Dr hab. inż. Robert Kupczyński, prof. nadzw.
Dr hab. inż. Antoni Szumny, prof. nadzw.
Dr Piotr Kuropka,
Dr Renata Nowaczyk

WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES

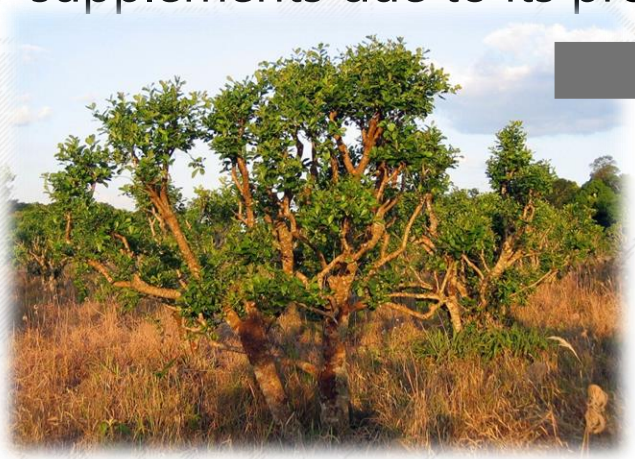
Influence of Ilex extracts and their fractions on kidney structure and performance

MSc. Eng. **Anna Zwyrzykowska-Wodzińska**
Department of Environment Hygiene and Animal Welfare

Ilex paraguariensis

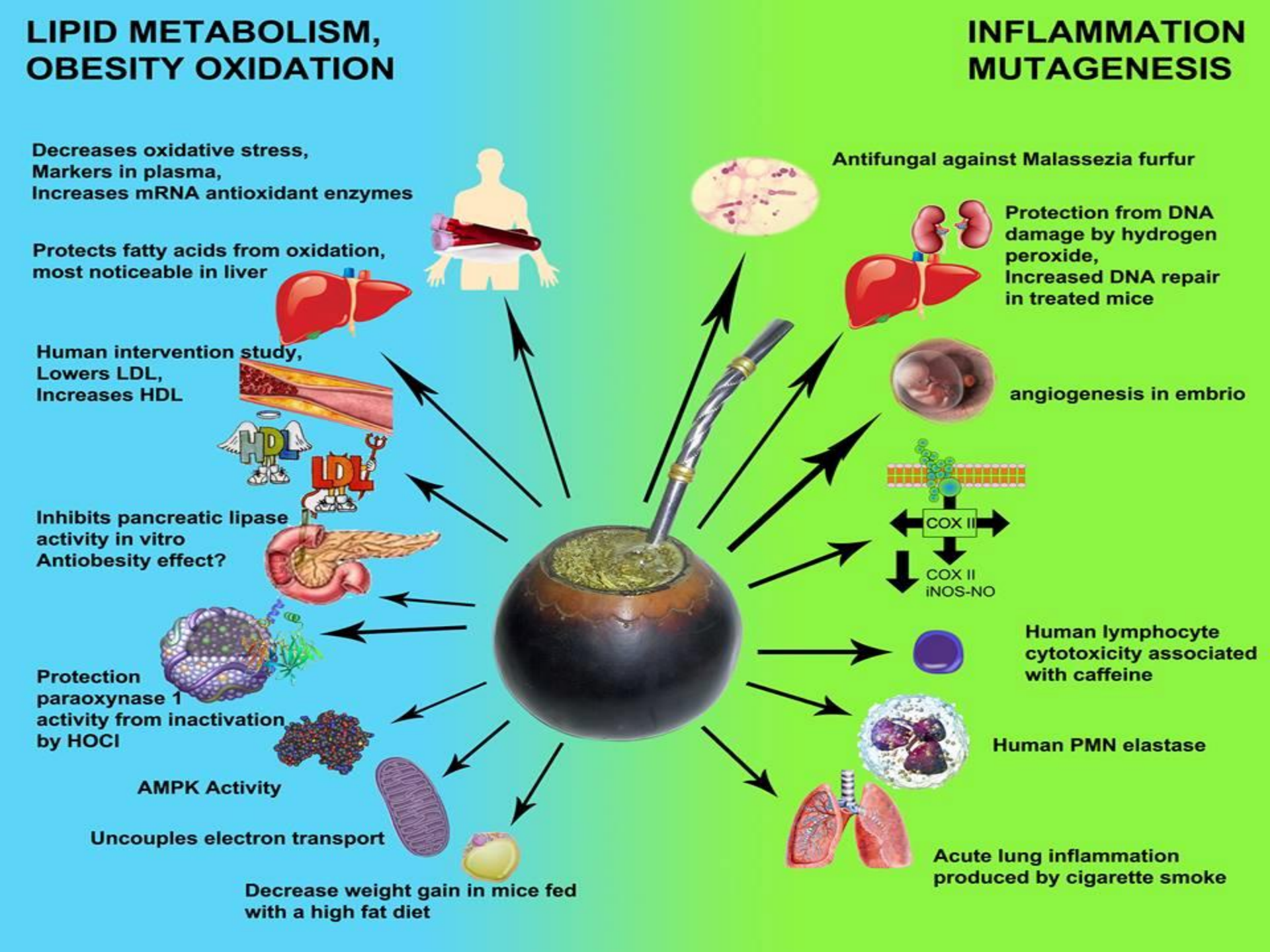
Ilex paraguariensis is a species of the holly family (Aquifoliaceae). It occurs in the wild in South America (Brazil, Argentina, Paraguay, Uruguay). Name (*Ilex paraguarensis*) of the plant gave the French botanist Auguste de Saint Hilaire in 1822.

Yerba Mate tea, an infusion made from leaves of tree *Ilex paraguariensis*, is widely consumed traditional beverage in South America. It is rapidly gaining world market, either as a tea or as ingredient in formulated foods or dietary supplements due to its properties.



Influence of Yerba Mate

Phenolic compounds present in the yerba mate leaves have anti-inflammatory properties, prevent obesity and diabetes, as well as reduce the oxidation of LDL cholesterol levels, which affects the increase in risk of heart disease (Heck et al., 2007, Burris et al., 2012, Valerga et al., 2013).


LIPID METABOLISM,
OBESITY OXIDATION
INFLAMMATION
MUTAGENESIS
Decreases oxidative stress,
Markers in plasma,
Increases mRNA antioxidant enzymes
Protects fatty acids from oxidation,
most noticeable in liver
Human intervention study,
Lowers LDL,
Increases HDL
HDL
LDL
Inhibits pancreatic lipase
activity in vitro
Antiobesity effect?
Protection
paraoxynase 1
activity from inactivation
by HOCl
AMPK Activity
Uncouples electron transport
Decrease weight gain in mice fed
with a high fat diet
Antifungal against Malassezia furfur
Protection from DNA
damage by hydrogen
peroxide,
Increased DNA repair
in treated mice
angiogenesis in embrio
COX II
COX II
iNOS-NO
Human lymphocyte
cytotoxicity associated
with caffeine
Human PMN elastase
Acute lung inflammation
produced by cigarette smoke

Aim of Study

It was observed that the infusion of leaf extracts increase urination in animal models. Based upon our earlier studies on detailed characterization of polyphenolic, saponin and terpenoid fractions from plants belonging to the *Ilex* sp. we decide to investigate on animal models the effects of *I. paraguariensis and Ilex x meserveae* Blue Angel extract on kidney structure in animals feed normal and hypercholesterolemic diet.

Extraction and quantitative and qualitative identification of biologically active compounds in Ilex sp.

Isolation of polyphenol, saponin and terpene fraction from Ilex sp.

Study of the effect of the extract and individual fractions biologically active substances from Ilex sp. applicated on animal models

Microscopic examination

The experiment:

Material and Methods -Plant material ILEX SP.

Ilex x meserveae **'Blue Angel'**

Ilex paraguarirnsis

Material and Methods - Extracts preparation

Collecting and buying leaves

- Leaves of *Ilex paraguariensis* bought in shop
- Laves of *Ilex meserveae* ‘Blue Angel’collected in University Herbarium

Preeliminary preparation of biological material

- Drying leaves
- Milling material

Water extraction

Extraction of poliphenolic fraction (Zwyrzykowska et al 2015)

Extraction of terpenoid fraction with Soxhlet apparatus

Extraction of saponin fraction

Material and Methods – *in vivo* study

1st group

I – rats fed a standard feed (SF),

II – rats receiving SF and water extract of *Ilex paraguariensis* (50 g/l),

III – rats receiving SF and water extract of *Ilex* × *meserveae* (50 g/l),

IV - rats receiving SF and polyphenol fraction from *Ilex* × *meserveae* (10 mg / kg of BW),

V – rats receiving SF and saponin fraction from *Ilex* × *meserveae* (10 mg / kg of BW),

VI – rats receiving SF and terpenoid fraction from *Ilex* × *meserveae* (10 mg / kg of BW),

The second group (Ia–VIa) was fed with hypercholesterolemic diet (20 g of cholesterol / kg standard feed) (HCHD) and was divided in the same manner as the first group.

Ia – rats receiving HCHD feed ,

IIa – rats receiving HCHD feed and the extract of *Ilex paraguariensis* (50 g/l)

IIIa –rats receiving HCHD feed and the extract from *Ilex meserveae* (50 g/l),

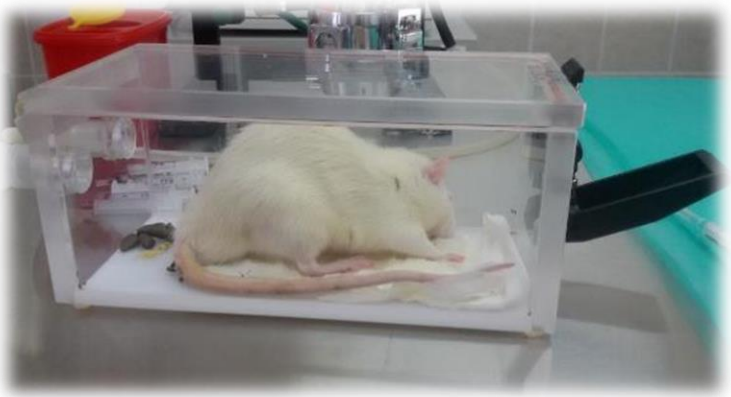
IVa – rats receiving HCHD feed and of polyphenolic fraction of *Ilex meserveae* (10 mg/kg BW).

Va rats receiving HCHD feed and saponin fractions of *Ilex meserveae (*10 mg/kg BW);

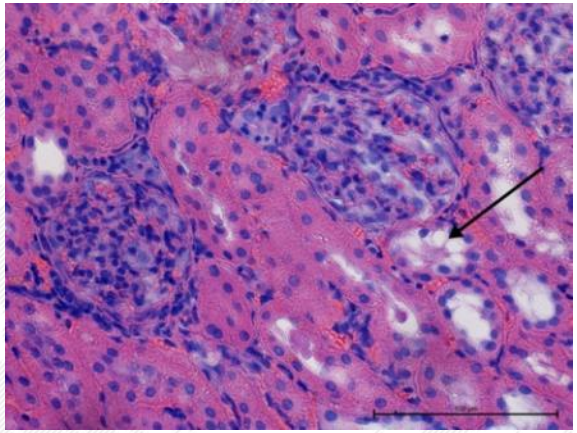
VIa – rats HCHD feed and terpenoid fractions of *Ilex meserveae (10 mg/kg BW)*,

Material and methods – histology and histochemistry

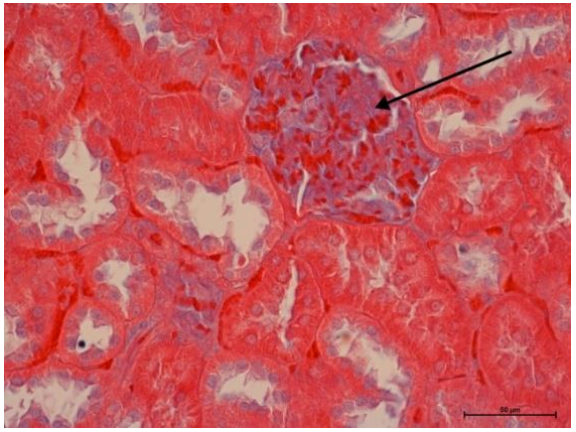
- After 8 weeks the rats were decapiated and material for histological examination was taken.
- The material was fixed in 4 % buffered formalin solution for 3 days, then washed in tap water for 24 h, dehydrated in alcohol series and embedded in paraffin.
- 5 µm thick sections were routinely stained with hematoxylin and eosin and Alcian blue and morphometry was performed.
- The study was carried out using a Nikon Eclipse 80i light microscope coupled with the Nis-Elements Ar software. Statistical analysis was performed in Statistica 6.0.



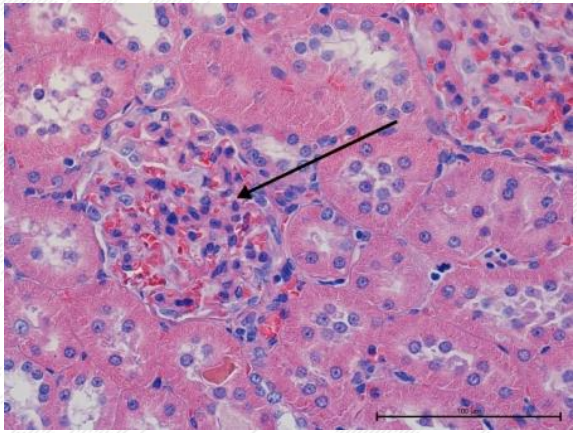
Results



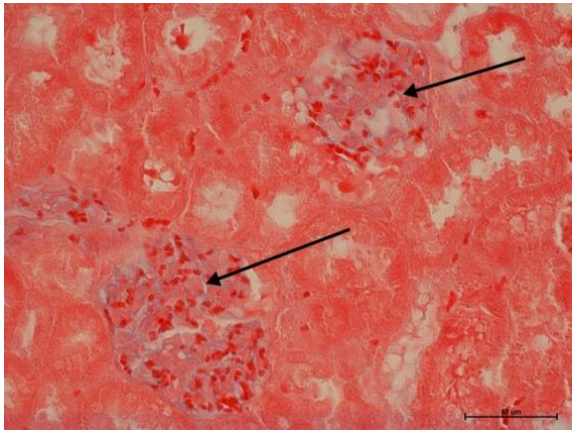
I



I



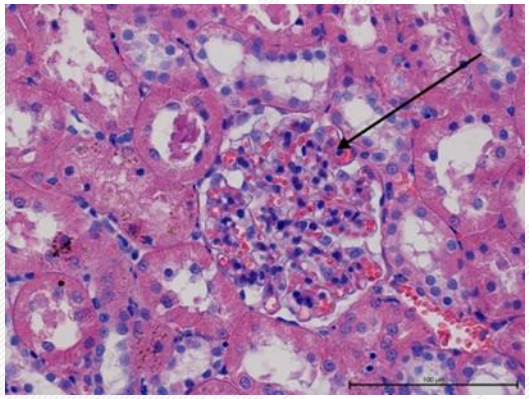
Ia



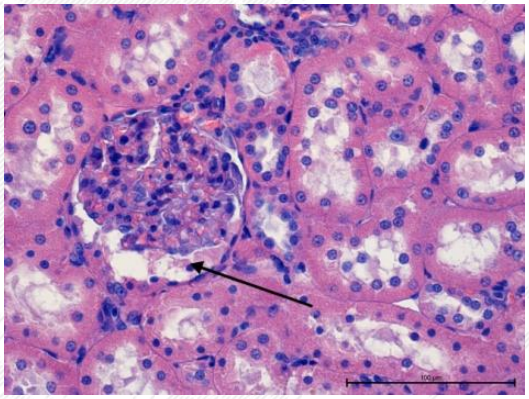
Ia

The changes in the structure of the kidney in control group in normal and HCHD diet. I- Note the lack of the urine in proximal tubules and slightly enlarged distal tubules. Note the high content of proteoglycans (blue color) in capillary tuft of the glomerulus. In group Ia glomerulus is filled with blood and filled with numerous erythrocytes. Mag 400x.

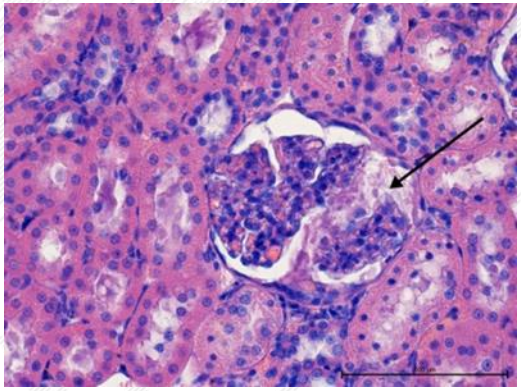
Results – histology Group1



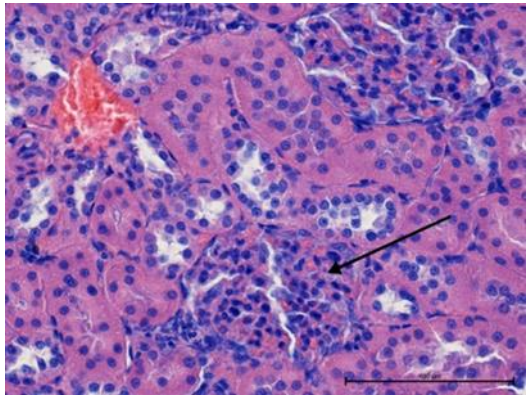
II



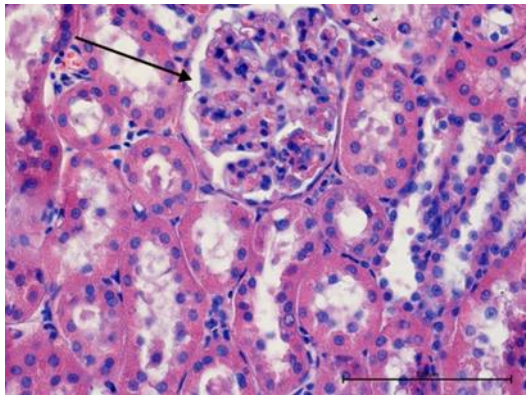
III



IV



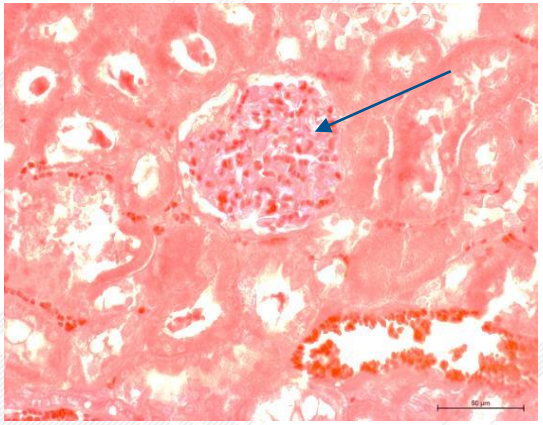
V



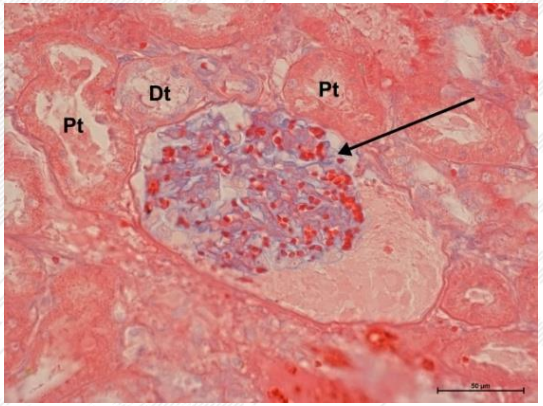
VI

The changes in the structure of the kidney II, III, IV, V and VI group in normal diet. Glomeruluses with different urine and blood content. Mag 400x.

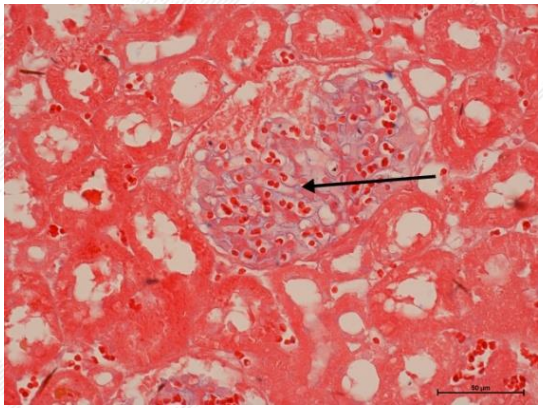
Results –histochemistry Group1



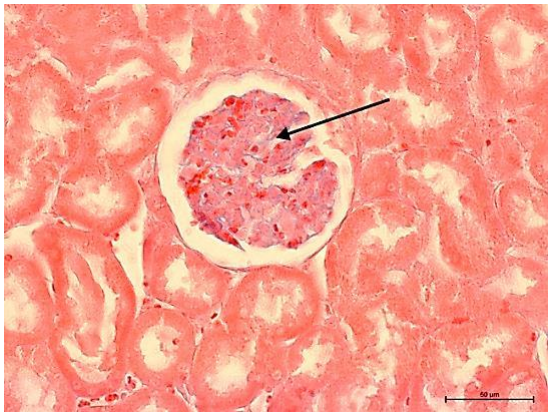
II



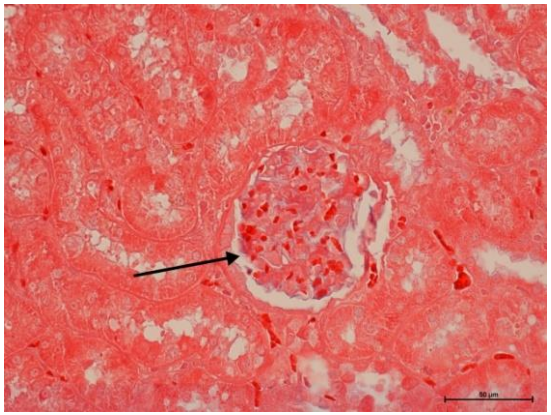
III



IV



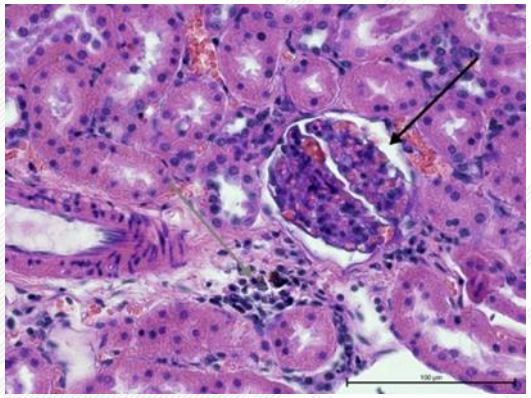
V



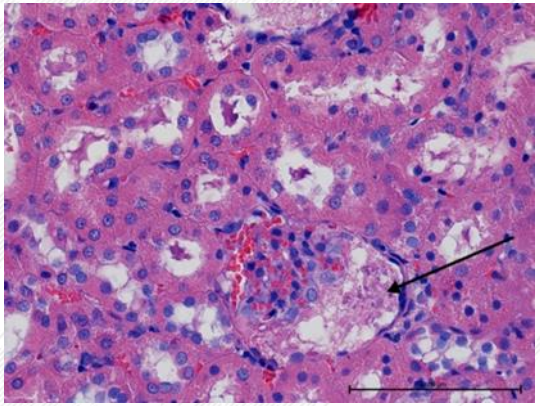
VI

The changes in the structure of the kidney II, III, IV, V and VI group in normal diet. Glomerulus with different proteoglycans content. Note the presence of urine in the glomerulus in II and III group. Different lumen of proximal and distal tubules caused by different level of diuresis. Alcian blue staining. Mag 400x.

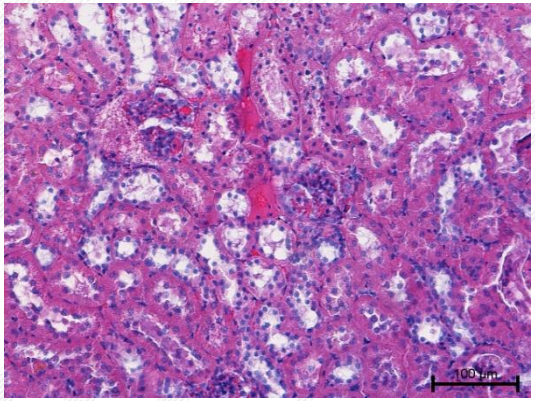
Results – histology Group 2



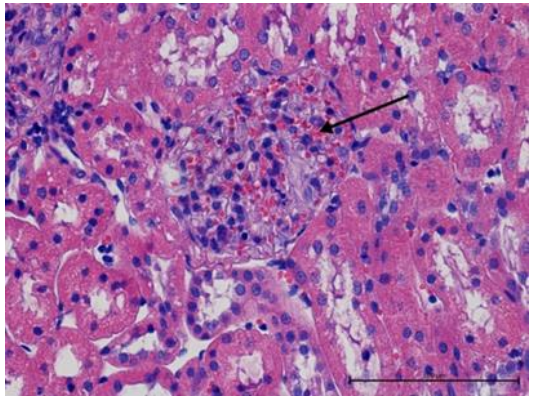
IIa



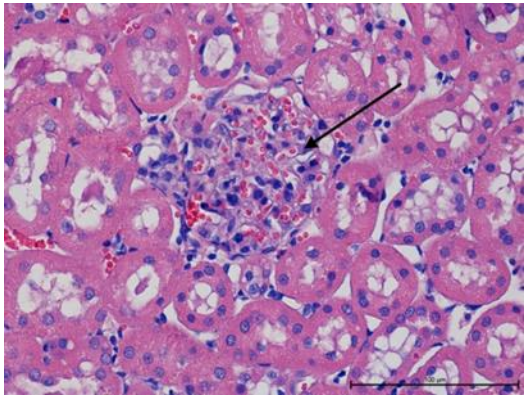
IIIa



IVa



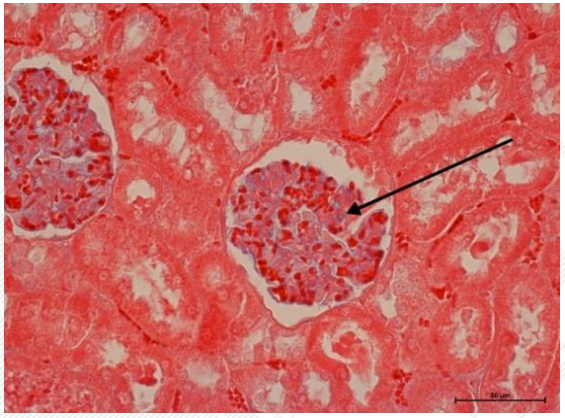
Va



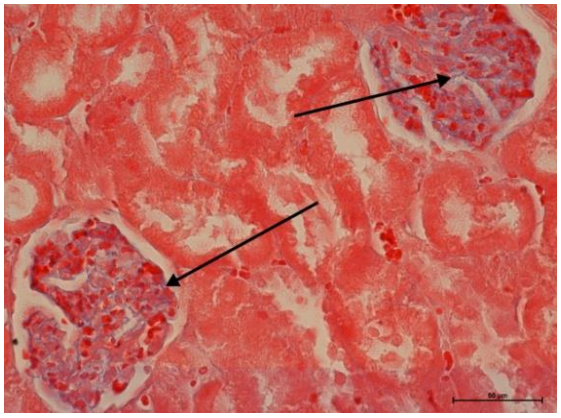
VIa

The changes in the structure of the kidney IIa, IIIa, IVa,Va and VIa group in HCHD diet. Different urine and blood content in glomerulus. Note the presence of leukocytes in the kidney parenchyma in IIa H&E staining. Mag 400x. (IVa-mag 200x)

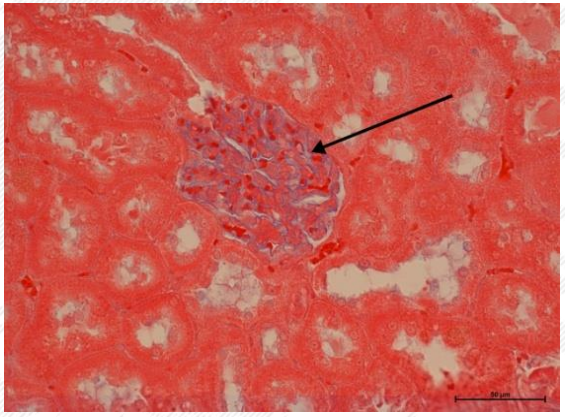
Results –histochemistry Group2



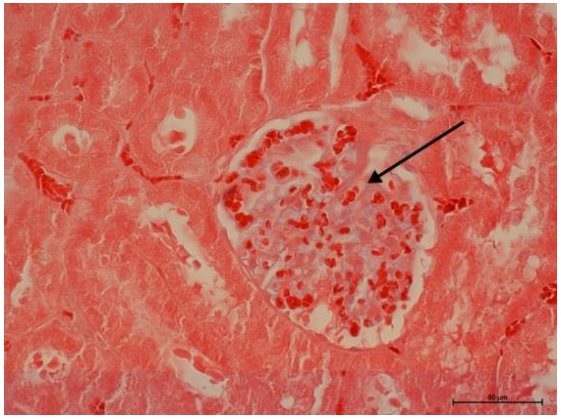
IIa



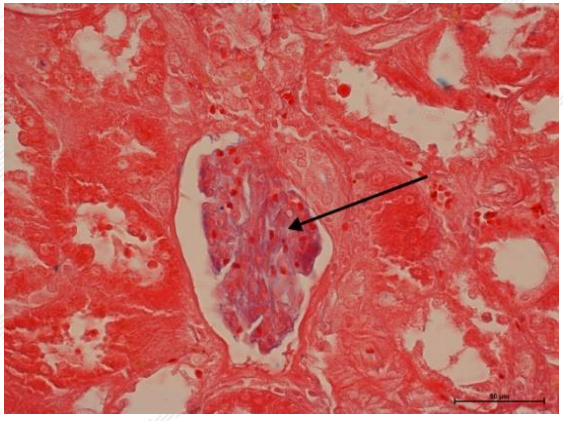
Va



IIIa



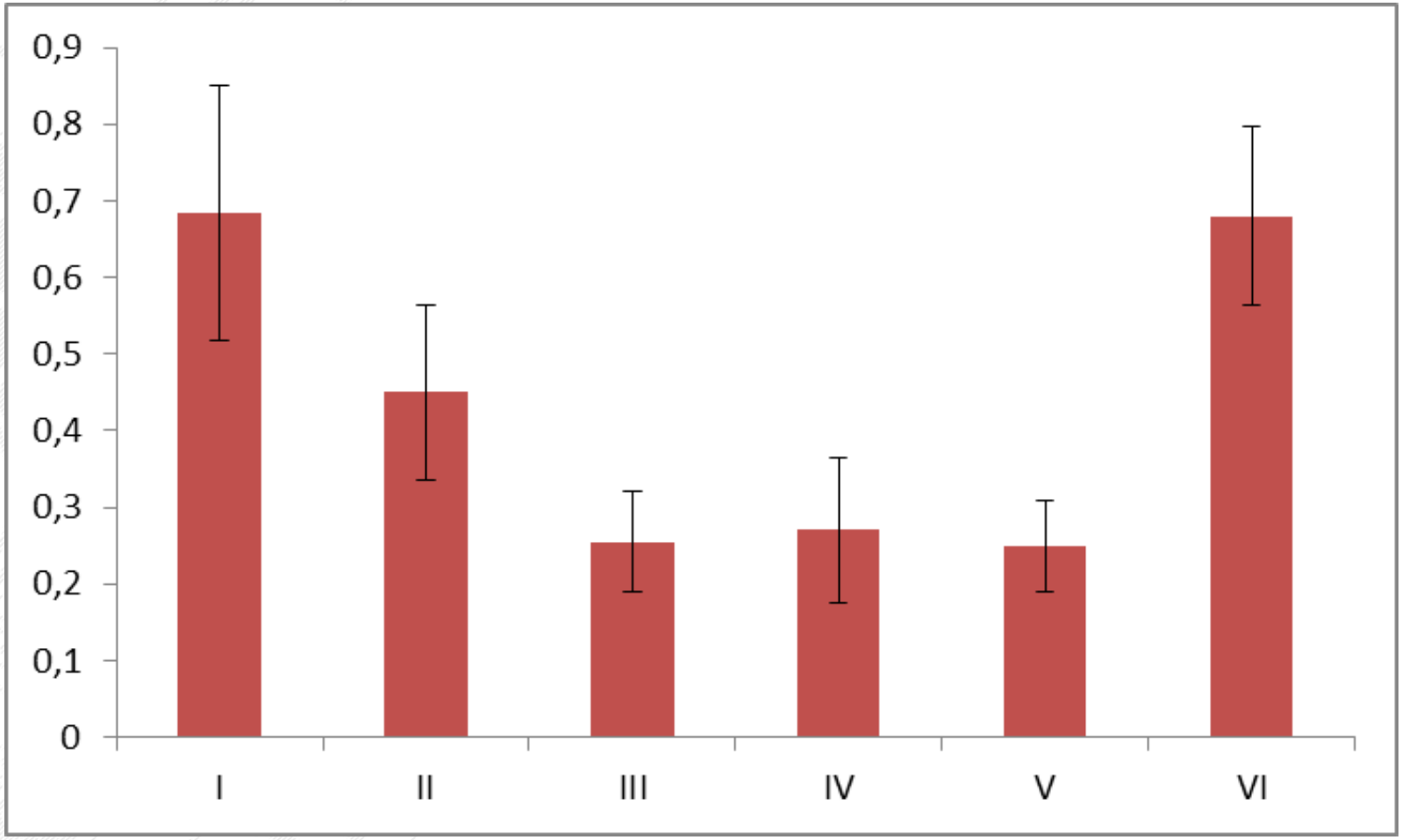
VIa



IVa

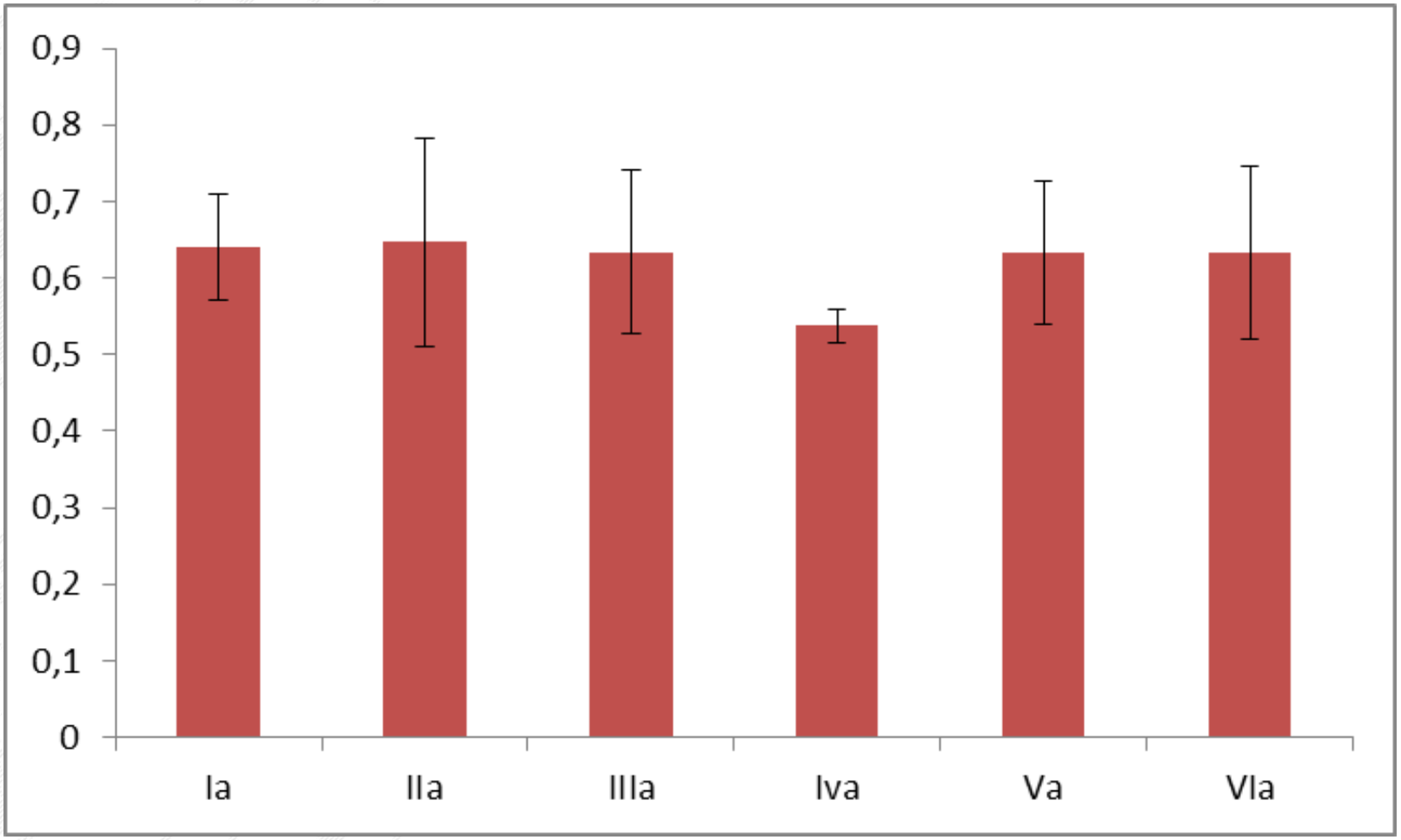
The changes in the structure of the kidney IIa, IIIa, IVa,Va and VIa group in HCHD diet. Glomerulus with different urine and blood content (black arrow). Different lumen of proximal and distal tubules caused by different level of diuresis. Alcian blue staining. Mag 400x.

Results – Polysaccharides content capilary tuft Group1



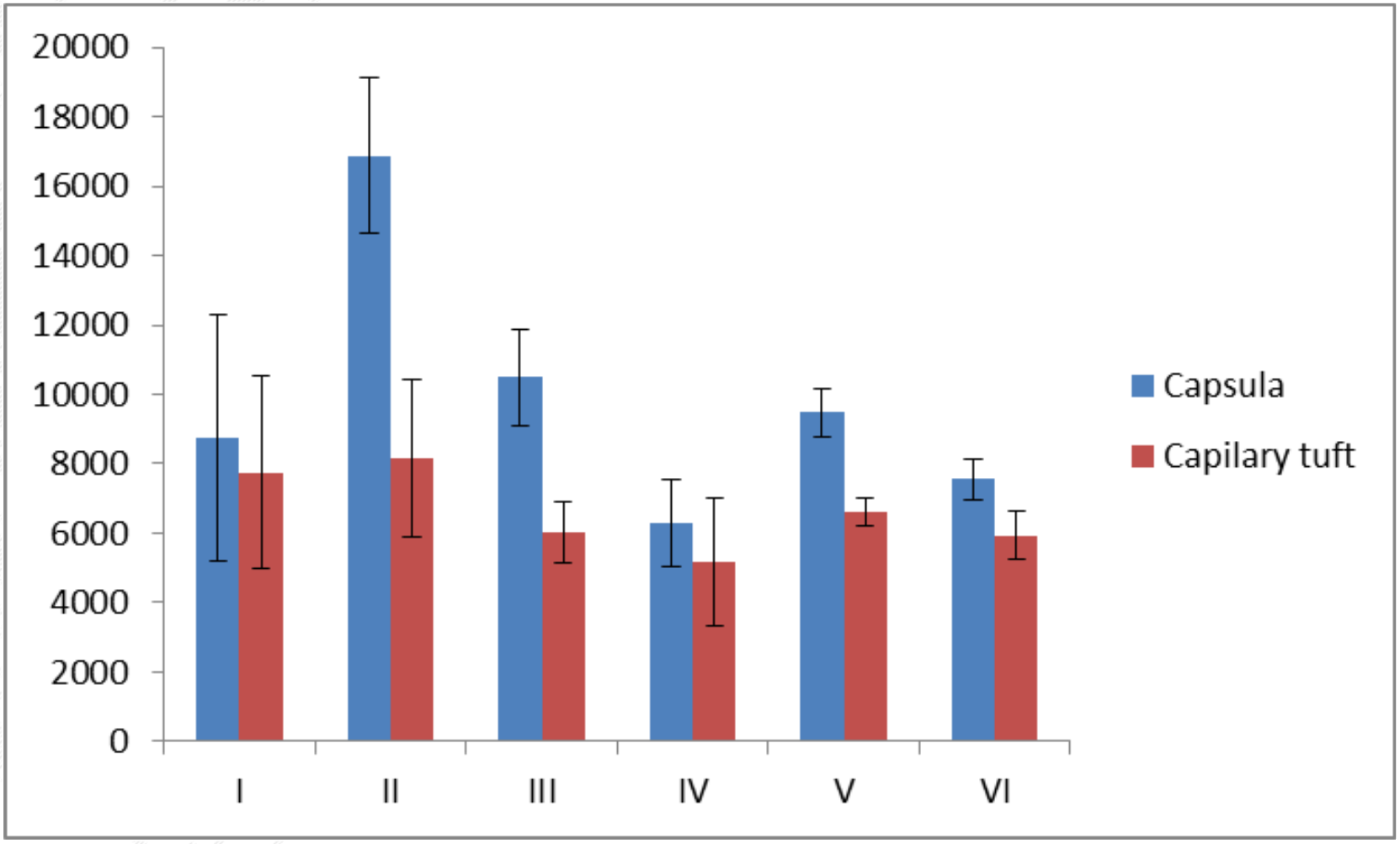
Polysaccharides content In capillary tuft. Differences between II, III, IV, V and VI group are statistically significant at alfa =0,05.

Results - Polysaccharides content in capilary tuft Group2



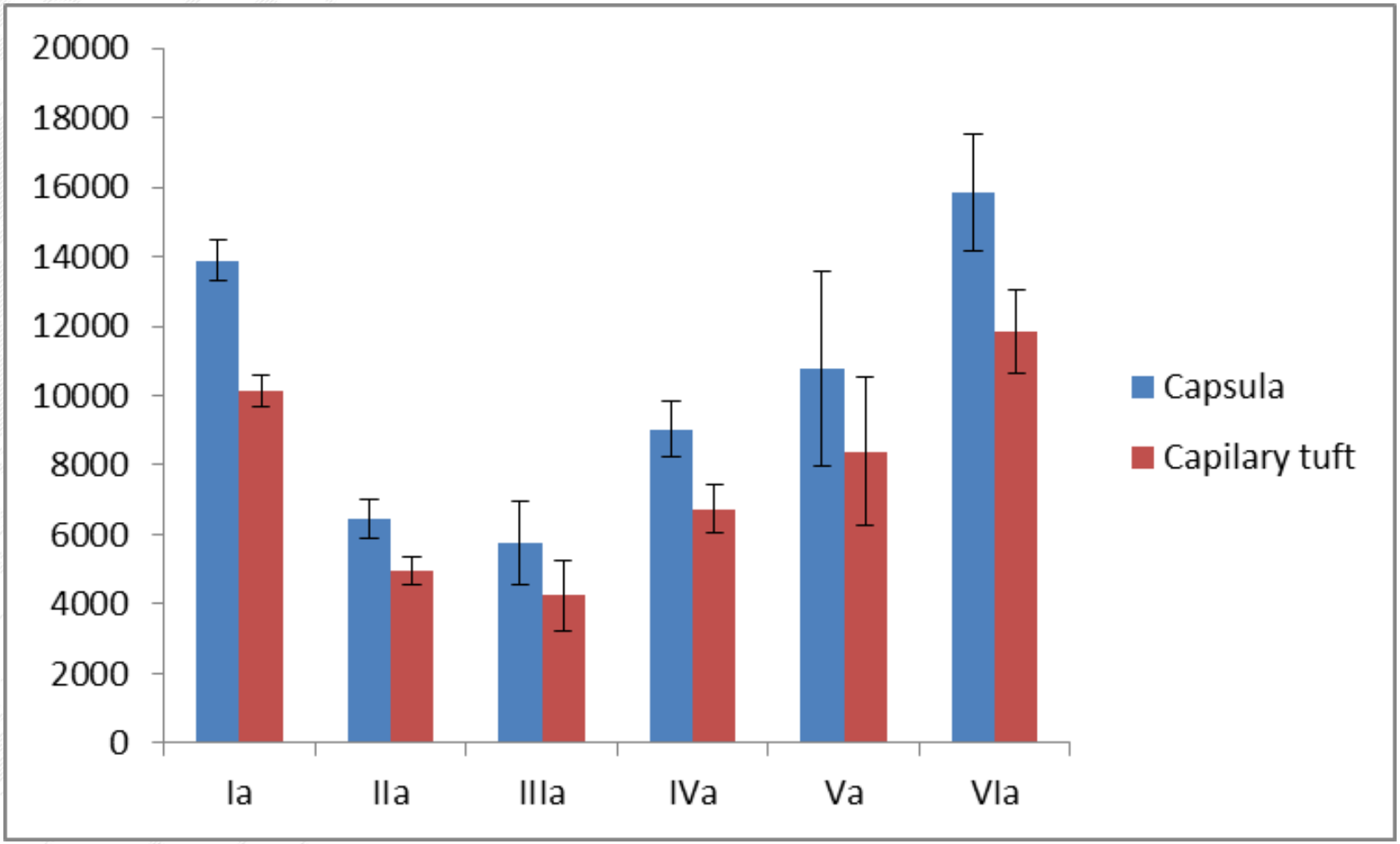
Polysaccharides content In capillary tuft. Difference between control and IVa group is statistically significant at alfa =0,05.

Results – Morphometry Group1



Surface area of capsula and capillary tuft in regular diet

Results- Morphometry Group2

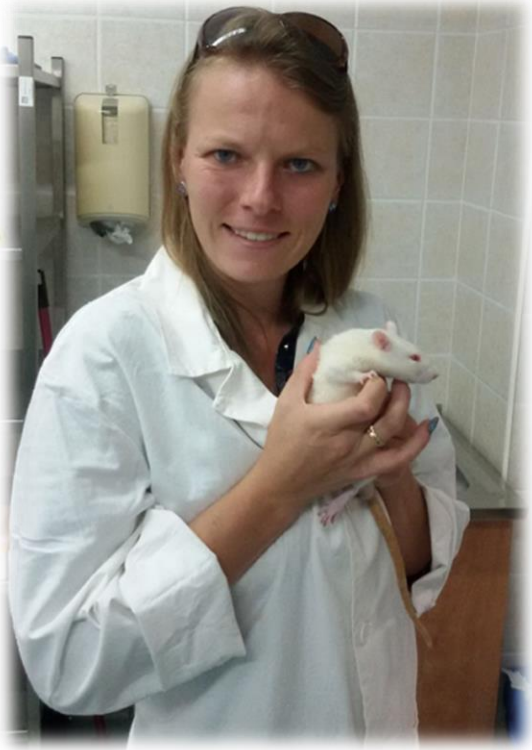


Surface area of capsula and capillary tuft in HCHD diet

Conclusion

In conclusion, extracts of terpenoids and saponins from *Ilex meserveae* seems to have no influence on kidney status. However, polyphenols present in extract as well as in the infusion from *Ilex meserveae and Ilex paraguaiensis* in normal diet have toxic effect which is decreased by high fat diet. This is synergistic effect and can be used equally for protection of kidney against polyphenols, as well as protection of the kidney against high fat diet.

WROCŁAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES



Thank you for attention

XIX International Congress of ISAH

ISAH 2019

September 8th - 12th 2019 - Wrocław, POLAND

Animal Hygiene as a Fundament of One Health and Welfare

improving biosecurity, environment and food quality

CONGRESS OVERVIEW

The ISAH Congress initiated in 1970 have been identified as an influential conference worldwide for professionals in animal hygiene, health and welfare which provides insights into the latest research results from many areas of animal science.

Congress is a unique opportunity for industry and scientists to meet and acquire new knowledge as well as to exchange experience. Carried out through many plenary meetings, poster sessions as well as technical tours discussions about scientific achievements in the world livestock production are also an opportunity for the application of new ideas in practice.

SCIENTIFIC TOPICS

- ANIMAL HEALTH, WELFARE AND BEHAVIOUR
- ANIMAL HYGIENE, DISEASE PREVENTION, BIOSECURITY AND ANTIBIOTICS RESISTANS
- FOOD QUALITY AND FOOD SAFETY OF ANIMAL ORIGIN
- ZOONOSES, VECTOR BORNE AND EMERGING DISEASES
- APICULTURE AND PROBLEMS OF BEEKEEPING
- INTERACTIONS BETWEEN ENVIRONMENT AND ANIMAL PRODUCTION
- NUTRITION, FEED AND ADDITIVES
- SUSTAINABLE ANIMAL PRODUCTION AND AGRO-BIODIVERSITY CONSERVATION: EFFICIENT AND ALTERNATIVE FARMING
- PRECISION LIVESTOCK FARMING: TECHNIQUES, RISKS AND BENEFITS
- HUSBANDRY OF FARMED ANIMALS, FISH AND AQUACULTURE

CONFERENCE TIMELINE

Sept. 10th 2018
Call for Abstract

Nov. 30th 2018
Submission of Abstract

March 15th 2019
Submission of Full Papers

April 30th 2019
Early Registration and Payment