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STRUCTURE AND FUNCTIONS OF KIDNEYS OF TWO GENERATIONS OF RATS FED ON GLYPHOSATE-RESISTANT GENETICALLY MODIFIED SOYBEAN AND ROUNDUP

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Given the broad use of transgenic soybean and Roundup, we have conducted histological studies of kidneys of two generations of rats fed on genetically modified Roundup Ready soybean not treated with the herbicide, the same genetically modified soybean treated with the herbicide and Roundup with potable water.

***The purpose** was to study an effect of Roundup and glyphosate-resistant soybean on morphology of kidneys of two generations of rats.*

***Materials and methods.** The object of the study was 4 months old Wistar rats (180-200 g). Experimental animals were allocated into 5 groups, 12 rats each (6 females and 6 males). Group I included intact animals (receiving standard vivarium food); group II included animals whose standard food contained up to 26 % of native soybean; group III included rats whose standard food contained up to 26 % of genetically modified soybean not treated with the herbicide; group IV included rats whose food contained up to 26 % of genetically modified soybean treated with Roundup; group V included rats fed on Roundup diluted in potable water (0.003 μg/kg body weight).*

In 42 days, rats of all groups were mated in order to obtain the second generation. At 12 months of age, rats of all groups were decapitated to collect fragments from the middle part of kidneys, which then were fixed for 48 hours in 10 % neutral formalin solution. Morphological studies were conducted using generally accepted histological methods [1].

***Results.** Histopathological changes in renal tubules were evaluated in experimental animals fed on transgenic soybean not treated with Roundup (group III), transgenic soybean treated with the herbicide (group IV), and animals fed on the herbicide with potable water (group V). Analysis of histological kidney sections of two tested groups of rats showed changes in renal tubules in groups IV and V. The experiment demonstrated swollen epithelium of renal tubules in these groups and signs of necrosis of individual cells in the first generation of rats, and destruction of tubule cells and their dysfunctions in the second generation.*

***Conclusions.** Feeding on genetically modified soybean treated with Roundup and the herbicide results in the loss of structure of renal glomeruli and their dysfunctions both in the first and the second generation. Such results suggest a negative effect of the herbicide on the rat body and its ability to accumulate in soybeans.*

***Keywords:** Biotechnology, conventional, transgenic, glyphosate-resistant soybean, kidneys, herbicide, glomeruli, hydropic swelling*

1. Introduction

Contemporary genetic engineering and biotechnology attained a level of development allowing genomes of living bodies to be modified by deleting or adding some genes. Artificially modified plants and animals became very popular and common worldwide. Such organisms are used so widely because they have a high yield, do not demand any special climate, are resistant to pests and herbicides. Most popular genetically modified plants are: soybean, corn, potato, rape, cotton plant, wheat, barley, etc. [2].

2. Literature review

Soybean is widely used in food industry to obtain a lot of foodstuffs due to its high protein content (38–42 %). [3] Amino acid composition of soybean protein is similar to amino acid composition of animal proteins, therefore it has better uptake by humans and animals.

Soybeans contain: carbohydrates, fats, phosphatides, and vitamins (β-carotene, pyridoxine, riboflavin, vitamin E, niacin, biotin, folic acid, pantothenic acid, thiamine, choline), macro- and microelements. Apart from nutrients, soybean also contains antinutrients: protease inhibitors (trypsin and chymotrypsin), lectins, saponins, antivitamins, urease, haemagglutinins, conglycinin (allergic substances), protein soyin, which are rendered harmless during heating. Soybeans heating leaves active such elements as isoflavons (genistein, daidzein), which subsequently are hydrolysed in intestines and undergo subsequent metabolism resulting in compounds having oestrogenic activity: formononetin, daidzein, genistein, biocanin A, etc. [4].

In agriculture and food industry, new transgenic Roundup Ready soybean is most widely used. Such soybean is resistant to Roundup containing glyphosate as the principal active substance whose effects on the human and animal body is unclear, especially as regards the structure and functioning of organs and organ systems.

The main function of kidneys is to remove excessive concentrations of toxins and foreign agents from endogenous medium in humans and animals. In urine, toxins and their biotransformation products can concentrate, as a consequence of reverse resorption of xenobiotics in renal tubules, which contributes to kidneys' high sensitivity to toxic agents. The structure of kidney cells can be affected by toxic agents, which can be present in soybeans treated with the herbicide and by the herbicide itself. Developing pathological processes lead to bilateral kidney damage and reduction of their functional activity. Pathomorphological picture and functioning of a kidney depends on chemical composition, properties of toxic substances and duration of kidney exposure.

3. Purpose and objectives of the study

The purpose was to study structural changes and functions of kidneys of two generations of rats fed on glyphosate-resistant soybean treated with the herbicide and effects of Roundup itself.

The following objectives served to attain the purpose:

1. To prepare and analyse histological kidney sections of rats fed on conventional and genetically modified soybean not treated with Roundup in two generations of rats;
2. To analyse histological data of kidneys of rats fed on genetically modified soybean treated with Roundup;
3. To analyse changes on kidneys of rats fed on Roundup with potable water (0.003 μg/kg body weight) administered orally based on a rat weight.

4. Materials and methods

The experiments were conducted using laboratory Wistar rats weighing 180-200 g given standard vivarium food. The researchers complied with provisions on handling laboratory animals in accordance with European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 1986) [5].

In the experiment, rats were allocated into five groups (12 rats per group):

I – Animals given standard vivarium food (intact animals)

II – Animals whose standard food contained up to 26 % of conventional soybean

III – Rats whose food contained up to 26 % of genetically modified soybean not treated with the herbicide

IV – Rats whose food contained up to 26 % of genetically modified soybean treated with Roundup

V – Rats given the herbicide with potable water (0.003 μg/kg body weight administered orally based of a rat weight), which is an allowable concentration within the EU requirements.

For research, transgenic GTS 40-3-2 soybeans (Monsanto Canada Inc.) were used [6]. GTS 40-3-2 soybean is resistant to glyphosate-containing herbicides due to insertion of a glyphosate-resistant gene, 5-enolpyruvylshikimat-3-phosphate synthase (EPSPS) enzyme. [6, 7]. In the experiments, we also used conventional domestic Chernivetska 9 soybean provided to us by Bukovyna Institute for Agro-industrial Production of the National Academy of Agrarian Sciences of Ukraine. Samples of the both soybean varieties (Chernivetska 9 and GTS 40-3-2) were controlled for the presence of a genetic modification as proven by Ukrainian Laboratory of Quality and Safety of Agro-Industrial Products with their Protocol No. 1691-H. In sample No. 2, target sequences of promoter 35S of cauliflower mosaic virus (CaMV), and terminator NOS (T-NOS) of T1 plasmid of Agrobacterium tumifaciens were detected. Based on the laboratory data, by its chemical composition (moisture, percentage of protein, fats, elements by weight) Chernivetska 9 is equivalent to genetically modified glyphosate-resistant GTS 40-3-2. The revealed differences in individual parameters are within acceptable biological limits. Based on nutritional value, 26 % of vivarium food was replaced with conventional and genetically modified soybean, based on the laboratory data (content of proteins, carbohydrates, fats, macro- and microelements, as well as vitamins).

In order to render antinutrients harmless and lower urease activity of soybeans, they were heated for 2 hrs at 140 °C before their adding to food.

After giving native and genetically modified soybean, females and males in experimental groups were mated in 42 days and continued to receive the same food depending on an experimental group. In 28 days, the second generation (F_1) was obtained, which received the same food as the first generation, depending on an experimental group. At 12 months of age, young females and males (F_0) were decapitated. Animals were decapitated after having been anaesthetised by brief exposure to ether, without breach of guidelines on humane handling of laboratory animals, based on generally accepted bioethics guidelines and in compliance with international provisions on experimental work [5]. Material for morphological analysis was collected from preliminarily weighed animals of all groups. Upon removal, a kidney was weighed, with cutting fragments about 5 mm thick from its middle for histological evaluations.

For histological evaluations, kidney fragments were fixed for 48 hours in 10 % neutral formalin solution. After fixation, the material was dehydrated in ethyl alcohol solutions (60 %, 70 %, 80 %, 96 %) and embedded in paraffin at 58 °C. Paraffin histological sections of kidney tissue 5-7 μm thick were prepared with MS-2 sledge microtome; after deparaffinisation the sections were stained with haematoxylin and eosine. Micropreparations were studied under a light microscope with 200-fold optical magnification, using an eye lens providing 10-fold and a lens providing 20-fold magnification.

Digital copies of optical image were obtained using Olympus SP550UZ camera. These copies were analysed using a licensed copy of ImageJ (version 1.48v, copyleft licence, W. Rasband, National Institute of Health, USA, 2015) [8]. Morphologically abnormal cells were counted using a licensed copy of ImageJ (version 1.48v, copyleft licence, W. Rasband, National Institute of Health, USA, 2015) [7]. The mentioned programme, among other things, makes it possible to calculate a percentage of cells in different states by marking and numbering them, which results in the count of cells in a certain state per field of vision, and total count of cells per field of vision, wherefrom a percentage of cells in a certain state is calculated (e.g., in a state of reversible swelling or necrosis). In each animal, 1,000 cells were evaluated [9, 10].

Morphological evaluations were conducted using generally accepted histological methods; histological sections were stained with Böhmer's haematoxylin and eosine using a standard method [1].

In order to be able to evaluate and compare the degree of kidney damage, morphometric methods were used. Morphometric analyses were conducted using ImageJ (version 1.48v, copyleft licence, W. Rasband, National Institute of Health, USA, 2015) and Microsoft Excel on a personal computer. Statistical processing of the quantitative data obtained was carried out using Excel and STATISTICA 6.0 and parametric techniques. For all parameters, the arithmetic mean (M), the error of the arithmetic mean (m) and the standard deviation (σ) were calculated. The confidence of difference between independent quantitative values was determined assuming a normal distribution using the Student's t-test. Differences are considered confident if $p<0.05$.

5. Results and discussion

Studies of the structure of kidneys of the two generations of rats fed on genetically modified soybean and Roundup revealed morphological changes in cortex and medulla of the kidney. Microscopic study of kidneys of intact animals of two generations showed that they have typical morphology of renal tissue. In cortex (Fig. 1), kidney glomeruli have usual structure, epithelium of proximal and distal tubules is also unchanged. In control animals (intact group), micrographs show a renal corpuscle formed of glomeruli, which covers a glomerulus with a two-layer capsule. The renal corpuscles are rounded in shape, proximal and distal tubules are also visible. Epithelium of collecting ducts in medulla is unchanged. The similar picture can also be seen in kidneys of rats of group II (Fig. 2) fed on conventional soybean.

In Fig. 2, a swelling in tubules can be seen, the tubules are non-patent almost at all.

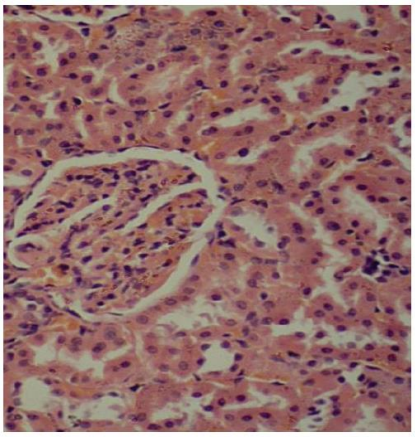

Fig. 1. Group I includes intact animals. Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

In Fig. 2, a Bowman's capsule can be seen, which is composed of a double layer of epithelial cells and has a cavity inside – the urinary space (Bowman's space) where urine is filtered. It can also be seen that the outer layer of the glomerular capsule is formed of a single layer of squamous cells lying on the basement membrane. The inner layer of the nephron capsule is formed of large epithelial cells.

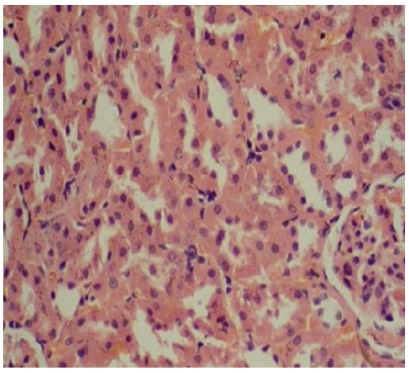

Fig. 2. Group II includes animals whose standard food contained 26 % of conventional soybean. Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

There are minor morphological changes in kidneys of rats fed on genetically modified soybean not treated with the herbicide (group III), both in the first and the second generations. In medulla, the structure of interstitium and medullary rays is the same as in intact animals.

Morphological structure of collecting ducts of renal papilla, as well as interstitium and blood vessels show signs characteristic of intact animals, a loss of structure in some glomeruli is observed, and openings of tubules are filled with fluid, which suggests compromised filtration in them (Fig. 3).

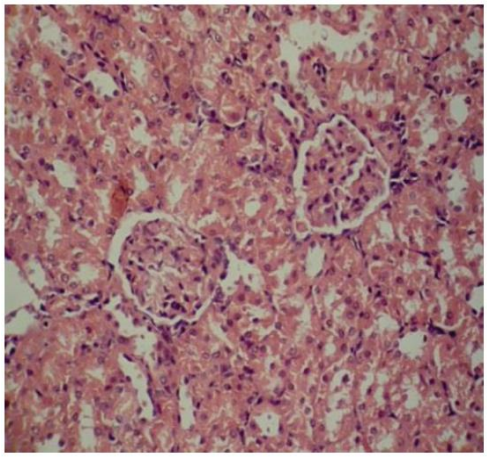

Fig. 3. Group III includes animals whose standard food contained 26 % of transgenic soybean. Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

Pathomorphological changes are observed in the structure of kidneys of rats fed on Roundup: Glomeruli in cortex show signs of intercapillary oedema, and tubular epithelium has signs of reversible cellular swelling. Some epithelial cells of proximal tubules show signs of necrosis (karyopyknosis and cytoplasmic condensation), 80±1.9 % of epithelial have signs of hydropic swelling, openings of these tubules are narrow, which is observed in group V (Fig. 4), and in (Fig. 5), atrophy of tubular and capsular epithelium can be seen.

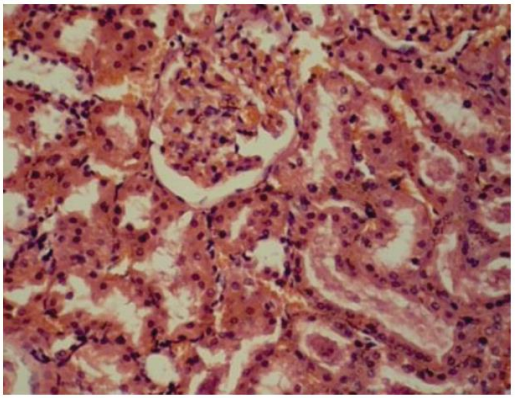

Fig. 4. Group V includes animals given Roundup with potable water (0.003 μg/kg body weight) (F_0). Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

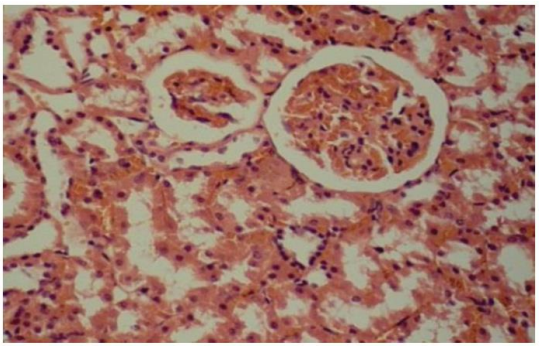

Fig. 5. Group V includes animals given Roundup with potable water (0.003 μg/kg) (F_1 generation). Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

Hydropic swelling is most pronounced in renal glomeruli of the second generation of rats fed on soybean treated with the herbicide (Fig. 6, 7) and Roundup with potable water; the effect of these factors is confirmed by previous studies [11, 12], though the combined effect of these factors was not studied. Micrographs show that epithelial cells of collecting ducts have signs of hydropic vacuolation. Swelling of epithelial cells of proximal tubules is also observed. In rats fed on genetically modified soybean treated with Roundup, also swelling of renal cells, marked signs of necrosis in individual cells are observed. Distal tubules have epithelial cells showing signs of hydropic swelling, openings of the tubules are filled with fluid, which suggests compromised filtration in them (Fig. 7). These results suggest structural changes and dysfunctions of kidneys in groups IV and V.

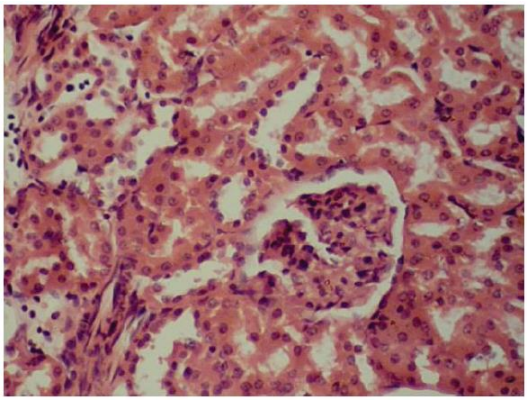

Fig. 6. Group IV included animals whose food contained up to 26 % of genetically modified soybean treated with Roundup (F_0 generation). Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens ×20. Eye lens ×10

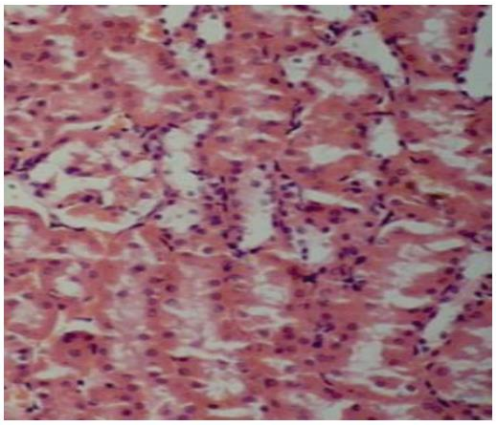

Fig. 7. Group IV included animals whose food contained up to 26 % of genetically modified soybean treated with Roundup (F_1 generation). Rat kidney. Micrograph. Staining with haematoxylin and eosine. Lens 20×. Eye lens 10×

The revealed morphological differences can provide a basis for further biochemical research into changes of functioning of kidneys in animals fed on glyphosate-resistant soybean treated with Roundup and effects of the herbicide itself. The results of the studies showed that the most significant histopathological changes are observed in experimental animals of groups IV and V, which can be caused by Roundup.

The structure of renal glomeruli in these experimental groups is compromised, cellular destruction can be seen in tubules, and openings of the tubules are filled with fluid; such changes suggest compromised renal filtration.

6. Conclusions

1. In experimental animals fed on conventional soybean, structural changes and dysfunctions of kidneys of rats of the first and the second generations are not observed. In animals fed on genetically modified soybean not treated with Roundup, loss of structure in some glomeruli is observed, and openings of the tubules are filled with fluid, which suggests compromised filtration.

2. When Roundup was given with potable water (0.003 μg/kg body weight), structural changes were revealed in kidneys of rats of the first and the second generations, there was hydropic swelling of epithelial cells of distal tubules, openings of these tubules are narrow, necrosis of individual cells is noted.

3. Giving transgenic soybean treated with Roundup to rats results in changes in structure of glomeruli, with cellular destruction observed in the tubules, and openings of the tubules filled with fluid both in the first and in the second generations. The above changes suggest compromised renal filtration.

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