

Histological changes in the internal organs of rats during the experimental study of the acute toxicity of the new G10 substance

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ABSTRACT

The article presents the results of experimental studies devoted to the study of morphological changes in the internal organs of rats after the introduction of Substance G 10. The purpose is to determine the toxicity of the new substance G10 extracted from the leaves of the Calligonum plant on the basis of morphological studies of the internal organs of laboratory rats under acute experimental exposure to the substance under study. The materials and methods used are, the object of the study is the substance G10 from the Calligonum plant, which is a brown powder, odorless, poorly soluble in water. The substance was provided by the research Institute of Bioorganic chemistry of the L. N. Gumilyov ENU. The work was carried out in accordance with the ethical and scientific requirements of preclinical studies on animals.

Key words: acute toxicity, hazard class, substance G10, preclinical study, submucosal layer, Calligonum plant, intraperitoneally, enteral substance

INTRODUCTION

Today, in the Republic of Kazakhstan, the science of human life and health is one of the priority areas of clinical and experimental research. At the same time, special attention is paid to the development of new, original medicinal and prophylactic drugs for medicine, from domestic herbal raw materials. A promising medicinal plant in this direction that grows on the territory of the Republic is a plant of the genus Calligonum of perennial branched shrubs of the Buckwheat family, from which the substance G10 was obtained. A number of researchers have found that this plant is a source of phenolic compounds, tannins, flavonoids, saponins, etc. The active compounds isolated from Calligonum aphyllum leaves include the substance G10, presumably possessing hepatoprotective properties [1]. All of the above determines the need to study the pharmacological effects of the substance G10 on the internal organs. It is known that an important and necessary requirement for preclinical research of new drugs, both synthetic and natural origin, is the experimental study of their toxicity [2].

PURPOSE:

To determine the toxicity of the new substance G10 extracted from the leaves of the Calligonum plant on the basis of morphological studies of the internal organs of laboratory rats under acute experimental exposure to the substance under study.

MATERIALS AND METHODS:

The object of the study is the substance G10 from the Calligonum plant, which is a brown powder, odorless, poorly soluble in water. The substance was provided by the research Institute of Bioorganic chemistry of the L. N. Gumilyov ENU. The work was carried out in accordance with the ethical and scientific requirements of preclinical studies on animals [3].

To study the acute toxicity of substance G10, we used adult white rats of both sexes with a body weight of 160–230 g, obtained from the vivarium of the NJSC “Astana Medical University”. The work was carried out in the "Educational and Research Pharmacological Laboratory" of the Department of General Pharmacology. The experiment was carried out in accordance with the methodological recommendations of the Organization for Economic Cooperation and Development (OECD) No. 423[3]. The toxicity class was determined in accordance with the international classification system for toxicity of substances according to the system of classification and labeling of chemicals and mixtures (GHS) [4].

To study acute toxicity in experimental animals, the substance G10 was injected into the stomach on an empty stomach, using a syringe with a probe in the form of a suspension in a volume of 1.8-2.0 ml, and also intraperitoneally parenterally. 1% starch mucus was used as a solvent; control animals were injected with physiological saline in the same volume.

The following doses of the substance have been tested: 2000, 3000 mg/kg of body weight.

All 35 animals were divided into 5 groups (in each group n = 7):

- I - animals that received saline;
- II - animals that received enteral substance G10 at a dose of 2000 mg / kg;
- III - animals that received enteral substance G10 at a dose of 3000 mg / kg;
- IV - animals receiving substance G10 intraperitoneally at a dose of 2000 mg / kg;
- V - animals receiving substance G10 intraperitoneally at a dose of 3000 mg / kg.

The animals were under constant observation, while the total duration of observation was 14 days. The general condition of the animals, the features of their behavior, the intensity and nature of motor activity, the presence and nature of seizures, coordination of movements, RR of movements, the condition of the hair and skin, the amount and consistency of feces, frequency of urination, food and water consumption and changes in body weight were regularly recorded. Hematological data (general, biochemical, coagulogram) were determined by generally accepted methods. Fragments of tissues of the stomach, liver, intestines and kidneys were subjected to pathomorphological examination. Fragments of tissues of the stomach, liver, intestines and kidneys were subjected to pathological examination.

RESULTS & DISCUSSION

When observing the animals of the first, control group, which received physiological saline, no pathological changes were noted in terms of their appearance, general behavior and activity. Feed and water consumption did not change. Macroscopic examination of the internal organs of the control group did not reveal significant changes. The arrangement of the internal organs is typical.

In the animals of the second group, which received enteral substance G10 at a dose of 2000 mg /kg, in comparison with the control group, there were no changes in their appearance, general behavior and activity. The consistency of feces and frequency of urination did not differ in comparison with the control group. During the experiment, the death of animals was not observed. The body weight of the rats did not change significantly. Primorphological examination of internal organs did not reveal any pathological changes, and their absolute and relative masses did not differ much from those of the control group.

In the third group of experimental animals, who received enterally substance G10 at a dose of 3000 mg/kg, the following were observed: insignificant passivity, depression of the animals, decrease in the frequency of respiratory movements, they were inactive, changes in the reflexes of position persisted. Clinical signs of intoxication in the form of lethargy, lethargy, rapid breathing and refusal of food and water were also noted. However, these signs of intoxication disappeared after 1.5-2 hours from the beginning of observations. By the end of the observations, the experimental animals did not outwardly differ from the control ones. Morphological examination revealed plethora of organs and moderately expressed dystrophic changes in the parenchyma and stroma of the studied organs.

Significant clinical and morphological changes were revealed in the studied organs of the fourth group of animals, to which the substance G10 was injected intraperitoneally at a dose of 2000 mg/kg. There was a decrease in their mobility, polydipsia, an increased reaction to sound stimuli (startle, jumping in place), frequent and shallow breathing (in one female in the group - deep and convulsive), tremor were observed.

THE STOMACH WALL:

At autopsy, their stomach was stretched with fodder, the stomach wall was thinned, pinpoint and spotted hemorrhages were determined on the mucous membrane. Microscopic examination against the background of signs of plethora and edema of the mucous membrane revealed single erosive defects and focal inflammatory infiltration. The basal lamina of the mucous membrane was infiltrated by plasma cells, lymphocytes, leukocytes, and eosinophils. Edema and plethora of blood vessels were observed in the submucosal layer (Figure-1).

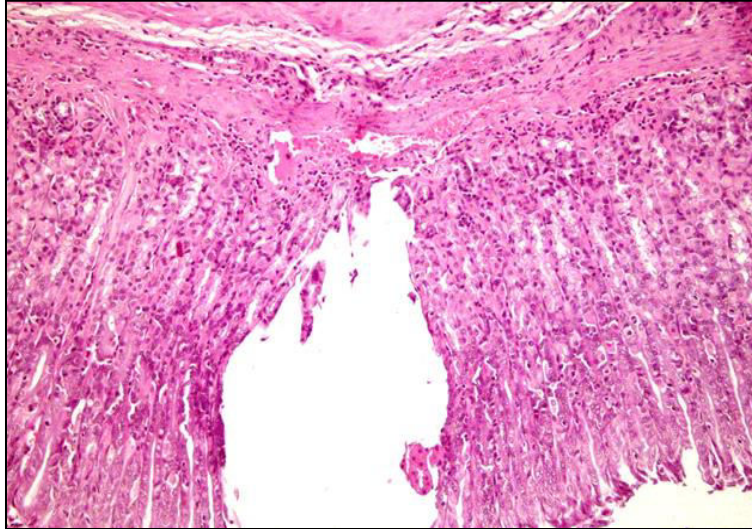


Fig-1. The gastric mucosa of the experimental rat of the 4th experimental group. Erosive defect of the mucous membrane against the background of plethora and edema. Lymphocytic-plasmacytic infiltration of the basal plate with an admixture of leukocytes and eosinophils. Staining with hematoxylin and eosin. Magnification x160.

THE SMALL INTESTINE:

Microscopic examination in the small intestine of experimental animals of the fourth group showed pronounced inflammatory changes, moderate hyperplasia of lymphoid follicles without a light center, located in the basal layer of the intestinal mucosa (Figure-2).

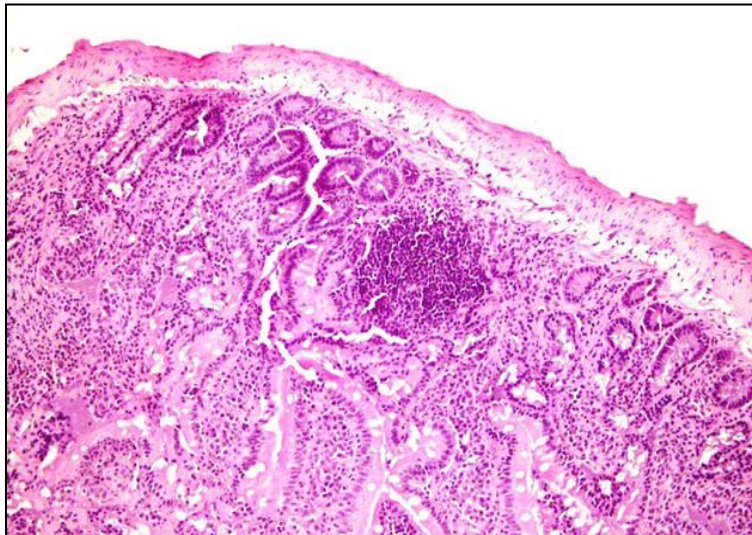


Fig-2. Small intestine of an experimental rat of the 4th experimental group. Pronounced inflammatory changes. Hyperplasia of lymphoid follicles without a light center in the basal layer of the mucous membrane of the small intestine. Staining with hematoxylin and eosin. Magnification x160

THE LIVER:

Microscopic examination of the liver of rats of this group revealed that the beam structure of the hepatic lobules is preserved. There is a moderate plethora of the central veins in the lobules of the liver, sinusoids, mostly slit-like, in places unevenly expanded. The lumen of the sinusoids contains erythrocytes and single lymphocytes. Hepatocytes are in a state of protein dystrophy. Isolated apoptotic cells are found among hepatocytes (Figure- 3).

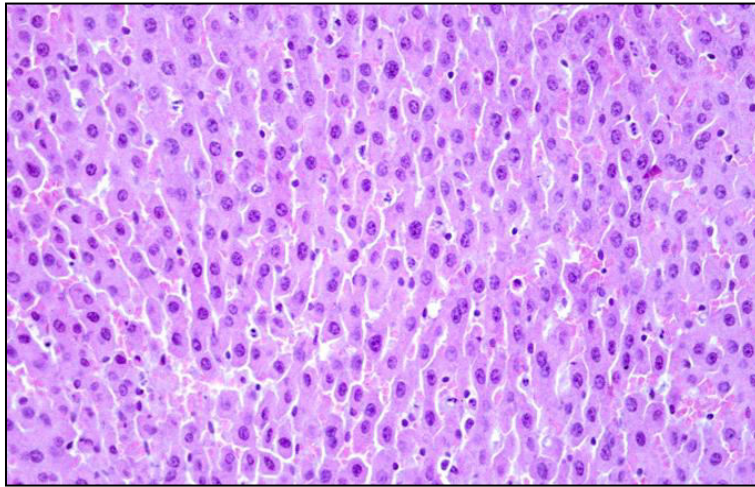


Fig-3. Liver tissue of the experimental rat of the 4th experimental group. The beam structure of the hepatic lobules is preserved. Sinusoids in lobules collapsed, in places unevenly expanded, moderately full-blooded. The sinusoids contain lymphocytes. Hepatocytes in a state of protein dystrophy. Staining with hematoxylin and eosin. Magnification x160

THE KIDNEY:

When examining the kidney tissue, in the stroma of the organ, perivascular inflammatory infiltrates from lymphocytes and plasma cells with an admixture of single leukocytes were determined (Figure 4).

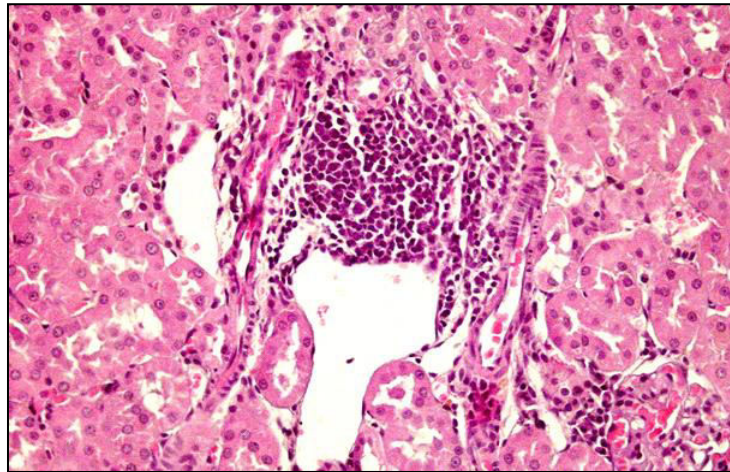


Fig-4. Kidney tissue of the experimental rat of the 4th experimental group. Perivascular inflammatory infiltrates from lymphocytes and plasma cells, with an admixture of single leukocytes in the renal stroma. Staining with hematoxylin and eosin. Magnification x160

Against the background of signs of plethora and edema in the glomeruli, there were signs of mucoid swelling of the basement membranes of capillary loops, and in the lumen of the capillaries, stasis and lysis of erythrocytes. There were unevenly expressed signs of focal hyperplasia of mesangiocytes. (Figure-5).

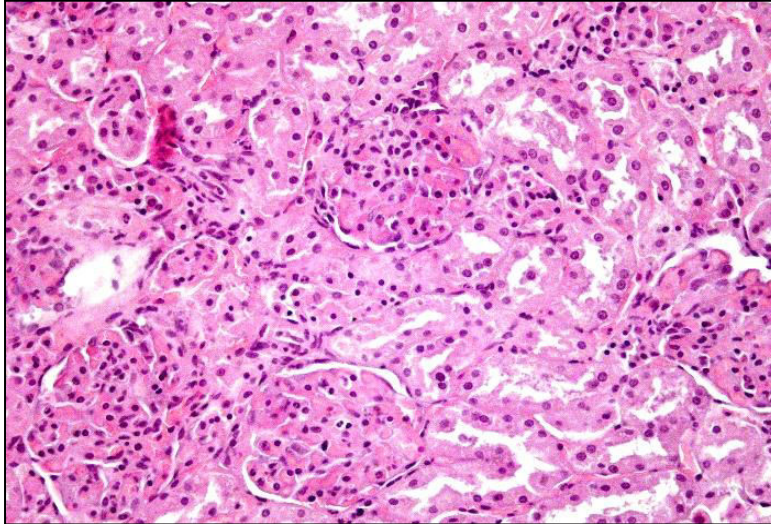


Fig- 5. Kidney tissue of the experimental rat of the 4th experimental group. Uneven plethora of capillary glomeruli. Swelling and protein dystrophy of the epithelium of the renal tubules. The nuclei of individual epithelial cells are pale, without clear contours. Some tubules contain proteins in the lumen. Staining with hematoxylin and eosin. Magnification x160

In the dynamics of observations of experimental animals of the fifth group, which were injected intraperitoneally with substance G 10 at a dose of 3000 mg/kg, there were signs of deterioration of the general condition, a delayed response to stimuli, aggressiveness and increased respiration. The animals were characterized by the retraction of the abdomen. This reaction is probably determined by a spasm of the oblique muscles of the abdomen and diaphragm. It is possible that the listed symptoms were the result of a direct irritating effect of the injected drug on the nerve plexuses of the abdominal cavity.

THE STOMACH:

Pathohistological examination of the internal organs of this group revealed the development of signs of general pathological processes. Thus, microscopic examination of the stomach wall revealed signs of smoothing of the villousness of the mucous membrane and its uneven thickening due to plethora of blood vessels of the microvasculature, edema and diffuse moderate lymphocytic infiltration. Signs of edema, vascular congestion and small focal perivascular lymphocytic infiltration were found in the submucosal layers. In some areas, erosive defects of the gastric mucosa were determined (Figure-6).

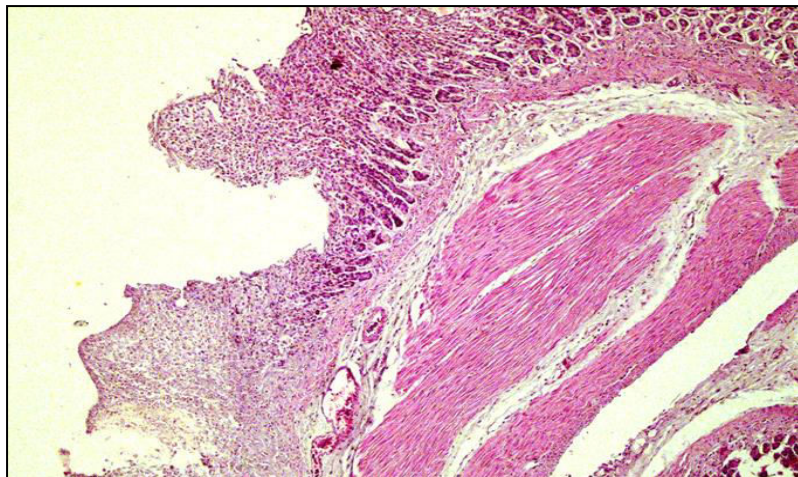


Fig-6. Stomach tissue of the experimental rat of the 5th experimental group. The mucous membrane with an erosive defect, with signs of plethora of blood vessels of the microvasculature, edematous, infiltrated by lymphocytes. Submucosal layers with signs of edema, vascular congestion and small focal perivascular lymphocytic infiltration. Staining with hematoxylin and eosin. Magnification x160

THE LIVER:

Changes in the liver of experimental rats were expressed in uneven edema of the reticular stroma, uneven plethora and small-focal hemorrhages, and in the presence of small lymphocyte-leukocyte infiltrates in the thickness of the hepatic lobules. In the cytoplasm of hepatocytes of the subcapsular zone of the liver, small fatty vacuoles were determined (Figure-7).

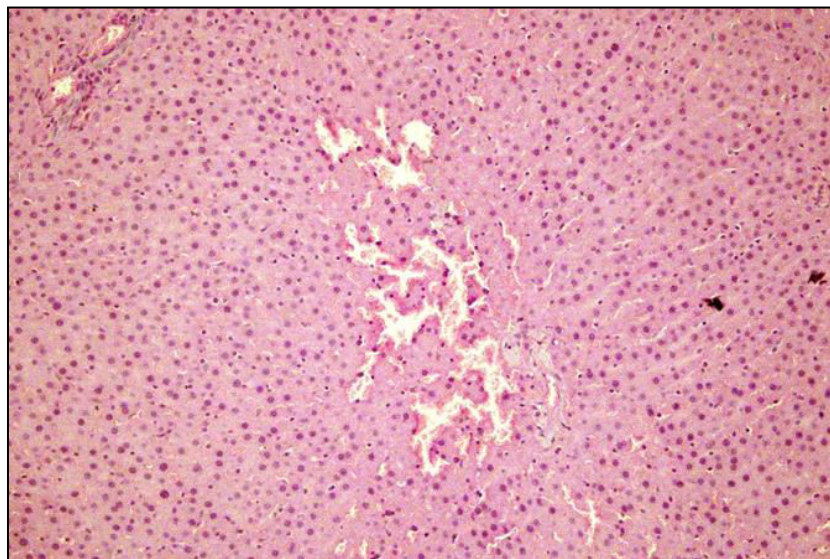


Fig- 7. Liver tissue of the experimental rat of the 5th experimental group. In the reticular stroma of the hepatic lobules, small focal diapedetic hemorrhages, small inflammatory infiltrates from lymphocytes and leukocytes. Staining with hematoxylin and eosin. Magnification x160

CONCLUSIONS:

The results of a pathomorphological study of the internal organs of laboratory animals (rats) with a single enteral and parenteral administration of a suspension of a new substance from the Calligonum plant allow us to conclude that the substance G10 is a low-toxic substance and can be classified as hazard class IV. The obtained data on the low toxicity of the G10 substance open up new prospects for further studies of its pharmacological properties.

HISTOLOGICAL CHANGES IN the internal ORGANS of RATS in the EXPERIMENTAL STUDY of ACUTE TOXICITY of the NEW SUBSTANCE G10: Experiments were conducted on 35 white mongrel rats. The animals were divided into 5 groups. The study of acute toxicity of substance G10 from the plant Calligonum, conducted by us in an experiment on rats, showed that a single intragastric administration of toxic doses of substance G10 does not have pronounced General toxic effects, which allows it to be assigned to class IV – a class of low-toxic substances.

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