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Morphological and Morphometric Changes in the Valves of the Heart in Experimental Toxic Myocarditis

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Abstract

The present study aims to identify postnatal development's morphogenesis characteristics while examining the rat heart's membranes and various departments' histological structures 1 to 22 days after birth. Fifty rats were chosen as the study's subjects, and the morphological changes in their hearts were observed at 1, 6, 11, 16, and 22 days after birth. Alternating periods of acceleration and deceleration of the growth rate with increasing thickness of atria and ventricles were noted. Pesticide toxicity caused specific pathological morphological changes in rat heart tissue, including nuclear-cytoplasmic ratio violations and myofibril loosening. Protein dystrophy, connective cell proliferation, and disorganization of the fibrous structures in the wall of capillaries and arterioles were also observed in the muscle fibers of the myocardium. Compared to controls, experimental animals' atrial wall layers, and ventricles exhibit a significant decrease in morphometric parameters. The myocardium underwent changes resembling necrosis in the latter stages of the experiment.

Keywords: Rat heart, Postnatal ontogenesis, Cardiomyocytes, Fibrous structure atria, and ventricles.

Introduction

Toxicology is one of the most prevalent etiological causes of heart disease. Various chemical products are currently used in agriculture to treat cotton, grape, and vegetable fields. Most chemical substances include pesticides, which have significant adverse health effects.[1,3,4] With their number growing yearly, there are currently more than 1000 different types of pesticides worldwide. Chronic exposure to pesticides, even in low doses, worsens and increases the frequency of diseases of the cardiovascular system, including myocarditis of various etiologies. Therefore, the study of pesticide effects on humans and animals, particularly on the cardiovascular system, is of great interest. The issue of pesticide influence on developing young organisms has gained significant relevance.[5,6,8] The variability of the heart is not only of general biological interest but also has some bearing on the disclosure of physiological processes that arise in it in response to environmental factors. The World Health Organization (WHO) reports that cardiovascular system diseases are the leading cause of death worldwide, frequently resulting in disability in the able-bodied population. About 29% of all deaths occur from cardiovascular diseases, which affect 17.5 million people annually. As a result, advanced treatment systems for diseases of the cardiovascular system have received particular attention in modern medicine. In the Republic of Uzbekistan, 8000 cases of myocardial infarction are reported annually; 60% of these cases result in pre-hospital death. Statistics show that 58% of deaths in Uzbekistan are caused by cardiovascular diseases, out of which 20 to 50% are unexpected, concluding that prophylactic measures are significantly vital.[2,7,9,10] It is crucial to conduct targeted studies on the use of pesticides, which have various chemical compounds, to achieve high efficacy in agriculture globally. The following are given special attention at that time: the creation of methods to prevent pesticides' harmful effects on humans; research into how long-term, low-dose pesticide use accelerates the occurrence of myocarditis and cardiovascular diseases and deepens their courses; a thorough examination of the effects of synthetic pyrethroid-kinks and herbicide cotoran on the human and animal cardiovascular systems, the essence of physiological processes and the changeability of the effects of peritroids and herbicides on the heart, and particularly the harmful effects of pesticides that enter a baby's system through the mother's milk. Therefore, the study aims to assess the microscopic and morphometric alterations in the different layers of the walls of the atria and ventricles of the heart during early postnatal ontogenesis under the influence of pesticides that have permeated the mother's milk.

Methods

The results of procedures conducted on 205 white laboratory rats during early postnatal ontogenesis provided scientific validation for the research. The experiments were conducted by the 'European Convention for the Protection of Vertebrate Animals and used for Experimental and other Scientific Purposes (Strasbourg, 1985).

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Animals were divided into three groups. Following the delivery of the baby rats, mothers in the first group were given a daily intragastrically administered dose of the pesticide cotoran at a concentration of 1/100 of the LD50. The infant rats were anesthetized during the age mentioned above ranges in the second group in the same manner as female rats were given kinmiks pesticide at the maximum permissible level 5 dose. Distilled water in 1 ml was given daily, first thing in the morning, to the female rats in the control group, depending on the period. No. 1 subclavian catheters were used as a probe on the female rats. At 1, 6, 11, 16, and 21 days after birth, rats underwent Rausch-narcosis anesthesia. It was used in complex morphological studies that included mathematical modeling and forecasting of toxic myocarditis, general histologic and histochemical techniques, organometry, morphometry, scanning and transmission electron microscopy, and ECG heart.

The rat heart's length, width, and thickness were measured after removing it from the thorax. Calipers with a 0.05 mm scale were used to measure the linear dimensions. The heart's length is the distance between the tip and the furthest point of the heart's base. The width of the heart is measured from left to right between the protruding portions at the atrioventricular sulci. The thickness of the heart is the distance from front to back between the most noticeable areas at the level of the atrioventricular sulci. Visual cues helped determine heart shape, and electronic scales were used to measure the mass and weight of the rat hearts.

Using a microtome, 8–10 micron-thick histological sections were created, and they were then stained using the conventional techniques of hematoxylin and eosin. Pikrofuksin by the Van Gieson method revealed collagen fibers in the connective tissue shell of the heart's walls, elastic fibers by the Weigert method, and reticular fibers by the Foote modification N.A. Yurina.

For scanning electron microscopy, infarction-sized pieces that are 1.5x1.5 mm were fixed in 2.5% glutaraldehyde solution after being pre-fixed in phosphate buffer with osmium tetroxide. They were then dehydrated in alcohol-acetone and dried by the apparatus NSR-2's critical point. Gold was deposited using the IB-2 apparatus, and the Hitachi-S405 and JEOL JSM-6010LV electron microscopes were used to examine it. In contrast, the newest Canon digital camera was used to take pictures of the monitor screen. The research findings were processed statistically using a Pentium-IV computer's Microsoft Office Excel-2012 package, including statistical processing functions. The drugs were photographed using the Mikromed-3 and Microscope 11 with photo and video equipment Tucsen Camera TCA-5.0C China.

Results

All stages of development (1, 6, 11, 16, and 21 days) saw a significant increase in the thickness of the left and right endocardial atriums, with the left atrium's thickness increasing by 29% on day six and the right atrium by 8% on day 16. The study's findings on myocardial thickness revealed that it increased rapidly across all study periods, from 13% to 29%, especially on the sixth day, when it increased by 25% in the top and bottom of the left ventricle and by 24% in the top and bottom of the right ventricle. The thickness of the myocardium in the left and fitting atriums increased significantly over time (1, 6, 11, 16, and 21 days), ranging from 12% in the right atrium at the 6th and 16th days to 22% in the left atrium at 11- and 21st day. According to the growth rate of the epicardium's thickness in the left and right ventricles, it is always slightly thicker than previously recorded, ranging from 8.2% to 11.5%. These percentages range from 9% to 18% in the left and right atriums.

The structural components of the cardiac atria and ventricle layers displayed a distinct pattern of development and differentiation in the dynamics of early postnatal ontogenesis check in intact rats. All layers of the heart wall's structural components are still undifferentiated at the 6th and 11th days of the study, which is best seen in the myocardium, where cellular components predominate over myofibrils. After 21 days, the heart wall's structural components fully develop and reflect their actual morphological and functional characteristics.

The left and right ventricles distinguish the subendocardial, subepicardial, and intermediate layers. The myocardium's subendocardial layer is more differentiated than the other layers and comprises parallel cardiac histocyte beams that run parallel to the endothelium. Cardiomyocytes in the subepicardial myocardial layer are more extensive, have a random arrangement, and have a loose structure. A vague and vacuolated myofibril in some cardiomyocytes is thicker in others (Figure 1a). Left ventricular myocardial muscle cells are arranged perpendicular to the subendocardial layer in the intramural layer. The denser, thicker interventricular septum myocardium forms cardiomyocyte parallel beams. Collagen and elastin fiber bundles are positioned around the vessels and cardiac histiocytes. Reticular fibers are arranged as dark brown fibrous structures between the cardiomyocytes that encircle the individual muscle bundles and form large loops (Figure 1b.). A small loop network also surrounds the vessels in the epicardium.

The intramural myocardial layer is shown parallel to the nuclear structures, eventually reaching myofibrils. Comparatively thin and transversely cut cardiac histiocytes make up the subepicardial layer sandwiched

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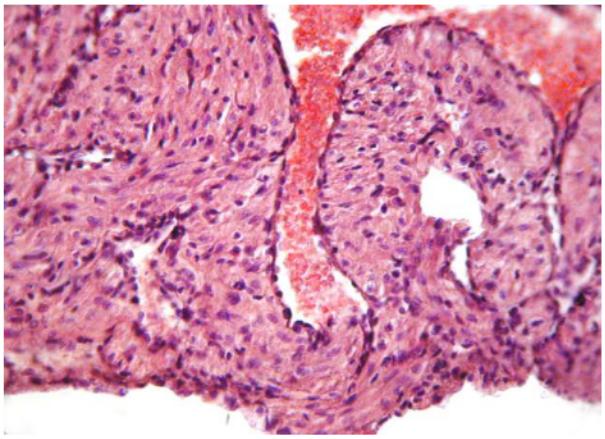
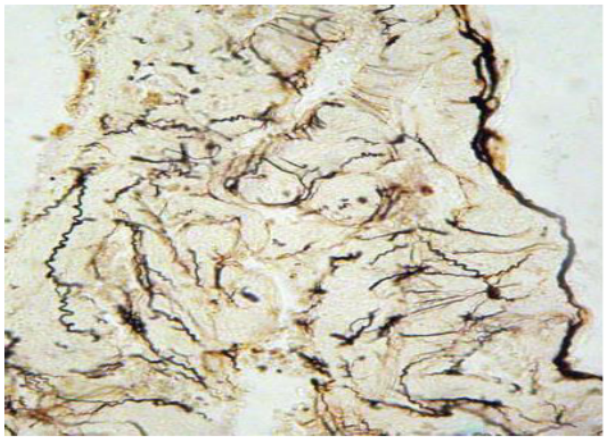
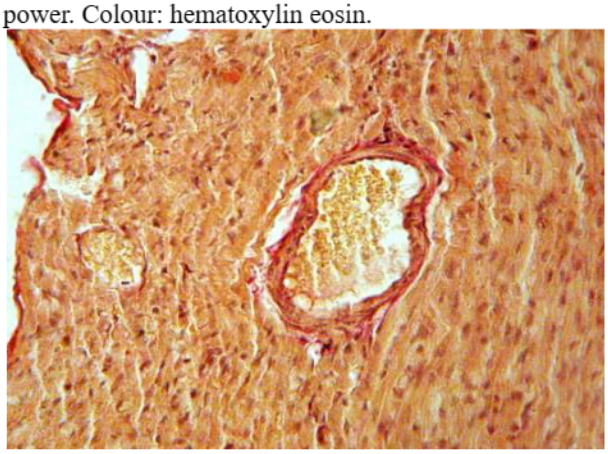
between the arterial and venous vessels. The size and shape of these venous sinuses vary; some create large, elongated blood lakes. Arterioles were found in the walls of arteries using Van Gieson's histochemical method to check for collagen fibers (Figure 1c). Additionally, there are fragile and broken collagen fibers in the stroma of the myocardium as well as the walls of the veins. In particular, it thickens the subendothelial inner elastic membrane, depicted by a thick, unevenly layered, deep purple material (Figure 1d). Small, bluish shadowy structures of elastic fibers of the muscular and adventitial layers of artery walls are visible.



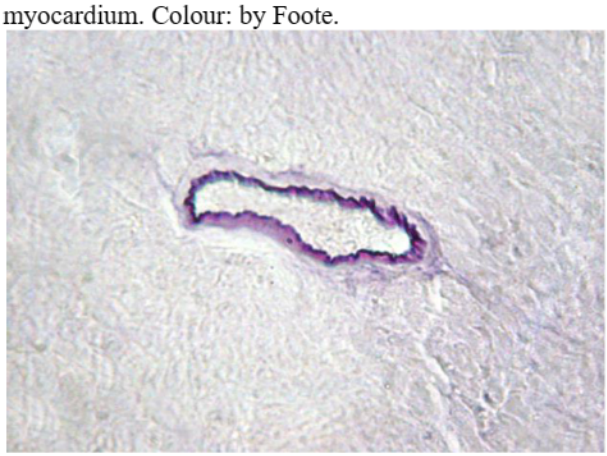
a) Cardiomyocytes different shapes and staining power. Colour: hematoxylin eosin.



b) Small loop wale network of reticular fibers of the myocardium. Colour: by Foote.



c) Collagen fibers in the vascular wall. Colour: Van Gieson.



g) Elastic fibers in the vascular wall. Colour: by Weigert.

Fig.1. Histology and histochemistry of rat's heart myocardium is in norm

A thin inner layer, a distinct middle layer, and an outer layer distinguish rat heart arterioles. Arterioles made up of nearby endothelial cell nuclei make up the inner layer. Muscle fiber bundles that are directed in a circle make up a middle membrane that has fully developed. T, and they are into two. The average inner diameter of the arterioles in the newborn rats in the control group is 11.7 mm. The most effective rate of arteriole diameter growth occurs up to day 6 when it is 40%, and the inner diameter of the arterioles grows slightly more slowly in the following age groups.

The average capillary diameter is 4.70.6 microns. Endothelial cells' capillary walls comprise the inner layer, and the basement and external elastic membranes comprise the middle layer. Mainly found in the myocardium's capillaries, revealed in the subepicardial layer. Blood vessels in the subendocardial layer are uncommon. Venular endothelial cells make up the wall and are spread far apart—underdeveloped venules in the muscular layer. The average venule thickness is 16.7 ± 1.2 m.

The findings of morphometric studies in nursing rats' ventricles revealed that all departments' walls were thinner at the beginning of the experiment than in the control group. It has been proven that a noticeable lag in ventricular wall thickness was seen on day 11; it was only 31% below the benchmark. The minor changes were discovered on day 21 when the differences between the experimental and control groups ranged from 4% to

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19%. When the left thickness and right atrium were compared to the control group, it was clear that the endocardium and myocardium were thinner in all experimental periods than the benchmark range of 6% to 23%. It is still being determined what the differences between the left and right atriums were, and Epicardium benchmarks range in thickness from > 2% to 11.5%.

Since the sixth day, a decrease in the internal diameter of arterial vessels has been observed, according to morphometric results of examinations of different diameter blood vessels of the rat heart wall while feeding breast milk under the influence of cotoran. At six days old, the average artery diameter is 49.8 ± 4.3 microns, which is 12.3% less than the reference index; at 11 days old, the average artery diameter is 52.5 ± 5.6 microns, which is 8% less than the average; at 16 days, the average artery diameter is 56.7 ± 6.3 m, which is also less than the average rate of 11.2%; and at 21 days, the average artery diameter is 61.3 ± 7.3 m. The diameter of the heart's artery grows with age, but compared to the average, it shows some of its backlogs. The dynamics of changing arteriolar diameters have been studied. However, it has only ever been a trend: the study of the figure consistently showed a decline compared to the control group.

Microscopic examinations have revealed that pathological changes in the structural elements of the heart manifest as edema-dys circulator processes and dystrophic degenerative -and-destructive inflammation in female rats that were poisoned with cotoran and kinmiks during early postnatal ontogenesis. Pathomorphological changes first exposed the microcirculatory system in the vessels, then the venous vessels, and finally, on day 21, changes included the artery. Hyperemia, stasis, and diapedetic hemorrhage were found in the small blood vessels of the heart, along with perivascular edema, swelling, and connective tissue stromal disorganization.

As a result of developing dyscirculation of microcirculatory vessel toxic venous plethora and increased vascular permeability, dyscirculatory violations extended to venous vessels in the form of enhancement and hyperemia, development of perivascular edema and hemorrhage, and development of diapedetic perivascular edema; the toxic effects of pesticides bring on these conditions. Increased venous vessel level and microvascular permeability were also accompanied by the release of the liquid component of the blood through the vessel wall to the surrounding connective tissue (Figure 2a) because the redistribution of glycosaminoglycans, hyperacidity, and hydrophilic fabrics in amorphous material alters tissue fluid quality and quantity. This disrupts cellular and fibrous structures in stromal and vascular tissue, which manifests as mucoid and fibrinoid swelling of fibrous structures. These modifications to the heart's structure specifically affect the perivascular connective tissue and vessel walls first. Alternative changes appear alongside fibrinoid swelling of the fibrous structures and advance inflammatory and hyperplastic processes.

In our study, the inflammatory process peaked on day 16 and manifested as an interstitial and perivascular lymphohistiocytic infiltrate (Figure 2b). Proliferative tissue infiltration, which is autoimmune, is a sign that chronic inflammation is developing as original granulation and connective tissue, and lymphohistiocytic cell proliferation is known to be primarily associated with the development of hyperplastic proliferation processes. This, in turn, causes a thickening of the vascular-stromal interstitial stroma, which is discernible by the Van Gieson histochemical method (Figure 2c). This fixed vacuum decay, intramuscular interstitial reticular fibers, and uneven vascular wall thickening were all observed (Figure 2d). The metabolic disorders in the parenchymal cells or cardiac histocyte are caused by the changes mentioned above in the vascular, stromal tissue of the heart. According to our observations, these disorders in the cardiomyocyte's morphology were seen as hyaline protein droplets and vacuolar degeneration, frequently localized in the myocardium's perivascular regions and subendocardial layer.

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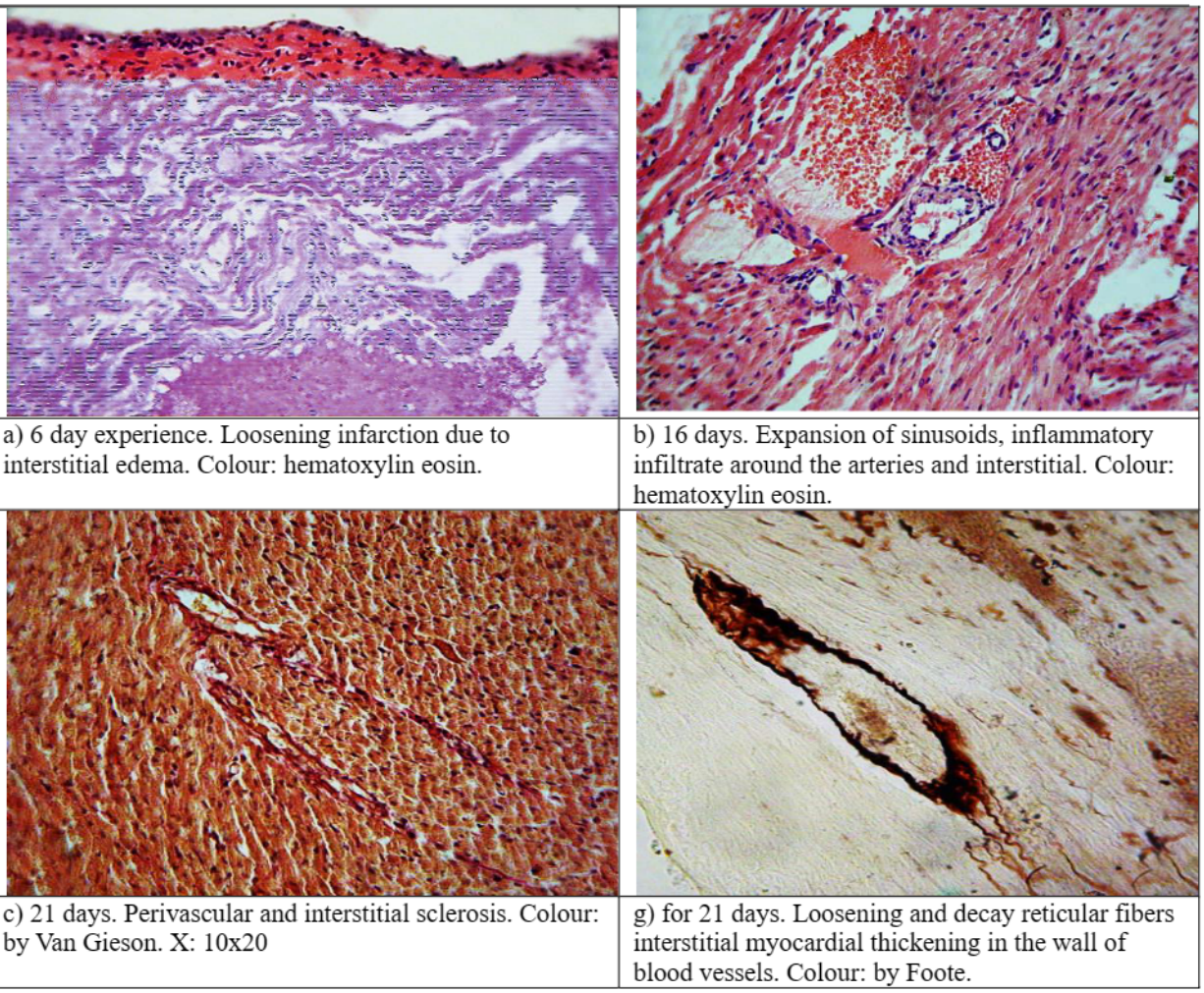

a) 6 day experience. Loosening infarction due to interstitial edema. Colour: hematoxylin eosin.

b) 16 days. Expansion of sinusoids, inflammatory infiltrate around the arteries and interstitial. Colour: hematoxylin eosin.

c) 21 days. Perivascular and interstitial sclerosis. Colour: by Van Gieson. X: 10x20

g) for 21 days. Loosening and decay reticular fibers interstitial myocardial thickening in the wall of blood vessels. Colour: by Foote.

Fig.2. Morphological changes of the myocardium of rats under the influence of cotoran

All areas of the heart wall decreased under kinmiks, according to the analysis of morphometric data from the left and right atria and ventricles of the experimental groups. It is known that a noticeable lag in ventricular wall thickness is seen on day 11; it was lower than the control index of 60%. The left ventricle's myocardium underwent the most significant change, found explicitly in the ventricles' base. Additionally, compared to the control group, the thickness of the endocardium in the right and left ventricles at the top and bottom is less than 20% to 35%. Rats exposed to kinmiks during the 16-day experiment showed the most pronounced growth lag behind the heart's walls, as revealed by comparing the left and right atrium thickness with the control group.

The left and right atrium myocardium's thickness decreased by 23-29%. Atrial endocardial thickness throughout all experimental periods was lower than the 6% to 19% control index, and Epicardium thickness was between 2% and 11.5% of the control index. In this series of experiments, pathomorphological changes in structural heart cells also manifested as edema-dyscirculatory, dystrophic, degenerative, and inflammatory-destructive phenomena. However, unlike the previous series, these processes were expressed more strongly. This was determined by microscopic examination of the infant rat heart during lactation under the influence of kinmiks to female rats. As a result of the toxic effects of pesticides, this group of researchers studied how dyscirculatory violations extended to venous vessels in the form of enhancement and hyperemia, the development of perivascular edema and diapedetic hemorrhage, as well as the decirculation of microcirculatory vessels, toxic venous plethora, and the rise in vascular permeability.

Alternative changes emerge along with fibrinoid swelling of the fibrous structures, while inflammatory and hyperplastic processes develop in some cases. The proliferative infiltrate's appearance in the tissues indicates a chronic autoimmune inflammatory nature. It is well known that the development of hyperplastic proliferation processes as initially granulated, then connective tissue, and ultimately led to thickening of vascular-stromal

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interstitial stroma, accompany the cell proliferation of lymphohistiocytic primarily obtained morphological data about violation of cardiomyocyte contractile function of the mitochondrial damage in violation of energy and metabolic processes that lead to necrobiosis, changes in microvessels with changes in the size of the blood vessels.

Studies to determine the effect of pesticides on myocardial ultrastructure using scanning and transmission electron microscopy on half-section slices revealed that the nature of myocardial changes in rats exposed to cotoran or kinmiks through the mother's milk is nearly identical. Early on (6–11 days), significant changes were observed in the cardiomyocyte myofibrils' ultrastructure, indicating that the cells' contractile function had been compromised. It causes the myocardial muscle fibers' three-dimensional structure to be violated.

Discussion

The presence of specific patterns in the histological structure, comparative perfection of the structural elements of the atrial wall, and ventricular dynamics control rats in early postnatal ontogenesis were revealed by the coefficient of relative changes in heart mass and body, as well as changes in heart shape and development of the chest. The toxic effects of pesticides on the heart of rats revealed specific pathological morphological changes, including the loosening of myofibrils and the violation of the nuclear-cytoplasmic ratio. There was also disorganization of fibrous structures in the wall of capillaries and arterioles, proliferation of one's connective cells, and protein dystrophy of the myocardium's muscle fibers.

It has been established that control baby rats exhibit morphometric and histotopographic variations depending on their developmental stages, particularly in the structural components of all layers of the heart's atria and ventricles' outer walls. However, experimental animals' atrial wall layers and ventricles significantly reduce morphometric parameters compared to controls. It was also discovered that the cotoran and kinmiks, which affect the ultrastructural components of the heart, cause a change rate in myofibrils, a breach in energy and metabolic processes that result in necrobiosis in the mitochondria, a disruption of the transport function of the endothelium and the inner layers of small blood vessels, and the development of intracellular and intermediate tumor tissue.

Numerous heart rhythm and conduction disorders emerged as a hallmark of ECG changes. In the later stages of the experiment, necrosis-like changes in the myocardium were seen, and T and the complex reflected necrosis. Based on morphometric data, the coefficient (the ratio of stroma to parenchyma infarction and mutual relations), and other factors, mathematical modeling was developed to predict toxic myocarditis heart. Scaled clinical symptoms are used to diagnose and assess the severity of myocardial diseases.

Although the present study is focused mainly on the cotoran, several previous studies discuss the cardiotoxic effect of various other pesticides. Under the influence of keenmax and cotorone, analogical changes in terms of vessels were observed.[2] Hyperemia, stasis, and diapedetic hemorrhage were found in the heart's tiny vessels, along with vascular edema, swelling, and connective tissue stromal disorganization. The toxic effects of pesticides that result from a violation of the microcirculatory channel, toxic venous plethora, and increased vascular permeability were distributed in the venous vessels as an expansion and plethora of them, the development of perivascular edema, and diapedetic hemorrhage. El-nahhal and El-nahhal[11] also found that pesticides from toxicity classes II and III are most commonly involved in cardiotoxicity. The drug "Bleocin" was used in an experiment to simulate the cardiotoxic effects of bleomycin.[12] Ten adult male and female rats were used in the investigation. Following two weeks, there was a 7–10% drop in myocardial weight from baseline, and the myocardium underwent severe dystrophic alterations. With significant cumulative dosages of bleomycin, repeated treatment was found to have a cumulative cardiotoxic impact that caused irreversible alterations in the myocardium and endothelial dysfunction clinically indicated by heart attack and vascular disease. Right ventricular morphofunctional alterations were also noticeable and thought pathognomonic of bleomycin poisoning.

Although based on rat models, these findings emphasize the need for more stringent public policy regarding pesticides, which are linked to serious health events like cancer, endocrine disorders, neurological disorders, and cardiovascular risk. In order to assess the effects of particular chemical types and the effects of multiple chemical exposures, future studies should characterize pesticide exposure in greater detail, specifying different forms of exposure, intensity, and duration. Standardizing the description of cardiovascular outcomes, measuring serum pesticide levels, and conducting robust analyses that account for confounding variables are also necessary to improve study comparability.

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