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EFFECT OF TAGETES PATULA L. EXTRACT ON LIVER AND PANCREAS HISTOSTRUCTURE IN MODELS OF ETHANOL-PARACETAMOL- INDUCED HEPATITIS AND STREPTOZOTOCIN-INDUCED DIABETES IN RATS

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Drugs are increasingly becoming predictors of the development of liver and pancreatic lesions. Drug-induced lesions are usually characterized by strong oxidative stress and destruction of hepatocyte and pancreatic cell membranes. Modern therapeutic strategies are primarily aimed at reducing clinical symptoms and are not able to exert an organoprotective effect. Medicinal plant raw materials with antioxidant and cytoprotective effects can be considered a promising object for reducing the toxic effect of some drugs on the liver and pancreas.

The purpose of this work was to evaluate the effect of extract herb *Tagetes patula* L (EHTP) on changes in histological parameters under conditions of ethanol-paracetamol hepatitis and streptozotocin diabetes.

Materials and methods. Experimental ethanol-paracetamol hepatitis and streptozotocin diabetes were modeled in rats using standard methods. The investigated EHTP and the reference drug Silymarin were administered at a dose of 25 mg/kg in a therapeutic and prophylactic regimen. Organ samples (liver in the first experiment and pancreas in the second) were fixed in 10% formalin, dehydrated in ascending grades of alcohol, embedded in paraffin. Sections 6–7 μm thick were obtained using a sliding microtome, mounted on glass slides, stained with hematoxylin and eosin.

Results. The EHTP has a noticeable protective effect under the conditions of ethanol-paracetamol hepatitis, which is manifested in a significant reduction in the degree of hydropic and fatty degeneration of hepatocytes, disorders of internal hemodynamics, inflammatory reaction; normalization of the structure and morphological integrity of cells. In the model of streptozotocin diabetes, the EHTP reduces destructively dystrophically altered pancreatic cells and exhibits anti-inflammatory effects, significantly reducing signs of insulitis and inflammatory reaction in the interacinar tissue.

Conclusions. The EHTP has a significant organoprotective effect, with a protective effect on hepatocytes in conditions of ethanol-paracetamol hepatitis and on pancreatic cells in conditions of streptozotocin diabetes.

Keywords: *Tagetes patula L herb extract,* ethanol-paracetamol hepatitis*,* streptozotocin diabetes, rat, histological examination.

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ВПЛИВ ЕКСТРАКТУ ТРАВИ *TAGETES PATULA* L. НА ГІСТОСТРУКТУРУ ПЕЧІНКИ ТА ПІДШЛУНКОВОЇ ЗАЛОЗИ НА МОДЕЛЯХ ЕТАНОЛ-ПАРАЦЕТАМОЛОВОГО ГЕПАТИТУ ТА СТРЕПТОЗОТОЦИНОВОГО ДІАБЕТУ В ЩУРІВ

[1] Комунальний заклад вищої освіти Львівської обласної ради «Львівська медична академія імені Андрея Крупинського», Львів, Україна,
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У роботі оцінено вплив екстракту трави чорнобривців розлогих (*Tagetes patula L)* на гістологічні зміни за умов етанол-парацетамолового гепатиту та стрептозотоцинового діабету. Досліджуваний сухий екстракт трави чорнобривців розлогих (ЕТЧР) та референс-препарат Силімарин вводили в дозі 25 мг/кг у лікувально-профілактичному режимі. Встановлено, що ЕТЧР чинить помітну захисну дію за умов етанол-парацетамолового гепатиту, зменшує ступінь гідропичної і жирової дистрофії гепатоцитів, розлади внутрішньої гемодинаміки, запальну реакцію; сприяє нормалізації структури і морфологічній цілісності клітин. На моделі стрептозотоцинового діабету ЕТЧР зменшує деструктивно-дистрофічно змінені клітини підшлункової залози проявляє протизапальну дію, тобто ЕТЧР чинить виразну органопротекторну дію на печінку за умов етанол-парацетамолового гепатиту та на підшлункову залозу за умов стрептозотоцинового діабету.

Ключові слова: екстракт трави *Tagetes patula L,* етанол-парацетамоловий гепатит, стрептозотоциновий діабет, щури, гістологічні дослідження.

Introduction

The use of any medicinal product (MP) may be associated with the risk of developing adverse drug reactions (ADR), including those critical to a patient's health. Approximately 4% of hospitalizations result from the negative effects of MPs [1]. According to the generally accepted definition, any effect of an MP in therapeutic doses that does not correspond to the intended therapeutic purpose is considered an ADR. That is, any undesirable or unexpected reaction to an MP that usually requires modification/discontinuation of pharmacotherapy and/or additional treatment, any effect of a drug at therapeutic doses that does not meet the goals of treatment is deemed an ADR.

Based on the nature of development, ADRs are classified as pharmacodynamic, toxic, allergic, non-IgE mediated hypersensitivity, idiosyncratic, secondary (e.g., superinfection), withdrawal syndrome, or drug interaction-induced [2, 3].

Based on mechanism of onset, ADRs may be predictable (pharmacodynamic, non-IgE mediated hypersensitivity, toxic, secondary, withdrawal, interaction-related) or unpredictable (allergic, idiosyncratic).

Therapeutic strategies for ADR prevention should primarily target predictable moderate-severity reactions. These ADRs are most often associated with the negative effects of MPs on the liver, kidneys, and pancreas [4–7].

According to Stine J.G. et al., 2017, drug-induced liver injury manifests in various forms of acute and chronic liver disease, characterized by cytolysis syndrome, disturbances in the cytochrome P450 enzyme system, excessive activation of lipid peroxidation (LPO), and hepatocyte destruction [8]. One of the mechanisms of drug-induced liver injury, as reported by Saran C. et al, 2023, involves disruption of bile acid exchange and impairment of hepatic bile acid transporters, which may lead to toxic accumulation of bile acids in hepatocytes and increase susceptibility to MP-induced liver injury [9].

Liver dysfunction manifested by activated lipid peroxidation, impaired pigment metabolism, reduced protein synthesis, and hepatocyte cytolysis is also supported by the findings of Koshurba I. et al., 2022, obtained in rats with D-galactosamine-induced hepatitis – a model that is morphologically and functionally similar to viral hepatitis in humans [10].

Regarding drug-induced pancreatic injury, more than 100 MPs are currently known to induce diabetes mellitus (DM) [11]. According to Sosnowski K. et al., 2022, the destructive impact of MPs on the pancreas represents a heterogeneous group of adverse effects, which, as a result of uncontrolled LPO activation, cause damage to pancreatic cells, leading to intrapancreatic activation of pancreatic enzymes, resulting in drug-induced acute pancreatitis and, in some cases, DM. The diabetogenic effect of MPs, understood as impaired insulin secretion, may arise from direct β-cell destruction, systemic toxicity affecting pancreatic islets and glucose transporters on the cell membrane, induction of a Th1-type autoimmune response, disruption of voltage-gated calcium channels in β-cells, and endoplasmic reticulum stress [12].

Drug-induced damage to the liver and pancreas is marked by pronounced oxidative stress, leading to structural membrane disruption in hepatocytes and pancreatic acinar cells. Current therapeutic approaches are primarily aimed at reducing clinical symptoms, and the MPs used in such cases do not possess organoprotective properties. There are data on the use of hepatoprotectors based on medicinal plant raw materials, such as milk thistle, to reduce the negative effects of xenobiotics on various organs, especially the liver [13–15].

A promising candidate for reducing the toxic effects of certain MPs on the liver and pancreas is medicinal plant raw material containing various groups of biologically active compounds (BACs), which exert antioxidant and cytoprotective activity and are effective when used in a therapeutic-preventive regimen.

Researchers at Ternopil State Medical University under the leadership of Prof. Marchyshyn S. M. have developed a new dry extract of *Tagetes patula* L. herb (hereinafter EHTP), proposed for investigation as a potential organoprotective agent for therapy of drug-induced lesions, including those of the liver and pancreas.

The **aim** of this study was to assess the effect of dry extract of *Tagetes patula* L. herb on histological parameters under conditions of ethanol-paracetamol-induced hepatitis and streptozotocin-induced diabetes.

Materials and Methods

Experiments were carried out on male albino non-linear rats weighing 180–220 g. The animals were kept under standard vivarium conditions. The research was conducted according to the National "General Ethical Principles of Animal Experimentation" (Ukraine, 2001), adopted by the V National Congress on Bioethics (Kyiv, 2013), in compliance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the methodological guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine, "Preclinical Drug Research" (2001). Ethics approval for the study was obtained from the Bioethics Committee of the National Pirogov Memorial Medical University (Vinnytsia) (Protocol No. 11 dated November 18, 2024).

Ethanol-paracetamol hepatitis was induced using a standard method: animals received daily intragastric administration of paracetamol suspension in water at a dose of 500 mg/kg and 1 ml of 40% ethanol (to potentiate the paracetamol effect) for 2 weeks.

According to the research design, animals were divided into groups of 8:

- Group 1: Intact control (received solvents);
- Group 2: Pathology control (no pharmacological correction);
- Groups 3 and 4: received intragastric administration of EHTP at 25 mg/kg (dose established as effective in previous studies [16]) or the comparator drug Silymarin at 25 mg/kg [17] (Darsil tablets, "Pharmaceutical Firm Darnitsa", Ukraine). Dose recalculation from average human therapeutic dose was made considering species sensitivity coefficient [17; 18]. The EHTP and the reference drug Silymarin were ground in a mortar, added with distilled water q.s. to form a suspension, and administered intragastrically.

EHTP and Silymarin were administered 7 days prior to pathology modelling and for 2 weeks during the experimental model induction. For histological analysis, rats were euthanized on the second day after the last paracetamol and ethanol administration.

Streptozotocin (STZ)-induced diabetes was modelled via a single intraperitoneal injection of STZ (Sigma, USA) at 55 mg/kg in 5% citrate buffer, pH 4.5, after 14 days of feeding on a high-calorie diet (protein – 20.0%, fat – 60.0%, carbohydrates – 20.0% of total caloric intake) [18]. After STZ injection and until sacrifice, the animals continued receiving the high-calorie diet for 14 more days. DM development was assessed 2 weeks after administration of the diabetogenic agent (STZ).

Groups (n = 8 each):

Group 1: Intact control (received solvents);

Group 2: Pathology control (high-calorie diet for 28 days + STZ on day 14);

Groups 3 and 4: on top of the modelled pathology, animals received EHTP or Silymarin at 25 mg/kg in a therapeutic-preventive regimen – for 2 weeks before and 2 weeks after STZ injection.

The selection of doses for the investigated drugs was based on data from previous studies and the results of recalculating the human dose according to the species sensitivity coefficient.

Organ samples (liver in the first experiment and pancreas in the second) were fixed in 10% formalin, dehydrated in ascending grades of alcohol, embedded in paraffin. Sections 6–7 μm thick were obtained using a sliding microtome, mounted on glass slides, stained with hematoxylin and eosin. Additionally, liver samples fixed in formalin were frozen and sectioned to identify general lipids using Sudan IV staining. Slides were examined under a Granum light microscope and photographed with a Granum DCM 310 digital camera. Images were processed using ToupView software.

Research results and their discussion

According to the results of light microscopy, the liver of intact rats exhibited the typical structure characteristic of this animal species. Connective tissue septa between lobules were not prominent. The boundaries of the lobules were defined by triads – portal tracts (areas containing branches of the hepatic artery, portal vein, and bile duct). Hepatocyte cords (hepatic plates) within the lobules showed a clear radial orientation. Hepatocyte nuclei were normochromatic, centrally located, and contained one to two nucleoli. Kupffer cells showed no abnormalities (Fig. 1).

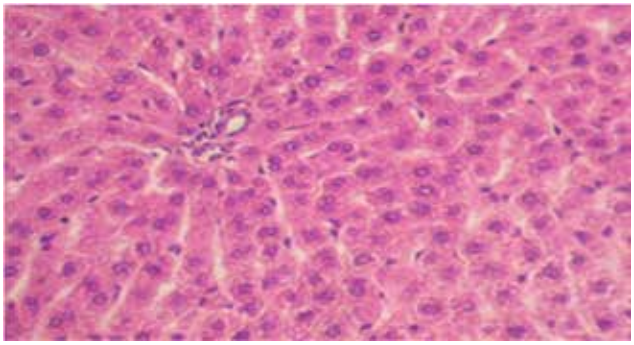
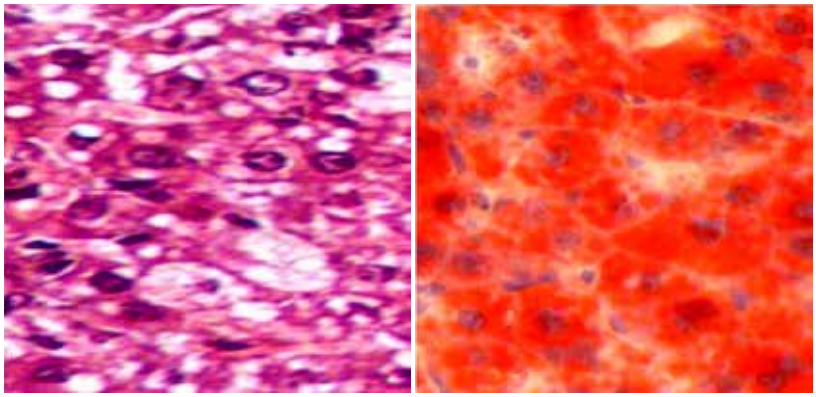


Fig. 1. Liver specimen of an intact rat. Normal condition of hepatocytes, triad area. Hematoxylin-eosin ×250

In the liver of rats from the control pathology group with ethanol-paracetamol-induced hepatitis, foci of vacuolar degeneration of hepatocytes were observed, along with necrotic changes in individual cells showing signs of karyopyknosis and plasmopyknosis. In areas of the most pronounced degeneration, disruption of the radial cord architecture was noted; hemocapillaries were compressed, and their lumens were not visible. A significant portion of hepatocytes were hypertrophied. The cytoplasm of the cells was filled with vacuoles of various sizes with irregular contours (hydropic degeneration), as well as medium- to small-sized, sharply demarcated, rounded inclusions (Fig. 2*A*), which showed a strong positive reaction for lipid (Fig. 2*B*).

The administration of EHTP at a dose of 25 mg/kg in a prophylactic and therapeutic regimen improved hepatic



A *B*

Fig. 2. Liver specimen of a rat with ethanol-paracetamol-induced hepatitis: A – a parenchymal area containing cells whose cytoplasm is filled with both irregularly contoured vacuoles and sharply demarcated rounded inclusions; disruption of the cord architecture is noted. Hematoxylin-eosin ×400; B – Medium- to small-droplet fatty degeneration of hepatocytes. Sudan IV–hematoxylin ×250

parenchymal morphology in rats with ethanol-paracetamol-induced hepatitis. In the majority of lobular zones, the typical architecture was preserved; hemocapillary disturbances were markedly reduced, and signs of inflammation were either absent or residual in nature (Fig. 3).

The organoprotective effect of EHTP on the liver was also confirmed by a marked reduction in fatty degeneration, which was of a small-focal nature, the absence of hepatocyte hypertrophy, and minimal pathological changes in liver cells (Fig. 4).

The comparator drug, Silymarin, under conditions of ethanol-paracetamol-induced hepatitis, was significantly inferior to EHTP. The same administration regimen and dosage of the reference drug Silymarin had virtually no effect on the condition of the hepatic parenchyma in rats compared to the control pathology group. This applies to the severity of fatty degeneration (Fig. 5*A*), and also to disturbances in local hemodynamics, inflammatory response, and manifestations of intrahepatic cholestasis (Fig. 5*B*).

Regarding the organoprotective effect on the pancreas, the results of the histological examination are presented in Fig. 6. In intact rats, the exocrine portion of the gland consisted of lobules separated by narrow connective tissue septa. The parenchyma of the lobules was composed of terminal secretory units (acini) with a very high packing density. The lumens of the acini were very small.

The islets were clearly demarcated from the surrounding exocrine parenchyma and consisted of clusters of light polygonal cells arranged in cords (see Fig. 6). This zonal distribution of alpha and beta cells is typical for this animal species and corresponds to the “mantle” type of islet structure.

Under conditions of streptozotocin-induced diabetes mellitus in rats of the control pathology group, significant changes were observed in the incretory apparatus. In some islets, a decreased number of beta cells was noted, and the islets themselves acquired ribbon-like and star-shaped forms. Numerous islets of different classes with more typical morphology showed signs of dystrophy (vacuolization) and necrobiotic changes in beta cells, resulting in visibly depleted areas in some cases (up to near-complete loss of cells). At the periphery of certain islets, focal proliferation of alpha cells and lymphohistiocytic infiltration was observed. Excretory ducts of all calibers were somewhat dilated, and clear proliferation of the ductal epithelium was seen (Fig. 7).

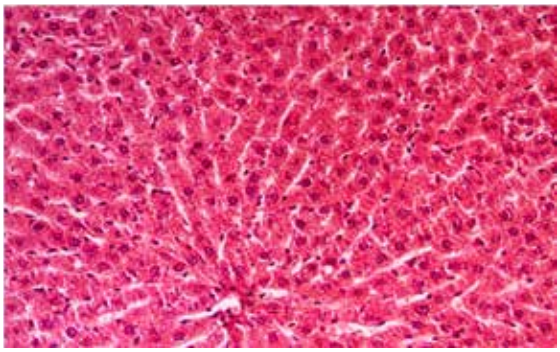


Fig. 3. Liver specimen of a rat that received EHTP (25 mg/kg) under conditions of ethanol-paracetamol-induced hepatitis. Normalization of the cord architecture, absence of inflammatory response. Hematoxylin-eosin ×200

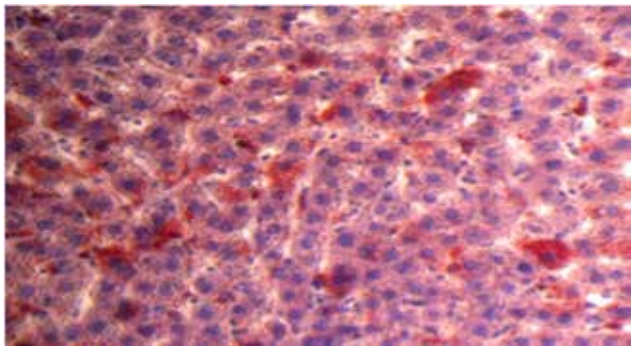
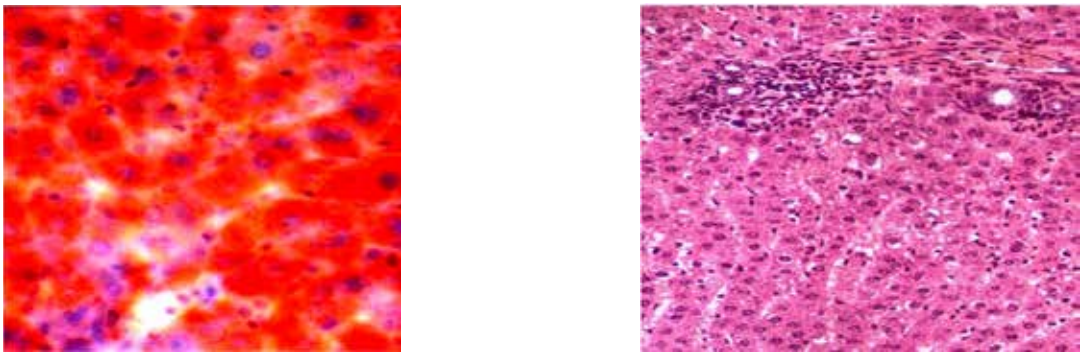


Fig. 4. Liver specimen of a rat that received EHTP (25 mg/kg) under conditions of ethanol-paracetamol-induced hepatitis. Marked reduction of hepatocyte fatty degeneration. Sudan IV–hematoxylin ×250



A *B*

Fig. 5. Liver specimen of a rat that received Silymarin (25 mg/kg) under conditions of ethanol-paracetamol-induced hepatitis: A – Pronounced fatty degeneration. Sudan IV–hematoxylin ×250; B – Uneven dilation of sinusoidal hemocapillaries, inflammatory response in the portal tract area, ductular proliferation. Hematoxylin-eosin ×200

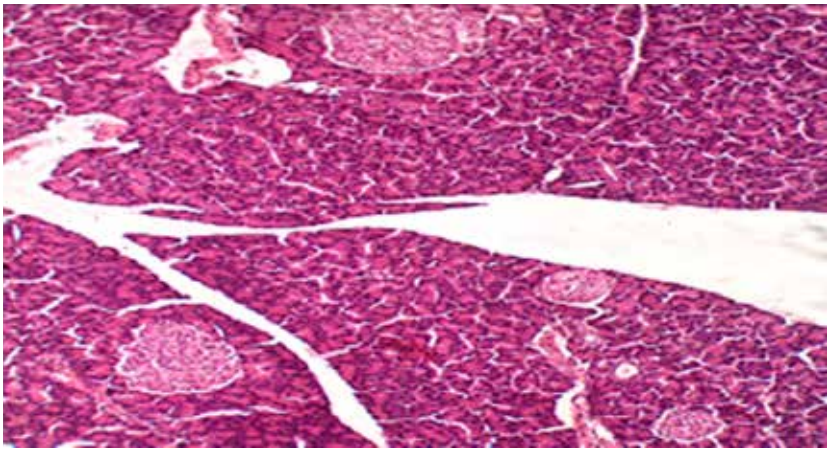
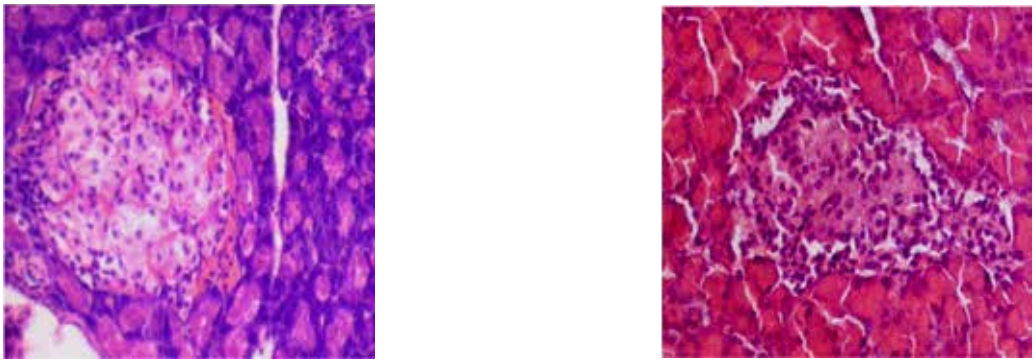
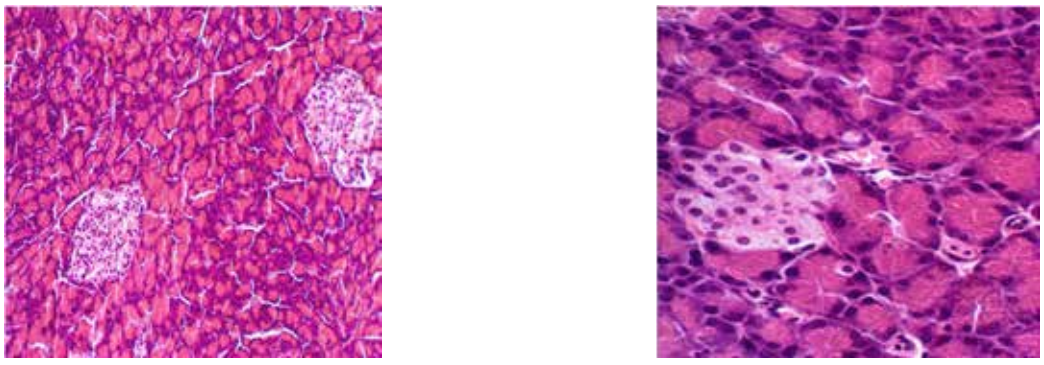


Fig. 6. Pancreas of an intact rat. Pancreatic islets of various classes; the condition of acinar pancreatocytes and insulin-producing cells is unaltered. Hematoxylin-eosin ×100



A *B*

Fig. 7. Pancreas of a rat with streptozotocin-induced diabetes mellitus:
***A* – Necrobiotic changes in a portion of beta cells with depletion of the pancreatic islet; *B* – Lymphohistiocytic peri-islet infiltration. Hematoxylin-eosin ×250**

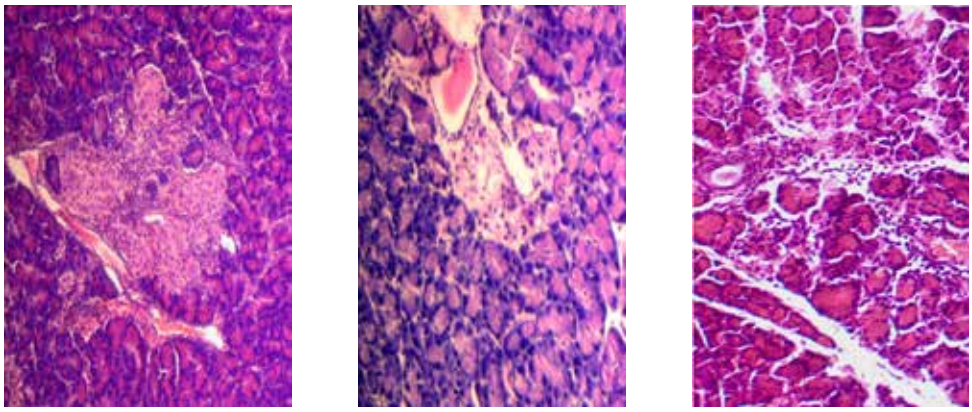


A *B*

Fig. 8. Pancreas of a rat with streptozotocin-induced diabetes mellitus + EHTP administration (25 mg/kg):
***A* – Increase in visually undamaged pancreatic islets (Hematoxylin-eosin ×100); *B* – Islet with dystrophic insulin-producing cells without proliferation of alpha and histiocytic cells at the periphery (Hematoxylin-eosin ×400)**

The organoprotective effect of EHTP under conditions of drug-induced damage to the pancreas is presented in Fig. 8. Administration of EHTP had a significant positive effect on the condition of the incretory apparatus compared to diabetic control rats, as more pancreatic islets were recorded in the specimens. Insulin-producing cells in many islets did not display dystrophic or destructive changes. The reduction in islets with depletion of central zones, the improvement in the uniform distribution of cells, and the proliferation of lymphohistiocytic and alpha cells at the periphery (Fig. 8) indicate the protective influence of EHTP on the pancreas. No pancreatic islets showed clearly distorted, atypical shapes or signs of insulitis (inflammation of islets).

The administration of the reference drug Silymarin at a dose of 25 mg/kg in streptozotocin-induced diabetes had a significantly lesser organoprotective effect on the state of the pancreatic islets. Islets with focal proliferation of alpha cells and lymphocytes in peripheral zones, along with changes in typical shape, were observed. Signs of insulitis and an inflammatory response in the interacinar stroma were also present (Fig. 9).



A *B* *C*

Fig. 9. Pancreas of a rat with streptozotocin-induced diabetes mellitus + Silymarin administration at a dose of 25 mg/kg: *A* – Change in the typical shape of the pancreatic islet; *B* – Vacuolization and destruction of beta cells; *C* – Proliferation of lymphocytic cells at the periphery of the islet, interacinar inflammatory response. Hematoxylin-eosin ×200 *(A, B)*; Hematoxylin-eosin ×100 *(C)*

Overall, the results indicate that the prophylactic-therapeutic administration of marigold extract has a noticeable protective effect under conditions of ethanol-paracetamol-induced liver damage, which manifests as a pronounced reduction in the degree of hydropic and fatty degeneration of hepatocytes, disturbances in internal hemodynamics, inflammatory reactions, and normalization of the structure and morphological integrity of liver cells.

In the context of drug-induced pancreatic damage, the marigold herb extract at the investigated dose prevents the development of structural changes in the islet apparatus of rats, enhancing the resistance of insulin-producing cells to the damaging effects of the diabetogenic agent, streptozotocin. Morphologically, this manifests as a reduction in destructively dystrophic changes and an increase in functionally intact insulin-producing cells.

The organoprotective effect of EHTP is likely due to the additive synergy of the main biologically active substances of the extract: hydroxycinnamic acids (5.07%), flavonoids (7.45%), and tannins (8.68%), which, according to many international studies, exhibit powerful antioxidant, cytoprotective, and consequently organoprotective effects [19; 20].

Conclusions

Under conditions of drug-induced liver damage (ethanol-paracetamol-induced hepatitis) and pancreatic damage (streptozotocin-induced diabetes), the administration of dry marigold herb extract in a therapeutic-prophylactic regimen at a dose of 25 mg/kg exerts a pronounced organoprotective effect, confirmed by the normalization of the morphological parameters of hepatocytes and pancreatocytes. The *Tagetes patula L* herb extract outperforms the reference drug Silymarin based on the investigated parameters.

Prospects for further research. The *Tagetes patula L* extract is a promising agent for further preclinical studies aimed at creating an effective phytomedicine for the prevention and treatment of liver and pancreatic injuries.

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