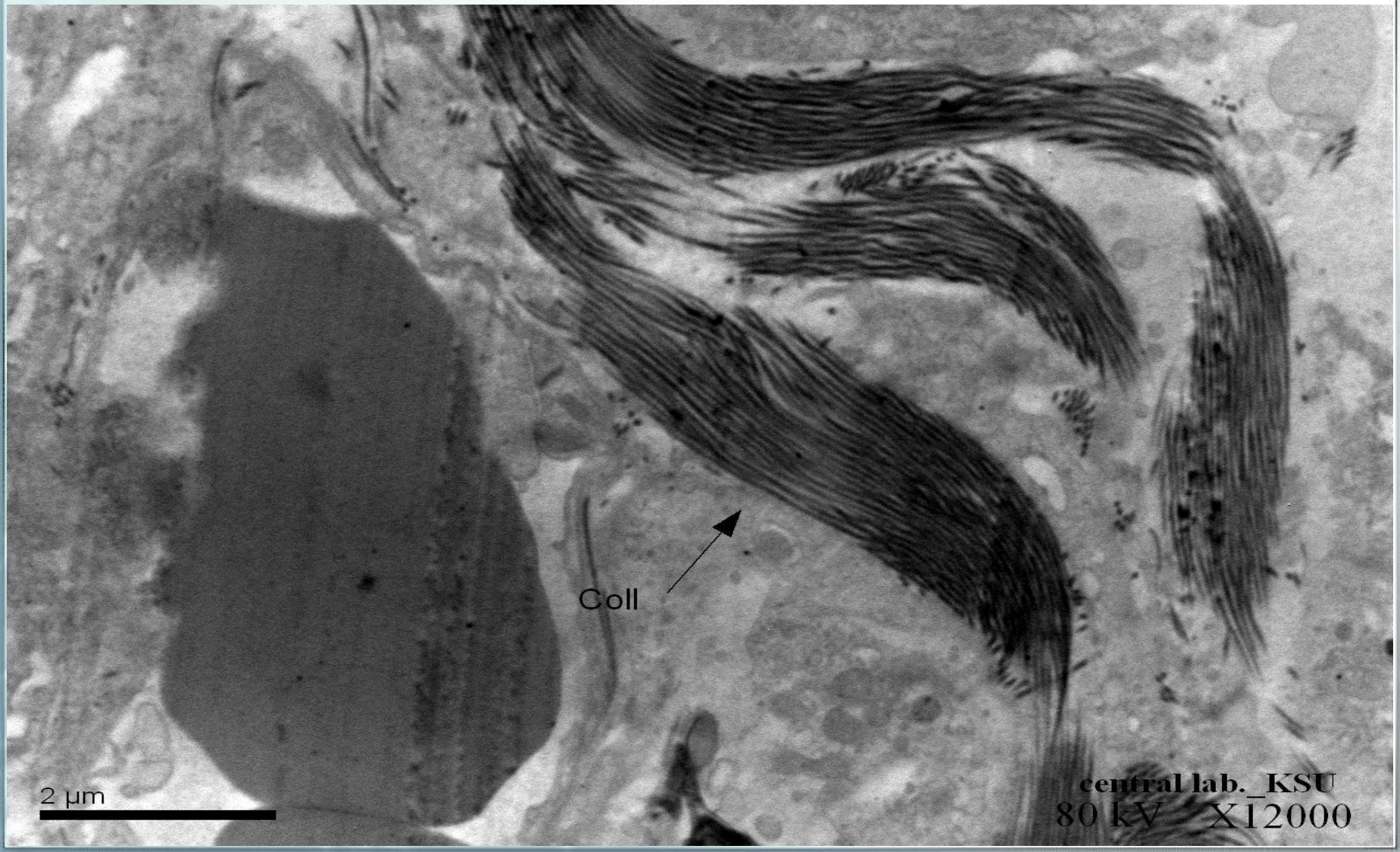
Electron micrograph of kidney from control animal



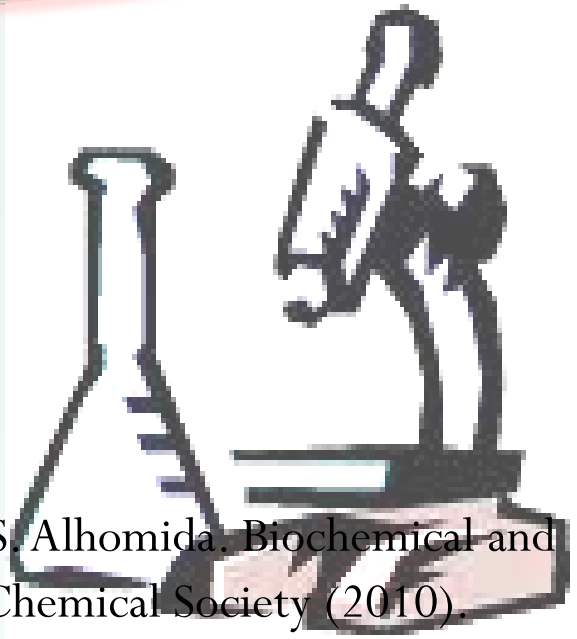
Control


central lab._Majdh
1011_JEOL
80 kV X15000
5/25/2009 10:41:39 AM
1 µm

Control

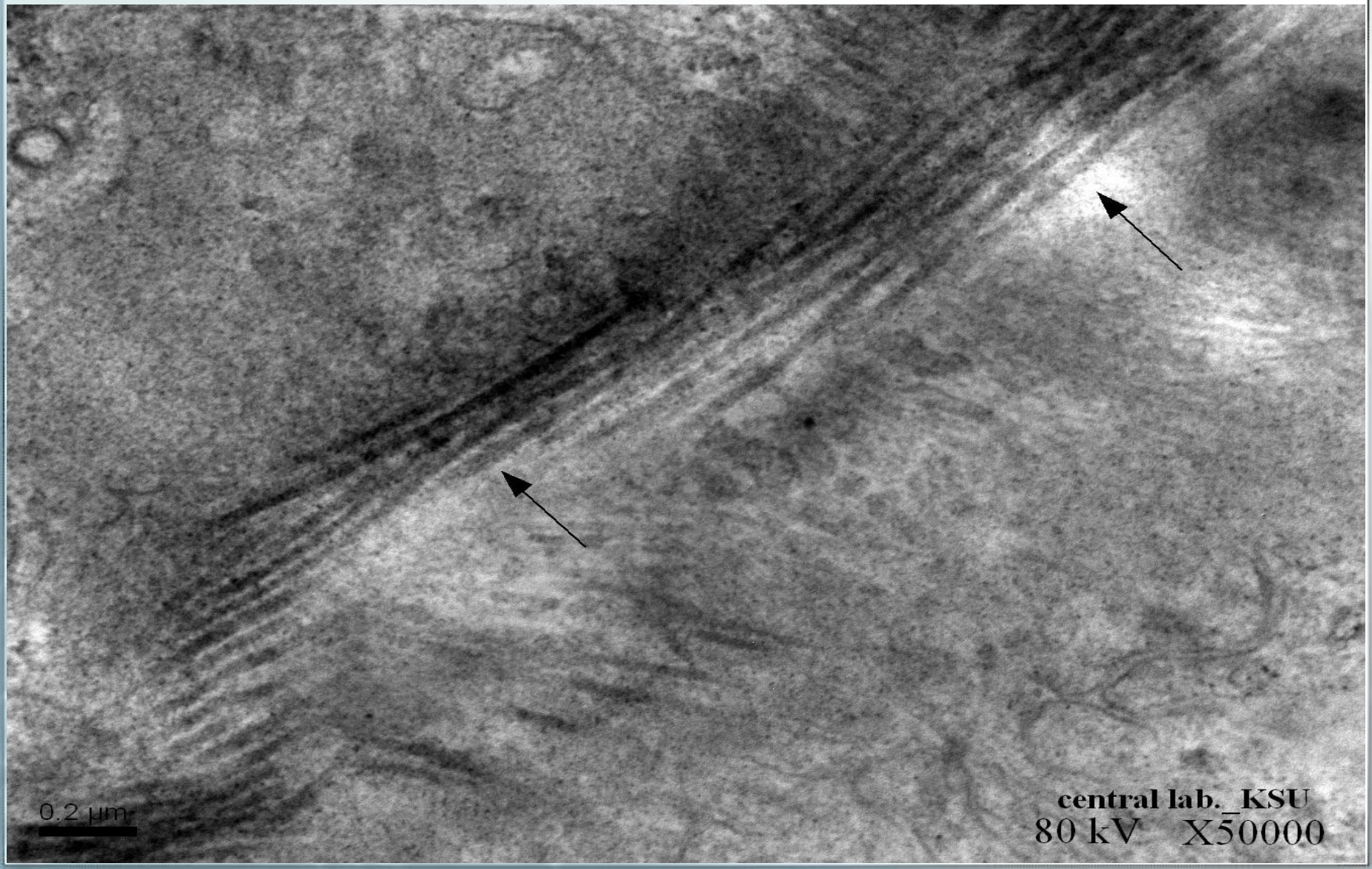


Electron micrograph of kidney collagen in rats injected with 5,10,20 and 30 mg/kg body weight of NaF*



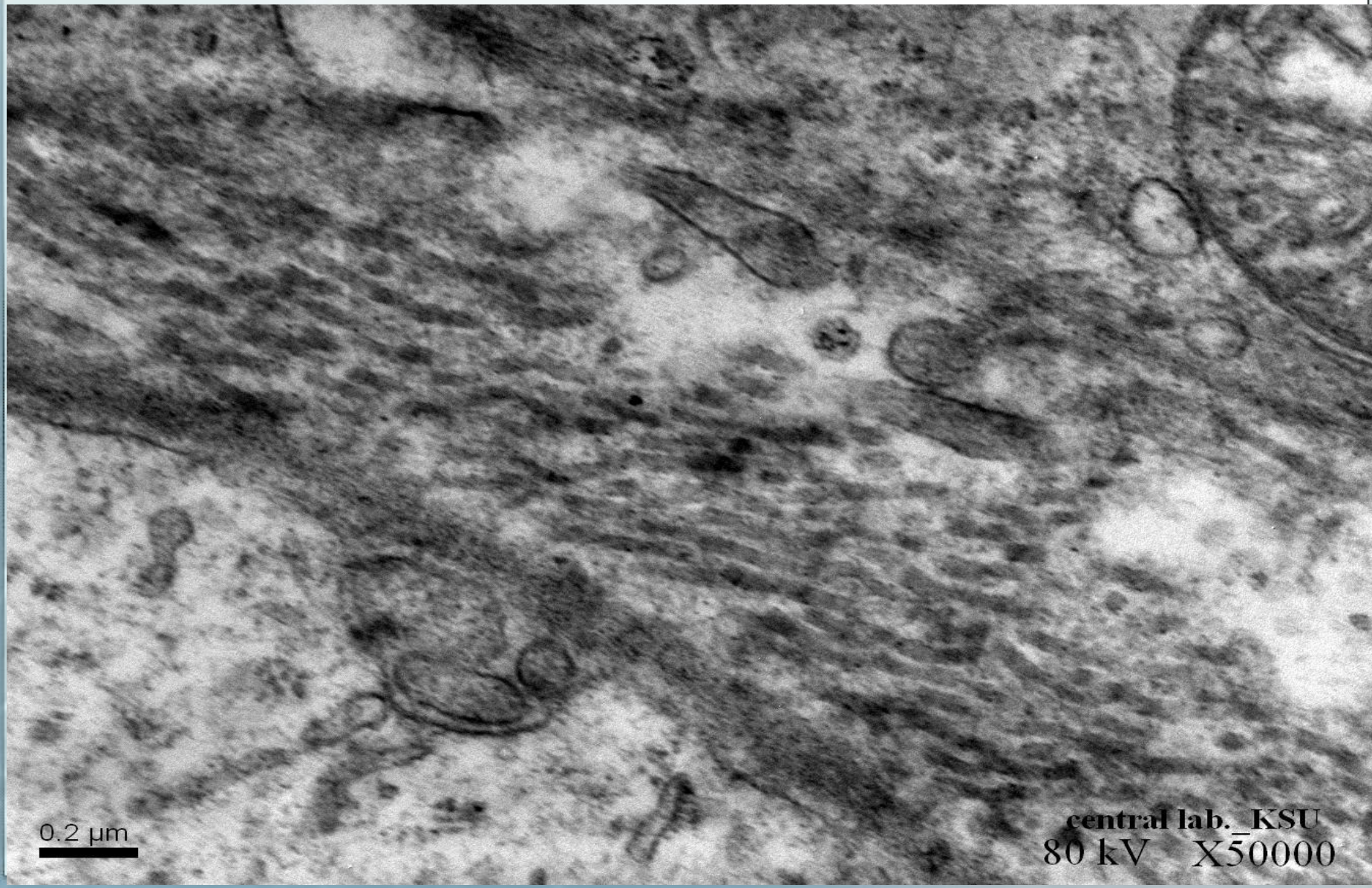
* Accepted for publication /E.A Alomireeni, N.J.Siddiqi, A.S. Alhomida. Biochemical and histological stuides on the effect of NaF on rat kidney. Saudi Chemical Society (2010).

5mg/kg body weight NaF

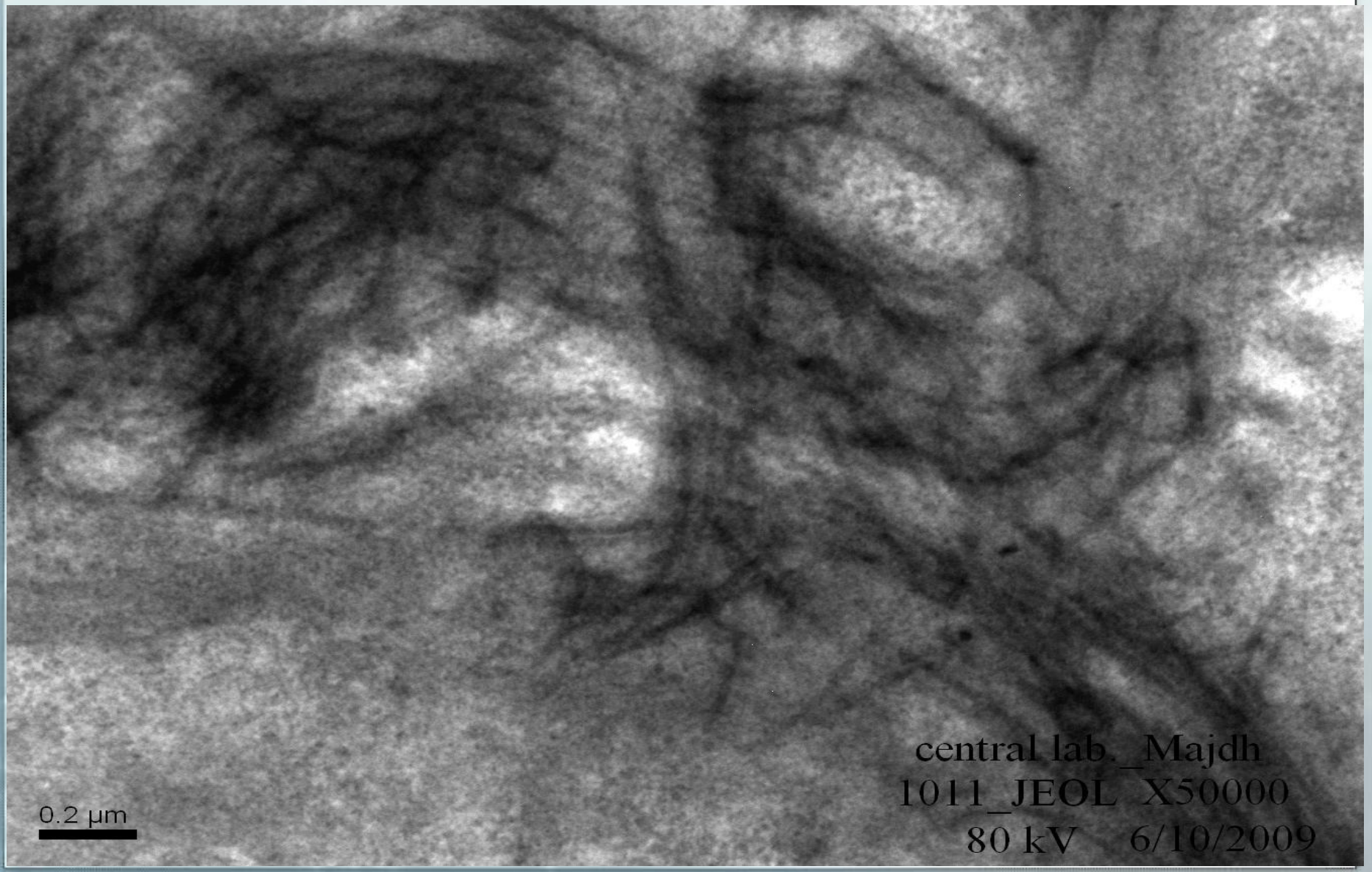


10mg/kg body weight NaF

20mg/kg body weight NaF



30mg/kg body weight NaF



Effect of $MgCl_2$ on NaF induced changes in renal hydroxyproline/collagen in rats*

* E.A. AlOmireeni, N.J. Siddiqi, A.S. Alhomida. Effect of magnesium chloride and sodium fluoride on various hydroxyproline fractions in rat kidneys. Canadian Journal of Pure and Applied Sciences,4(2) ISSN 1715-9997, (2010).

Effect of $MgCl_2$ and NaF treatment on body weight, kidney weight and protein content in rats

Experimental groups	Body weight (grams)	Kidney weight (grams)	Kidney protein (mg/gram tissue)
Control	228.33 ± 3.20	1.07 ± 0.97	150.1 ± 37.5
$MgCl_2$	200.83 ± 13.67**	0.97 ± 0.10^{ns}	45.61 ± 2.04***
NaF	183.83 ± 16.91***	1.09 ± 0.17 ^{ns}	113.7 ± 22.64*
$MgCl_2$ + NaF	178.83 ± 7.78***	0.76 ± 0.06 ^{ns}	54.93 ± 2.88***

Effect of $MgCl_2$ and NaF treatment on organosomatic index in rat kidney

Serial number	Groups	Organ somatic index
1	Control	0.58 ± 0.72
2	NaF	0.62 ± 0.11^{ns}
3	$MgCl_2$	0.49 ± 0.04^{ns}
4	NaF+$MgCl_2$	0.41 ± 0.05^{ns}

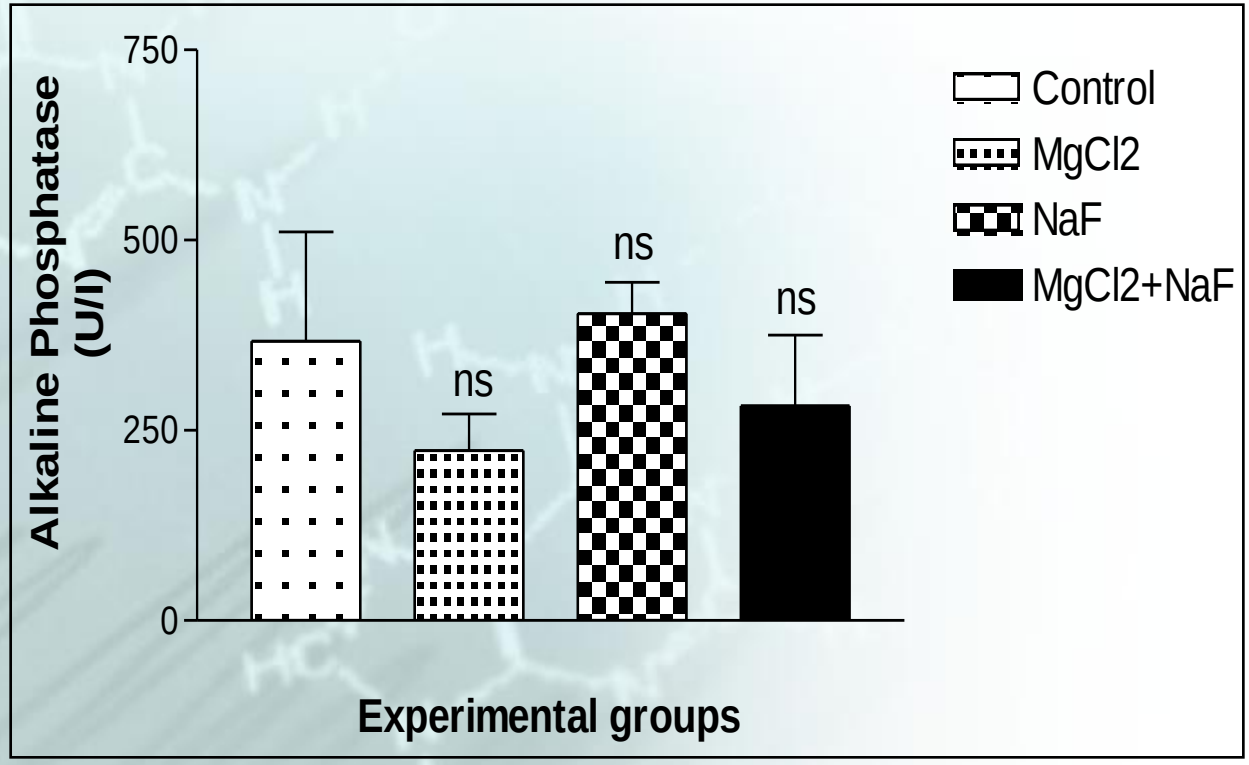
Effect of $MgCl_2$ and NaF treatment on serum biochemical parameters in rats

Biochemical Parameters	Control	$MgCl_2$	NaF	$MgCl_2$ + NaF
Urea (mg/dl)	33.5 ± 3.12	42.50 ± 4.80^{ns}	62.0 ± 9.31***	48.75 ± 4.35*
BUN(mg/dl)	17.75 ± 3.30	22.75 ± 2.75^{ns}	17.25 ± 1.5^{ns}	22.75 ± 2.22 ^{ns}
Creatinine (mg/dl)	0.37 ± 0.07	0.47 ± 0.02 ^{ns}	0.36 ± 0.06 ^{ns}	0.46 ± 0.03 ^{ns}
Uric acid (mg/dl)	1.33 ± 0.21	0.85 ± 0.13*	1.63 ± 0.15 ^{ns}	1.5 ± 0.29 ^{ns}
Albumin(g/dl)	3.56 ± 0.45	3.94 ± 0.28 ^{ns}	3.97 ± 0.15 ^{ns}	4.32 ± 0.15*
Protein(g/dl)	6.20 ± 0.45	7.62 ±0.22***	7.63 ± 0.60***	7.28 ± 0.23**

Effect of $MgCl_2$ and NaF treatment on serum electrolytes concentration in rats

Serum Electrolytes	Control	$MgCl_2$	NaF	MgCl2 + NaF
Sodium (mEq/I)	142.6 ± 4.56	138.8 ± 3.77^{ns}	142.8 ± 3.0 ^{ns}	132.2 ± 2.95**
Potassium (mEq/I)	5.58 ± 0.96	5.6 ± 0.36 ^{ns}	11.75 ± 0.87***	5.85 ± 0.35 ^{ns}
Calcium (mg/dl)	12.34 ± 0.66	10.81± 0.12**	11.99 ± 0.25 ^{ns}	10.78 ± 0.45**
Magnesium (mg/dl)	2.45 ± 0.05	2.37 ± 0.14 ^{ns}	2.44 ± 0.22 ^{ns}	3.05 ± 0.20**
Phosphorous (mg/dl)	10.24 ± 1.64	8.78 ± 0.31 ^{ns}	10.80 ± 0.86 ^{ns}	11.36 ± 0.60 ^{ns}

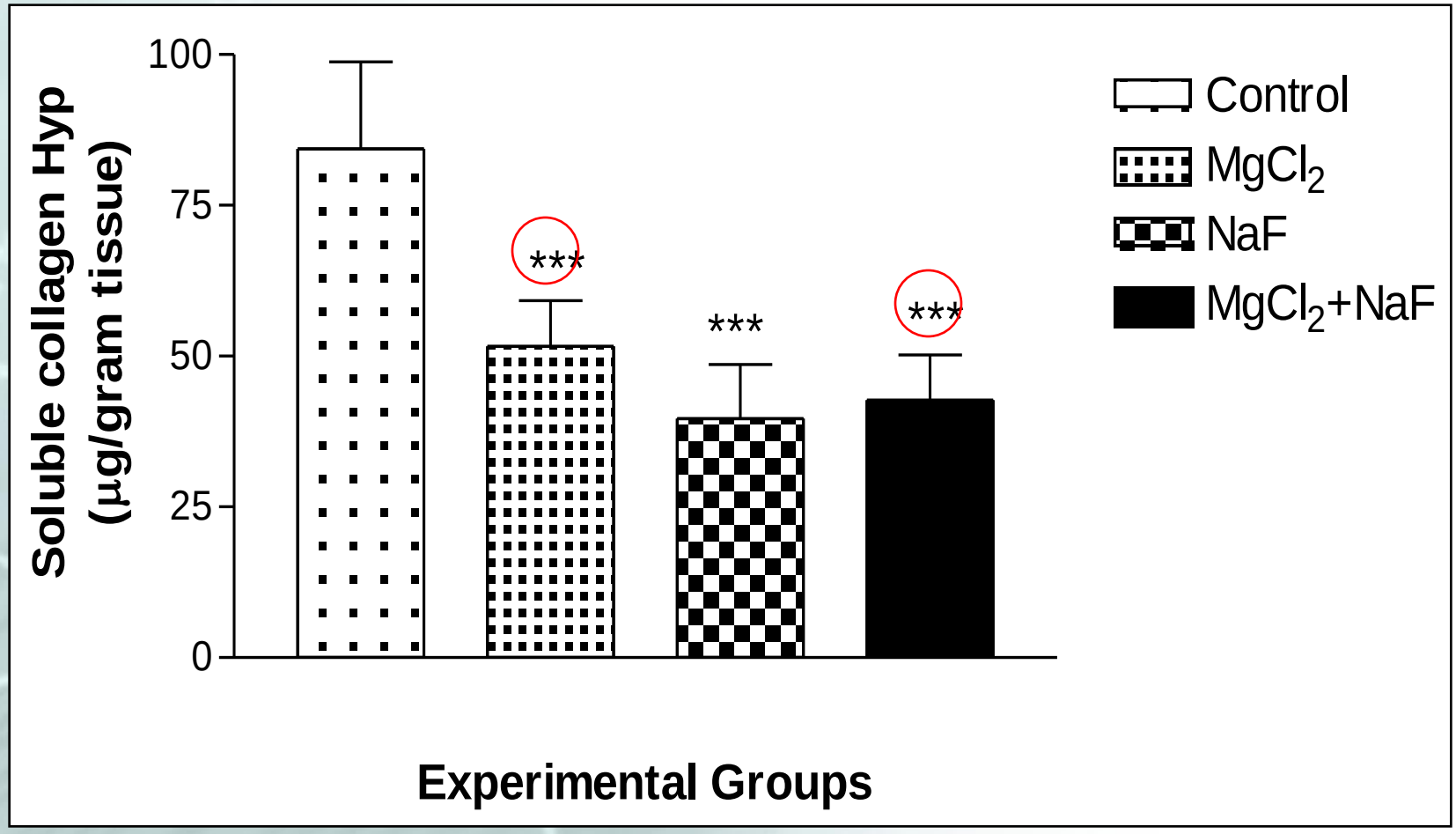
Effect of $MgCl_2$ and NaF on alkaline phosphatase in rat serum



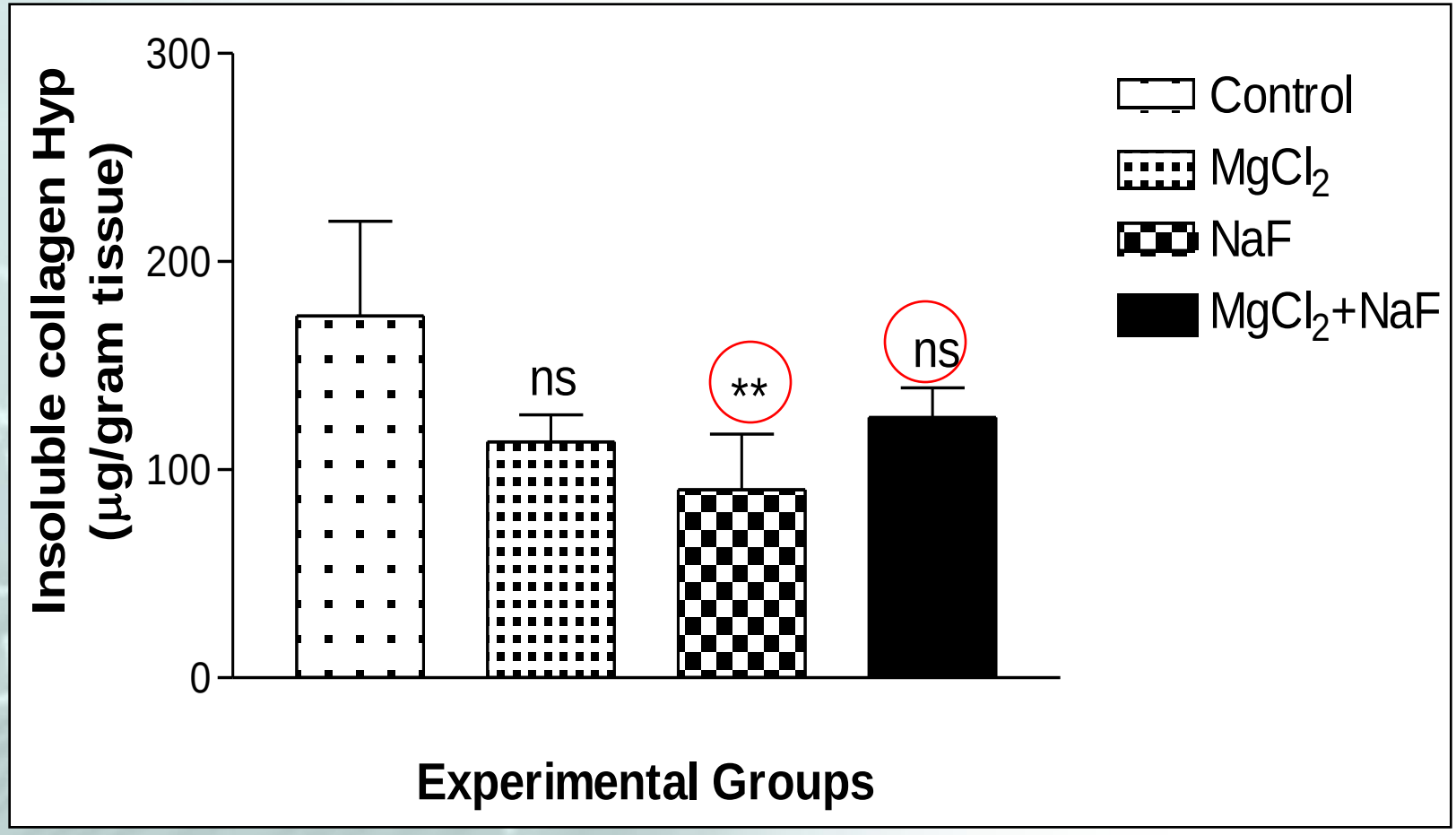
Effect of $MgCl_2$ and NaF treatment on various hydroxyproline fractions in rat kidneys

Experimental Groups	Free Hyp (µg/gm fresh tissue)	Protein-bound Hydroxyproline (mg/gm fresh tissue)	Peptide – bound Hydroxyproline (mg/gm fresh tissue)	Total Hydroxyproline (mg/gm fresh tissue)
Control	329.3 ± 54.65	1.04 ± 0.15	11.29 ± 0.88	12.67 ± 0.96
$MgCl_2$	149.6±22.77***	1.73 ± 0.17***	1.99 ± 0.47***	3.87 ± 0.48***
NaF	125.3±15.94***	1.26 ± 0.23 ns	1.61 ± 0.91***	3.44 ± 0.79 ***
$MgCl_2$ +NaF	218.1±11.16***	2.41 ± 0.33***	6.67 ±1.23***	9.19 ± 1.21 ***

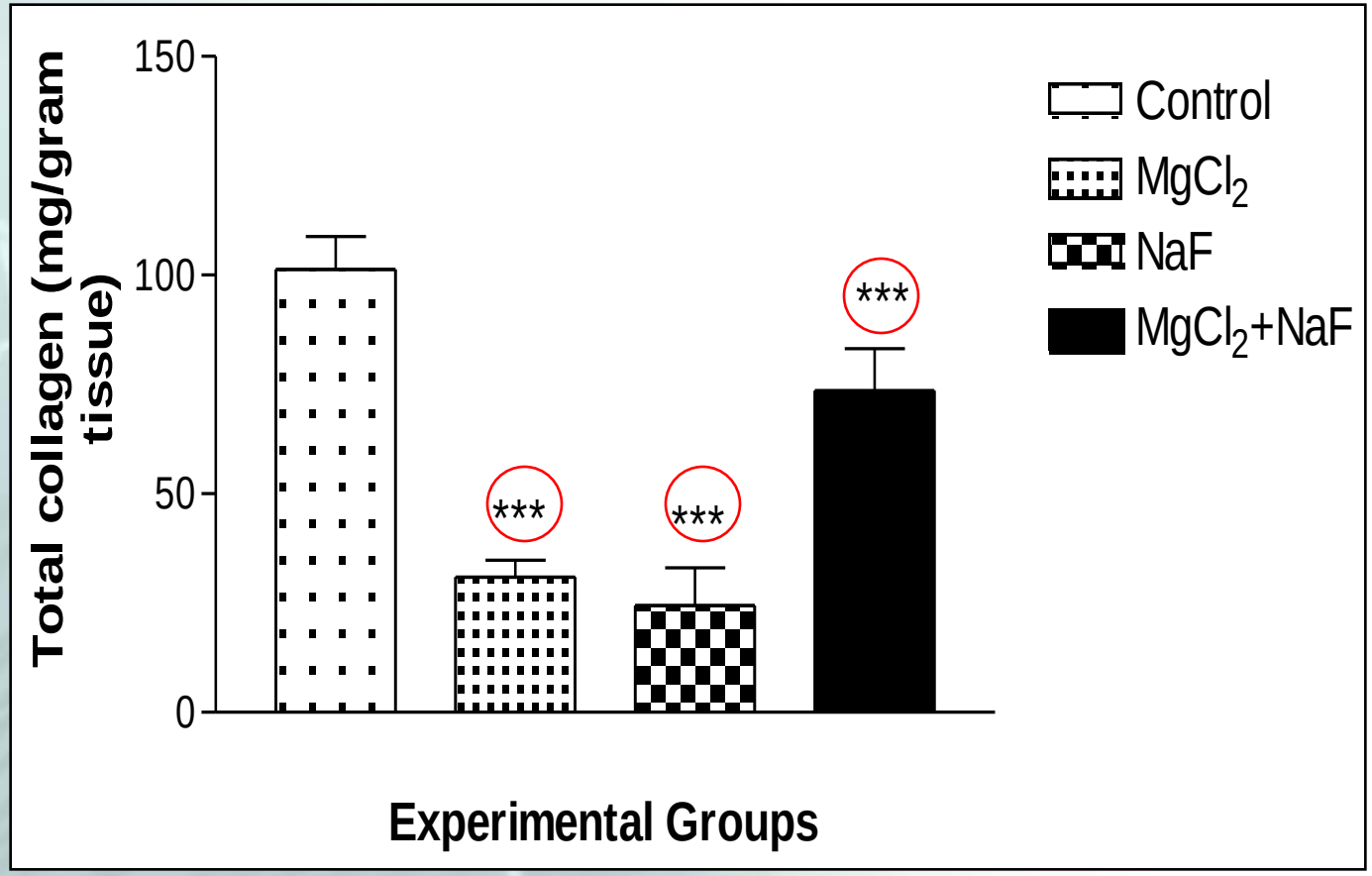
Effect of $MgCl_2$ and NaF on soluble collagen hydroxyproline in rat kidneys



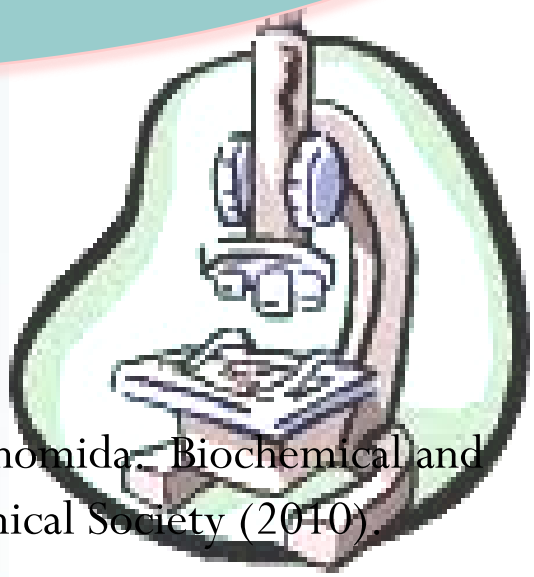
Effect of $MgCl_2$ and NaF on insoluble collagen hydroxyproline in rat kidneys



Effect of $MgCl_2$ and NaF on total collagen hydroxyproline in rat kidneys

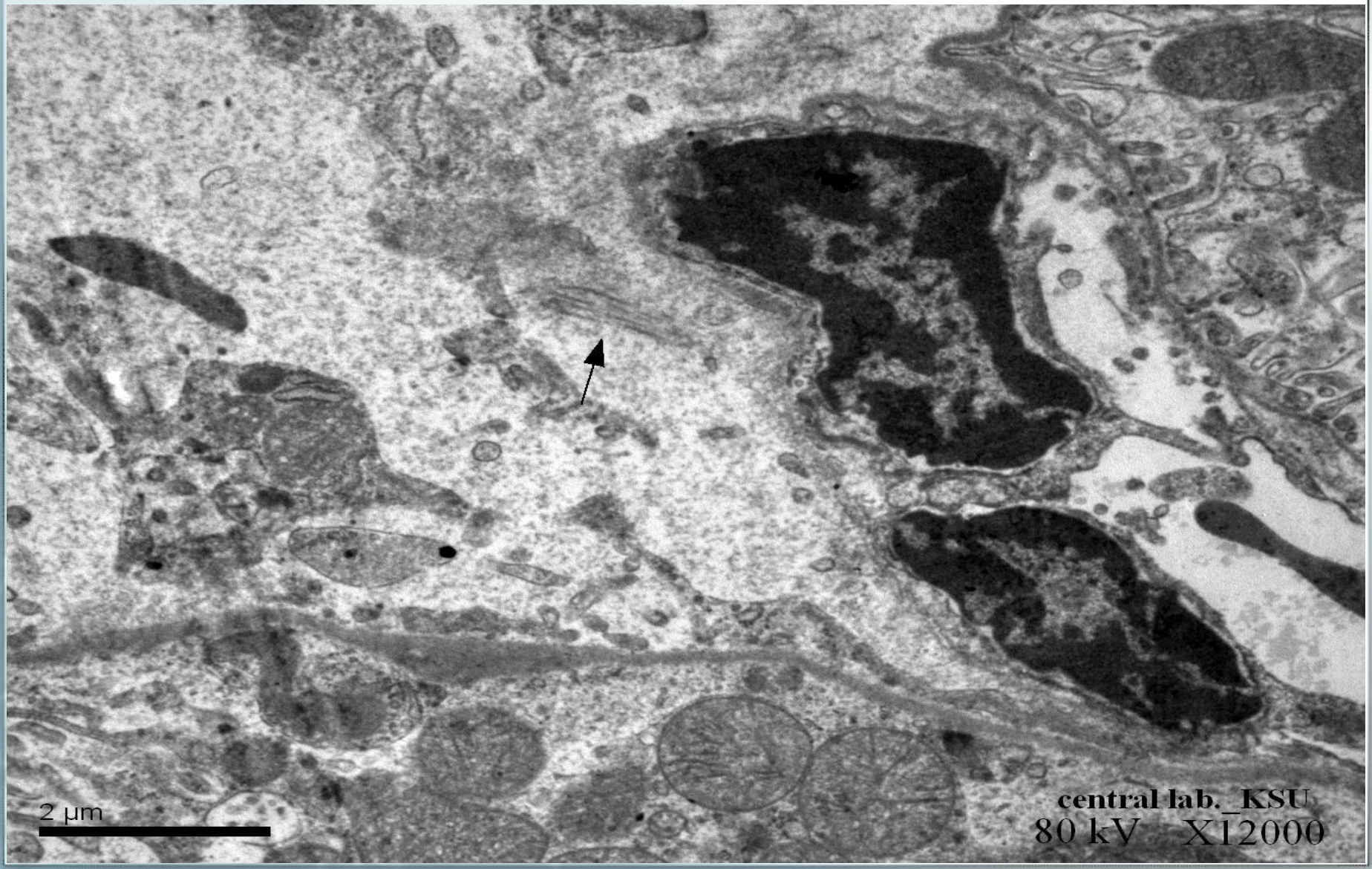


Electron micrograph of kidneys from rats treated with 30 mg/kg body weight of $MgCl_2$ alone and followed by NaF 10mg/kg body weight /24 hours, 30 minutes after $MgCl_2$ injection*

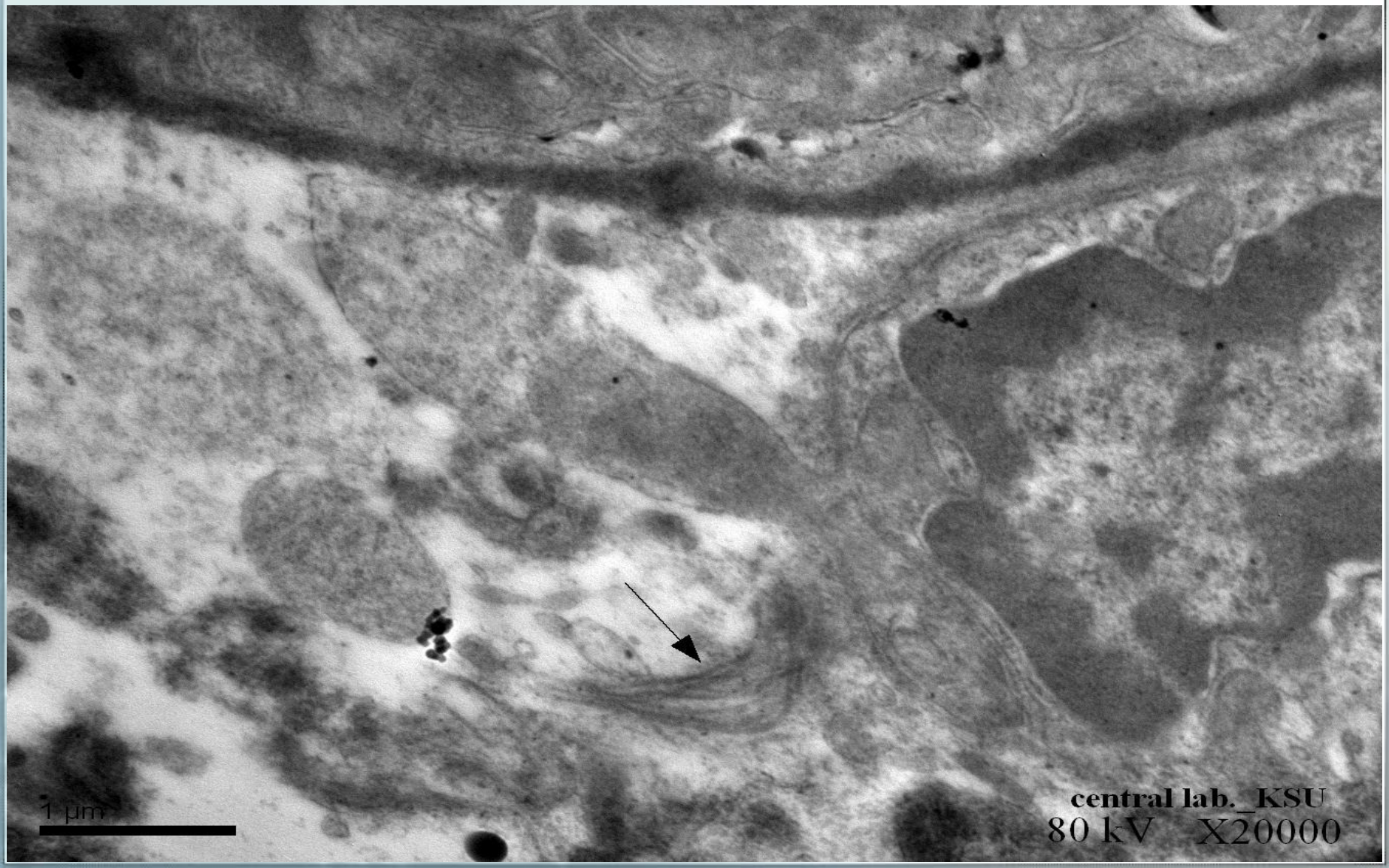


*Accepted for publication /E.A Alomireeni, N.J. Siddiqi, A.S. Alhomida. Biochemical and histological stuides on the effect of NaF on rat kidney. Saudi Chemical Society (2010).

30mg/kg body weight $MgCl_2$



30mg/kg body weight $MgCl_2$ Followed by NaF 10mg/kg body weight, 30minutes after $MgCl_2$ injection



Statistical Analysis

The hydroxyproline content was expressed as mean ± SD μg/gram fresh tissue. Hydroxyproline levels in various groups were compared using one-way ANOVA analysis followed Tukey's multiple comparision test.

Conclusion

- These results suggest that NaF disrupts the connective tissue matrix of the kidneys.
- Though $MgCl_2$ has been reported to be protective against the toxic effect of NaF it had no significant effect on NaF induced changes in kidney hydroxyproline content.
- Pretreatment of rats with $MgCl_2$ however restored insoluble collagen hydroxyproline to near normal levels though it had no significant effect on soluble collagen hydroxyproline.

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Thank you for
attention