REPUBLIC OF AZERBAIJAN

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ABSTRACT

of the dissertation for the degree of
Doctor of Philosophy

INVESTIGATION OF THE CHEMICAL COMPOSITIONS OF CERTAIN TROPANE ALKALOID-CONTAINING SPECIES FROM THE FLORA OF AZERBAIJAN

Speciality: 3400.02 – Pharmaceutical Chemistry, Pharmacognosy

Field of science: Pharmacy

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GENERAL CHARACTERISTIC OF THE WORK

Relevance and development of the topic

Plants are widely used in the treatment of various diseases since ancient times. Despite the extensive use of numerous synthetic preparations in medicine, the medical significance of plants and plant-based preparations remains relevant. The detailed investigation of the chemical composition of plants, by using modern analytical methods, has opened new directions for their successful application in the treatment of various diseases. In this regard, the comprehensive investigation of the chemical composition of plants, which abundant in biologically active substances and naturally distributed in our republic is a current issue.

Plants belonging to the *Solanaceae* family, such as *Datura*, *Hyoscyamus* and *Atropa* are considered as source of tropane alkaloids and have been used in the folk and traditional medicine of various countries, primarily for the treatment of asthma, cough, spasms and pain, neurological disorders, nausea and vomiting [1,2]. Medically important alkaloids, such as atropine, scopolamine, and anisodamine are still obtained from plants. With the assistance of modern analytical methods, more than 300 tropane alkaloids have been identified in different plant species [3, 4].

Flavonoids, phenolic compounds, saponins, fatty acids, sterols, amino acids, vitamins and other biological active substances have also been identified in the *Datura, Hyoscyamus,* and *Atropa* species [3, 4].

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[1] Benouadah, Z. Evaluation of acute and sub-acute toxicity of alkaloids from *Datura stramonium* sp. in Mice, Z Benouadah, N Mahdeb, A Bouzidi, International Journal of Pharmacognosy and Phytochemical Research, – 2016, 8(11), p. 1759-1766

[2] Kartal, M. Quantitative analysis of l-hyoscyamine in *Hyoscyamus reticulatus* L. by GC-MS / M. Kartal, S. Kurucu, L. Altun [et al.] // Turkish Journal of Chemistry, – 2003, Vol. 27, p. 565-569.

[3] Kohnen-Johannsen, K. L. Tropane alkaloids: Chemistry, pharmacology, biosynthesis and production / K. L. Kohnen-Johannsen, O. Kayser // Molecules, – 2019, Vol. 796, p. 1-24.

[4] Valiyeva, A. Analysis of fatty acids of some *Hyoscyamus, Datura*, and *Atropa* species from Azerbaijan / A. Valiyeva, E. Garaev, A. Karamli [et al.] // Turkish Journal of Pharmaceutical Sciences, – 2022, 19(4), p. 442-446.

Semi-synthetic tropane derivatives (tiotropium-bromide, *N*-butylscopolamine, ipratropium-bromide, oxitropium-bromide, tropisetron, trospium-chloride, benztropine) have been synthesized with reduced toxic and additional effects while preserving their biological activity and videly used in medicine for the treatment of spasmolytic pain, asthma, cardiac arrhythmias, enuresis and Parkinson's disease [3].

Poisoning with tropane alkaloid-containing plants results in the inhibition of muscarinic receptors in the peripheral and central nervous systems, which produces anticholinergic syndrome. Dry skin, blurred vision, photophobia, fever, hallucinations, urinary retention, tachycardia are observed in poisoned patients. The determination of chemical compounds with toxic effects in plant material, and their identification using modern analytical methods, is also essential for establishing treatment directions during incidents of poisoning involving these plants [5, 6, 7].

It is noteworthy that, a few reference materials are found regarding the investigation of the chemical composition of *Datura, Atropa* and *Hyoscyamus* species distributed in the flora of Azerbaijan [8, 9]. Tropane alkaloid-containing plants, particularly species of *Datura, Atropa* and *Hyoscyamus* are widely used in traditional medicine for the treatment of various diseases, and their beneficial effects have been identified. *In vitro* and *in vivo* studies have been demonstrated

[5] Ally, F. An overview of tropane alkaloids from *Datura stramonium* L., F. Ally, V. Mohanlall, Journal of Pharmacognosy and Phytochemistry, – 2020; 9(3), p. 5-13.

[6] Benítez, G., The genus *Datura* L. (*Solanaceae*) in Mexico and Spain – Ethnobotanical perspective at the interface of medical and illicit uses / G. Benítez, M. March-Salas, A. Villa-Kamel // Journal of Ethnopharmacology, – 2018, Vol. 219, p. 133–151.

[7] Diaz, G. J. Toxicosis by plant alkaloids in humans and animals in Colombia // Toxins, – 2015, Vol. 7, p. 5408–5416

[8] Флора Азербайджана. [в 8-и томах]. – Баку, – 1958, том. 7, с. 402-414.

[9] Юсифова, Д.Ю. Новые источники получения цинарозида и кверцимеритрина флоры Азербайджана // Ümummilli lider Heydər Əliyevin anadan olmasının 92-ci il dönümünə həsr olunmuş "Təbabətin aktual problemləri – 2015" mövzusunda elmi-praktik konfransın materialları. – Bakı, – 2015. – s. 232.

theirantimicrobial, antioxidant, anticancer, gastroprotective, immuno-protective, neurotropic, anxiolytic and other effects.

Additionally, these plants are recognized as raw materials with hallucinogenic and toxic properties. Therefore, the investigation of their chemical composition is considered a significant issue in pharmaceutical science [10].

Object and subject of research. The study focuses on species from the flora of Azerbaijan, including *Datura innoxia* Mill., *Datura stramonium* L. and its variety *Datura stramonium* var. *tatula* L., *Hyoscyamus niger* L., *Hyoscyamus reticulatus* L. and *A. belladonna* subsp. *caucasica* (Kreyer) Avet[8]. Plant materials were collected from different regions of Republic, mainly at the end of the vegetation period: *Datura* species from the villages of Ramana and Pirshaghi in Baku, also arounds of Gabala and Zagatala districts between June and September (2018-2021), *A. caucasica* from the village of Yukhary Chardaklar in the Zagatala district in July 2019, *H. niger* from the areas of Lerik, Nakhchivan, Gusar and Gadabay districts between May and July (2019-2020), *H. reticulatus* from the areas of Lerik district during June and July 2019. The subject of presented study is the investigation of alkaloids, phenoloids, sterols, fatty acids, triterpenic acids, polyamines, amino acids, organic acids, and macro- and microelements in plants. Additionally, study involves the examination of the antimicrobial and antioxidant effects of plant extracts, the chronic toxicity of the alkaloid-rich extract of *Datura innoxia* seeds, and its impact on behavioural adaptation using *in vivo* methods. To research the poisoning cases involving *Datura, Atropa* and *Hyoscyamus* species from 2010 to 2021, archival materials from the Toxicology Department of the №1 CMC were used and 46 cases of *Datura* poisonings were recorded and retrospective study conducted with obtained data.

[10] Lunga, I. Chemical structure of hyoscyamosides *D* and *D1* - spirostanol glycosides from the seeds of *Hyoscyamus niger* L. / I. Lunga, P. Kintia, S. Shvets [et al.]. // Planta Medica, – 2008, 74(09), p. 1-4.

The aim and objectives of the study. The aim of the study is to investigate the chemical composition of *D. innoxia, D. stramonium, D. stramonium* var. *tatula, H. niger, H. reticulatus* and *A. caucasica* species from the flora of Azerbaijan, and to study the preliminary pharmacological activity and toxic effects of plant extracts. To achieve this aim, the following **objectives** have been defined:

- The obtaining of individual alkaloids from plant extracts and their identification through modern analytical methods (chromatography, UV-, NMR- and mass spectrometry). The quantification of perspective alkaloids (atropine and scopolamine), which are considered important for the production of medical preparations from plant material;
- The investigation of other biological active substances in the composition of plant materials includes fatty acids, sterols, flavonoids, phenolic compounds, essential oils, triterpenes, amino acids, polyamines, and the examination of macro- and microelements;
- Determination the antimicrobial and antioxidant activity of plant extracts, the investigation of the chronic toxicity, toxic effect on liver tissue, and impact on behavioural responses of the alkaloid-rich extract of *D. innoxia* seeds through *in vivo* experiments.

Research methods. The presence of alkaloids, flavonoids, and triterpenic acids in plant extracts was determined by using TLC, LC-MS and GC-MS methods, while qualitative and quantitative analysis of sterols and fatty acids was carried out using GC-FID method. The quantitative analysis of atropine, scopolamine was performed HPLC-UV method, while the total phenolics and flavonoids, as well as the DPPH activity, were determined with UV-VIS spectrophotometric methods, macro- and microelements in plant materials analyzed with ICP-MS method. The quantitative analysis of amino acids of *D. innoxia* were analyzed by amino acid analyzer. The chemical structures of isolated compounds from plant extracts were identified based on LC-MS and NMR spectroscopy data. The antimicrobial activity of plant extracts was tested using disc diffusion and agar well diffusion methods. The chronic toxicity of the alkaloid-rich extract of *D. innoxia* seeds was tested on white laboratory rats,

and its impact on behavioural responses was examined in white laboratory mice. Concurrently with the investigation into chronic toxicity, histopathological changes in the hepatic tissue of rats were assessed using both light and electron microscopy techniques. Moreover, samples of blood, urine, and feces were obtained, and the alkaloidal metabolites were identified through the application of the GC-MS method. The retrospective study of poisoning incidents associated with *Datura* species was undertaken using archival materials obtained from the Toxicology Department of the №1 CMC covering the years 2010-2021, the comparative analysis of different data as the clinical symptoms of poisoning, patients' blood analyses and changes in liver enzymes was conducted.

The main provisions submitted to the defence:

- The investigation of alkaloids and other biological active substances, such as tyramine derivatives, phenoloids, triterpenic acids, amino acids, polyamines, organic acids, fatty acids, sterols, essential oil components, as well as macro- and microelements of *D. innoxia, D. stramonium, D. stramonium* var. *tatula, H. niger, H. reticulatus* və *A. caucasica* species from the flora of Azerbaijan, is conducted using modern analytical methods;
- The determination of the quantity of atropine and scopolamine alkaloids in the aerial parts of plants results in the assessment of their potential application in the pharmaceutical industry as medicinal compounds;
- The development and optimization of an efficient extraction method for scopolamine alkaloid from the seeds of *D. innoxia*, a plant species belonging to the flora of Azerbaijan;
- The determination of the antimicrobial and antioxidant activity of plants;
- The investigation of the chronic toxicity of *D. innoxia* seeds, their induced pathological changes in liver tissue, and their effects on behavioural responses through *in vivo* studies.
- The retrospective study on the toxic effects of *Datura, Hyoscyamus,* and *Atropa* species on the human body is based on archival materials of poisoning cases from №1 CMC, covering the period from 2010 to 2021.

The scientific novelty of the Study. A chemical investigation of the species *D. innoxia, D. stramonium, D. stramonium* var. *tatula, H. niger, H. reticulatus* and *A. caucasica* from the flora of Azerbaijan has been carried out. The chemical structures of the alkaloids atropine, scopolamine, apoatropine, methylhyoscyamine and anisodamine, as well as tyramine derivatives, methyl tropate and ursolic acid, which were individually isolated from *D. innoxia, H. reticulatus* and *A. caucasica*, have been identified based on UV-, MS-, and NMR spectroscopy data. The quantities of the alkaloids atropine and scopolamine in the aerial parts of the plants have been determined. A more efficient method for the individual extraction of scopolamine alkaloid from the seeds of *D. innoxia* has been developed and prepared for the first time. Flavonoids and phenolic compounds, essential oils, polyamines, triterpenic acids, and organic acids have been detected in different extracts of plants. Sterols and fatty acids in the seed oils, macro- and microelements of plants, as well as the total quantities of phenols and flavonoids in the aerial parts of *Datura* and *Hyoscyamus* species, were determined. The antimicrobial and DPPH activities of plant extracts, as well as the toxic effects of the alkaloid-rich extract of *D. innoxia* seeds on white laboratory rats, also pathological changes in the liver tissues of animals, have been determined using light and electron microscopy methods. Poisoning cases with the species of *Datura, Hyoscyamus* and *Atropa* have been searched based on the archive materials of Toxicological Center № 1 CMC, and a retrospective study has been conducted specifically on 46 poisoning cases associated with *Datura* species identified between 2010 and 2021.

Theoretical and practical importance of the research. The findings of the research have been published in different scientific journals, presenting information on the composition of *Datura, Hyoscyamus* and *Atropa* species from the flora of Azerbaijan. Applied methods of investigation can be employed for extracting and determining alkaloids and other substances from plant extracts, and would be beneficial for practical courses, scientific research, and the extraction processes of alkaloids and other components from plants for industrial purposes. The extraction and identification methods

submitted and experimentally developed during the study would be applicable in the teaching process at the Departments of Pharmaceutical Toxicology and Chemistry, Pharmacognosy, and Pharmaceutical Chemistry.

Author's personal contribution. All results presented in the dissertation were obtained through the author's direct involvement. The formulation of the issues, the conduct of experiments and preliminary tests, as well as the analysis, systematization, and generalization of the obtained experimental and scientific findings, were conducted with the author's personal involvement.

Approbation and application. The findings of the research have been presented at academic conferences, and abstracts have been published: "Scientific conference dedicated to the "90th anniversary of corresponding member of ANAS, honored scientist, prof. Damir Vahid oghlu Hajiyev"(Baku, 2019); "Current Issues in Medicine, dedicated to the 100th anniversary of the establishment of the Faculty of Medicine at Baku State University", international scientific-practical conference (Baku, 2019); "The 1st International Congress on Mediterranean Multidisciplinary Studies" (Mersin, 2019); "XIII International Symposium on the Chemistry of Natural Compounds" (Shanghai, 2019); "13th World Drug Delivery Summit" scientific conference (Montreal, 2019); "4th International Anatolian Agriculture, Food, Environment and Biology Congress" (Afyonkarahisar, 2019); "Current Issues in Medicine dedicated to the 90th anniversary of the Azerbaijan Medical University" (Baku, 2020); "Planta+" (Kiev, 2020 and 2021); V International Scientific Congress the topic of "Modern Problems of Pharmacy" dedicated to the 90th anniversary of the Azerbaijan Medical University and the 80th Anniversary of higher Pharmaceutical Education in Azerbaijan (Baku, 2021); "10th International Conference on Scientific Advances and Challenges in Biology, scientific conference" (Baku, 2021); "14th International Symposium on the Chemistry of Natural Compounds*"* dedicated to the 30th anniversary of the Independence of the Republic of Uzbekistan (Taskent, 2021); *"*The 7th International Mediterranean Symposium on Medicinal and Aromatic Plants" (İzmir, 2021); "International Congress on Biological and Health Sciences"(Turkiye,

2021 və 2022); International scientific-practical conference on “Current Issues in Medicine dedicated to Shusha year" (Baku, 2022); “International Conference on Biomedical and Pharmaceutical Sciences” (Pakistan, 2022); “International Conference of Technovation on Production and Processing of Medicinal plant” (Isfahan, 2023); International scientific-practical congress on “Current Issues in Medicine - 2023 dedicated to the 100th anniversary of the birth of National Leader Heydar Aliyev” (Baku 2023).

Regarding the dissertation, 22 abstracts (15 international, 7 national) and 9 articles (6 in abstracting and indexing databases, 3 national, 3 international) have been published. Application Certificates for various parts of the research have been obtained from the Departments of "Pharmaceutical Toxicology and Chemistry" and "Pharmaceutical Chemistry".

Name of the organization where the dissertation work is carried out. The dissertation work has been carried out according to the plan of scientific research activities at the Department of Pharmaceutical Toxicology and Chemistry of Azerbaijan Medical University.

The volume and structure of the dissertation. The dissertation consists of a title page, table of contents, introduction, five chapters, summary, conclusion, practical recommendations, references, appendices and abbreviations. The I Chapter provides a literature review focusing on alkaloids, mainly tropane alkaloids, their extraction and identification methods, other biologically active compounds in plants, as well as the distribution of plants, and literature information regarding their medical application. In II Chapter, information regarding research objectives and research methods has been presented. In III Chapter, the investigation of plant alkaloids is presented, while in IV Chapter, the research on other biologically active compounds and the elemental composition of plants is provided. In V Chapter, findings are presented related to the antimicrobial and antioxidant activity of plants, the investigation of the toxic effects of *D. innoxia* seeds through *in vivo* experiments, and the poisoning cases involving of *Atropa*, *Hyoscyamus* and *Datura* species. The dissertation includes 66 Tables, 53 Figures and 5 graphs.

In the references, 254 sources are presented, of which 18 are in Azerbaijani, 2 in Turkish, 5 in Russian, 215 in English and 14 are from scientific websites.

The dissertation consists of 247 pages. Excluding the title page, figures, tables, graphs, appendices and references, the number of characters is 227821. Table of contents 4840, Introduction 13433, Chapter I 58468, Chapter II 13037, Chapter III 44031, Chapter IV 32410, Chapter V 42431, Summary 14506, Practical recommendations 548, Conclusions 2815, The list of abbreviations 1302 characters.

MATERIALS AND METHODS OF RESEARCH

Plant materials were collected from various regions of Azerbaijan between May and October. The identification of the specimens was carried out by Sh.N. Mirzayeva, a staff member of the Institute of Botany of the ANAS.

Various types of extracts were obtained from selected plants, and the biologically active compounds contained therein were determined using analytical methods and compounds considered prospective for research were individually isolated using chromatographic methods. The purity of the individual substances was examined using chromatographic and spectroscopic methods. TLC, HPLC-UV, HPLC-MS, GC-MS methods have been applied in the analysis of alkaloids present in plant extracts. Silica gel plates, mobile phases, chloroform-methanol (8.5:1.5), ethyl acetate-methanol (9:1) and Dragendorff's reagent were employed for the analytical TLC of alkaloids. The identification of the individually obtained substances were carried out with LC-MS ions and NMR data of the molecules. Flavonoids and phenolic compounds in plant extracts were determined using the LC-MS method, while the total content of phenols and flavonoids, as well as the determination of antioxidant activity using the DPPH reagent, were performed with the UV-spectrophotometric method. The analysis of fatty acids and sterols were carried out using the GC-FID method in comparison with standard samples. Essential oils were investigated using the GC-MS method, compared with the

NIST database of the instrument. The amino acids of the aerial parts of *D. innoxia* were determined using an amino acid analyzer equipped with an ion-exchange column and a UV-VIS detector. The qualitative and quantitative analysis of macro- and microelements in dried plant organs was conducted using the ICP-MS method, compared with standard samples. The antimicrobial activity of plant extracts was evaluated using disc diffusion and agar well diffusion methods on Nutrient, Meat-peptone, and Sabouraud-dextrose agar.

The toxic effects of the alkaloid-rich extract of *D. innoxia* seeds were studied on white laboratory rats over periods of 14 and 30 days, administered orally at a dose of 5 mg/kg. The changes in the activity of liver enzymes and some haematological parameters were measured and metabolites of alkaloid-rich extract occurred in the blood, feces and urine samples identified by using the GC-MS method. Araldite-Epon blocks were prepared from the liver tissues following established protocols for electron microscopy, semi-thin and ultrathin sections were obtained using a Leica EM UC7 ultramicrotome. The impact of the alkaloid-rich extract of *D. innoxia* seeds on the behaviour of mice was studied at doses of 5, 15, 30, and 50 mg/kg, by intraperitoneal administration.

In a retrospective study of 46 poisoning cases by *Datura* species, the most frequently observed clinical symptoms, changes in liver enzyme activity and blood test parameters, as well as the months, locations, age groups, genders, *etc*. parameters in which poisonings occurred were comparatively analyzed.

Reagents: Dragendorff's reagent, a 25% ethanolic solution of phosphotungstic acid, Ninhydrin reagent, a 20% solution of sulfuric acid, and a 1% solution of iodine in chloroform were used for investigations. The solvents used in the research – dimethyl sulfoxide, ethanol, xylene, petroleum ether, diethyl ether, acetone, toluene, glycerin, and ethyl acetate were manufactured by the Vekton company in Russia. Methanol, chloroform, acetonitrile, hexane, dichloromethane and methanol-d_4 (99.8% D atom) and chloroform-d_1 (99.95% D atom) for NMR studies were produced by the Merck company in Germany. The deionized water used in the HPLC analyses was obtained using a Merck Millipore Direct Q5 system (USA).

Standards: Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), gallic acid, quercetin, atropine sulfate, a mixture of methyl esters of 37 fatty acids, 5-α-cholestane, campesterol, stigmasterol, and β-sitosterol were produced by Sigma-Aldrich. The hyoscyamine and scopolamine $HBr \cdot 3H_2O$ standards used for the determination of *H. reticulatus* and *A. caucasica* alkaloids by the LC-MS method were obtained from Merck (Germany), while 6β-hydroxyhyoscyamine was acquired from Chengdu Cogon Bio-tech (China). The standard sample, containing of a mixture of 18 amino acids (AA-S18-5 ml), was manufactured by the Sigma company in Germany.

Apparatus: For the analysis of alkaloids, essential oils, and organic acids in the extracts obtained from the investigated *Datura, Hyoscyamus* and *Atropa* species, a Shimadzu GC-MS QP-2010 Ultra system was employed (Japan). The essential oil sample of *D. innoxia* leaf was analyzed using the GC-MS system by Agilent, model 5977A/7890B (USA), equipped with a HP-MS detector.The analysis of fatty acids and sterols in the plant oil of *D. innoxia, D. stramonium* var. *tatula, D. stramonium, H. niger, H. reticulatus* and *A. caucasica* seeds was carried out using an Agilent Technologies 7820A GC-FID system. The detection of alkaloids and polyamines in the extracts of *A. caucasica* and *H. reticulatus* was applied using a UHPLC apparatus equipped with Xevo-Q-TOF-MS/MS and DAD detectors (Waters Corporations, USA). For the analysis of phenoloids in *H. reticulatus* leaf and fruit extracts, a HPLC-UV-MS system (Agilent 1100 HPLC, USA) was used. The methanolic extracts of the aerial parts of *D. stramonium, D. stramonium* var. *tatula, D. innoxia,* and *H. niger* were analyzed using LC-MS apparatus (Agilent Infinity 1260, USA*).* A flash chromatograph (CombiFlash NextGen 300+, USA) and silica gel columns (Teledyne Isco, USA) were used to fractionate the alkaloid-rich extracts obtained from *A. caucasica* and *H. reticulatus.* A preparative HPLC-UV system (Hanbon Newstyle NP7000, China) and a semi-preparative HPLC-DAD system (Waters 2690, USA) were employed for the isolation of pure substances from *H. reticulatus* and *A. caucasica* extracts. The characterization of isolated compounds from *H. reticulatus* and *A. caucasica* was conducted using a *Varian DDR NMR* spectrometer

(600/150 MHz, USA). The chemical structure of scopolamine and ursolic acid extracted from *D. innoxia* seeds was identified based on spectra obtained using an NMR spectroscopy system from the Bruker Company (*600 MHz,* USA). The *Agilent Cary 100 UV-VIS Spectrophotometer* was used for the quantification of DPPH activity, as well as the total amount of phenols and flavonoids. The investigation of the amino acids of *D. innoxia* was performed using an amino acid analyzer (*Hitachi L-8800,* Japan) equipped with an ion-exchange column (*Hitachi, 2622SC*). The macro- and microelement compositions of the plants have been determined using the Agilent Technologies ICP-MS 7700e instrument (USA).

Other equipments and tools: Chromatographic camera, Soxhlet extractor, autoclave, water pump, porcelain crucible, pipette, filter paper, cotton, 0.45 μm membrane filters, FN 5 chromatography paper (Filtrak, Germany), Silufol UV-254 and Silica gel UV-254 plates (Merck, Germany) were used for differents analysis.

Microbial strains were obtained from the American Type Culture Collection (ATCC). Strains and growth mediums were provided by the Azerbaijan Food Safety Institute. Nutrient agar (NA) is manufactured by Sifin Diagnostics (Germany), while Brain Heart Infusion broth, meat-peptone broth, and Sabouraud agar are produced by Merck (Germany).

Toxicological investigation: The activity of ALT, ASP, AST in blood samples, uric acid and creatine levels in the urine samples were determined using a kinetic photometric colorimeter (Analytica, BioScreen MS-2000, USA). For the research, reagents were manufactured at the facilities of "Linear Chemicals S.L.U." in Spain and "PZ Cormay S.A." in Poland. The comprehensive analysis of blood was conducted using the Auto Hematology Analyzer Ratyo RT 7600 (China). The pH of the urine was determined using the "Universal Indicator" (Whatman, UK).

The microscopic investigation of liver toxicity was conducted using Primo Star light microscopy (Zeiss, Germany) and JEM-1400 electron microscopy (Joel, Japan). The quantification of atropine and scopolamine in plant extracts, as well as the investigation of the chronic toxicity of alkaloid-rich extract of *D. innoxia* seeds on rats and

its impact on behavioural reactions in mice, was evaluated for integrity using various statistical methods. The statistical analysis, including the application of variance and dispersion analysis methods, was performed using the *IBM Statistics SPSS-26* statistical package. For the description of quantitative indicators, the mean of variation ranks (M), the standard error of the mean indicator (±m), median (Me), and quartiles (Q_1, Q_3) have been calculated. For the comparison of ranks, the *t-Student-Bonferroni* test was used, depending on the number of compared groups, along with the non-parametric *U-Mann-Whitney* and *H-Kruskal-Wallis* measures. In order to evaluate the effect of the studied factors on the final outcome, a variance analysis (Anova test) was performed.

RESULTS OF THE RESEARCH AND DISCUSSION

The alkaloids and other compounds, antimicrobial and antioxidant activity of *D. innoxia, D. stramonium, D. stramonium* var. *tatula, H. niger, H. reticulatus* and *A. caucasica* species from the flora of Azerbaijan have been investigated in our study. Additionally, the chronic toxicity of alkaloid-rich extract of *D. innoxia* seeds and its impact on behavioural reactions have been studied. Tropane, nortropane and pyrrolidine derived alkaloids have been identified in the composition of the plants. Using the GC-MS method, 118 alkaloids have been identified in the samples, of which 75 are tropane, 5 are nortropane, and 38 are pyrrole and pyrrolidine derived alkaloids. During the investigation of the alkaloid compositions of *H. reticulatus* and *A. caucasica* plants using the LC-MS method, in *H. reticulatus*, 17 tropane, 1 nortropane, 1 pyrrolidine-derived alkaloids, while in *A. caucasica*, 15 tropane and 1 nortropane-derived alkaloids were determined based on characteristic MS fragments compared with reference materials.

Using the LC-MS method, based on characteristic MS fragments, 2 tropane and 1 nortropane alkaloid were identified in *D. innoxia* species, 6 tropane alkaloids in *D. stramonium*, 11 tropane alkaloids in *D. stramonium* var. *tatula*, and 3 tropane alkaloids in *H. niger* species.

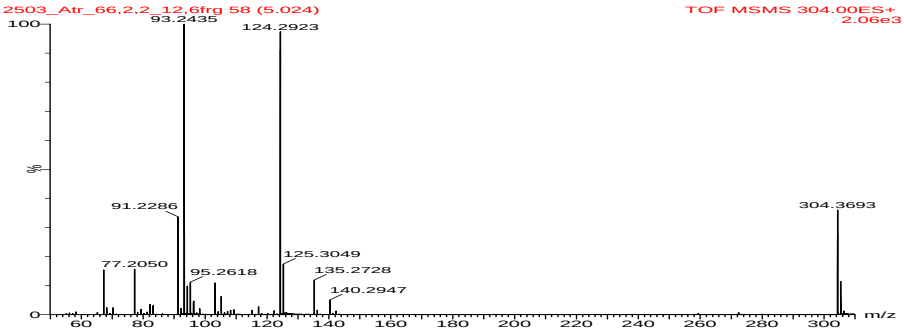

Figure 1. LC-MS fragments of the Methylhyoscyamine molecule identified in the *A. caucasica* extract.

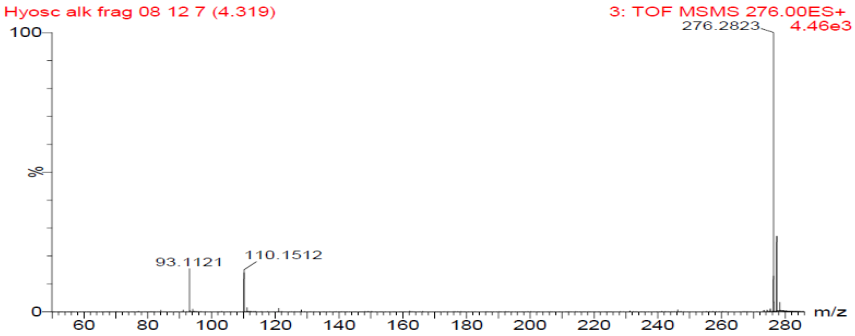

Figure 2. LC-MS Fragments of the Norhyoscyamine molecule identified in *H. reticulatus.*

Atropine, scopolamine, and anisodamine were identified in all samples. Individual samples of scopolamine, hyoscyamine, *L*-anisodamine, and apoatropine alkaloids obtained from *H. reticulatus* species, hyoscyamine, apoatropine, and methylhyoscyamine alkaloids obtained from *A. caucasica*, and scopolamine alkaloid obtained individually from *D. innoxia* were subjected to NMR spectroscopic analysis, and their chemical structures were determined based on their characteristic NMR data.

Hyoscyamine (*m/z* – 290/124; λ_{max}. 257 nm) was obtained from both *H. reticulatus* and *A. caucasica,* and MS, UV, ^{1}H, ^{13}C, 2D NMR spectroscopic data were used to identify its chemical structure.

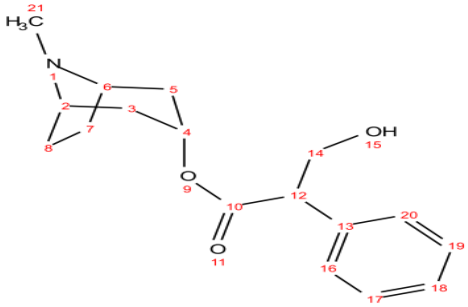

Figure 3. Structural elucidation of Hyoscyamine based on ^{1}H and ^{13}C NMR spectra.

The ^{1}H NMR spectrum of hyoscyamine showed aromatic resonances at δ 7.32 ppm (m, 2H, H-14, H-16), 7.23 ppm (m, 2H, H-13, H-17), 7.26 ppm (m, 1H, H-15), a methine resonance at δ 3.78 ppm (1H, H-10) and methylene resonance at δ 4.14 ppm (1H, H-11) and 3.80 ppm (1H, H-11'). ^{13}C NMR and the HMBC spectra supported the presence of a carbonyl group in C-9 position at δ 171.9 ppm, therefore this moiety was identified as tropic acid. Another spin system was identified as follows: four methylene signals δ 2.53 ppm (d, J = 15.6 Hz, 1H, H-4), 1.68 ppm (d, J = 15.6 Hz, 1H, H-4'), 2.62 ppm (d, J = 15.6 Hz, H-2), 1.88 ppm (d, J = 15.6 Hz, H-2'), 1.95-2.08 ppm (br m, 2H, H-7, H-7'), 1.79 ppm (m, 1H, H-6), 1.41 ppm (m, 1H, H-6') and three methine resonances at δ 3.45 ppm (br m, 1H, H-5), 3.58 ppm (br m, 1H, H-1), 5.06 ppm (t, J = 4.1 Hz, 1H, H-3) were assigned. The latter showed a three-bond correlation in the HMBC spectrum with the carbonyl carbon (δ 171.9 ppm C-9). In addition, an *N*-methyl group was also identified at δ 2.54 ppm (s, 3H, H-8).

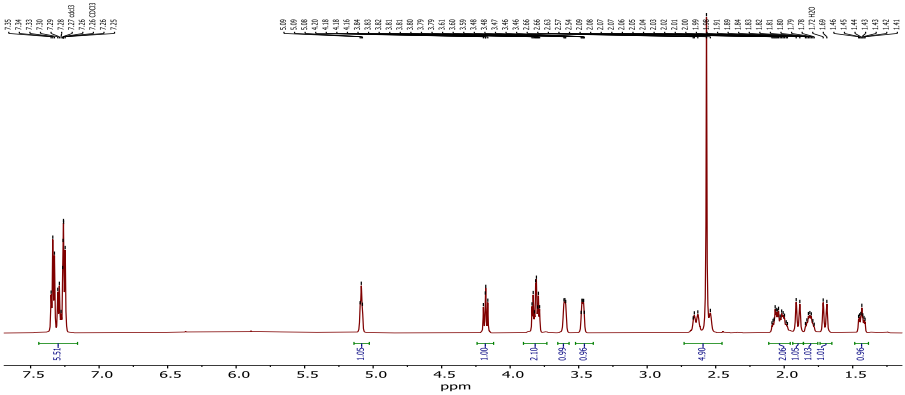

Figure 4. ^{1}H NMR spectrum of hyoscyamine

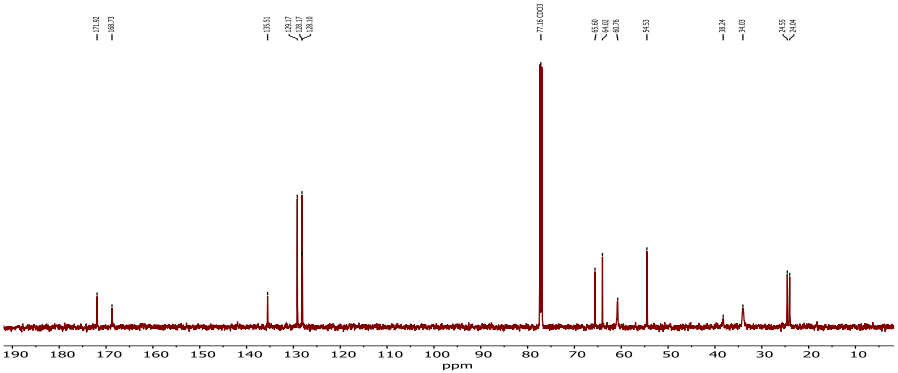

Figure 5. ^{13}C NMR spectrum of hyoscyamine

Scopolamine was determined based on UV, MS, NMR spectroscopy data (*m/z* – 304/138; λ_{max}. 257 nm). The ^{1}H NMR spectrum of scopolamine showed analogous resonances with hyoscyamine however instead of the two methylene units of the *N*-methylpyrrolidine ring two new methine resonances at δ 3.73 ppm (d, J = 3.3 Hz, 1H, H-7) and 3.18 ppm (d, J = 3.3 Hz, 1H, H-6) appeared. In the ^{13}C NMR spectrum these methylene resonances appeared at δ 54.8 ppm (C-7) and at 54.5 ppm (C-6) ppm indicating the presence of an epoxy group.

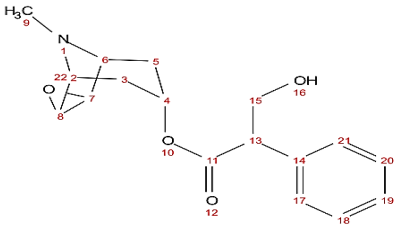

Figure 6. Chemical composition of scopolamine obtained *from H. reticulatus* based on ^{1}H and ^{13}C NMR spectra

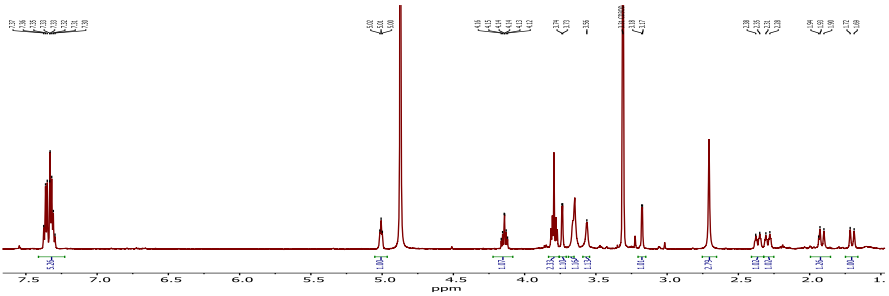

Figure 7. ^{1}H NMR spectrum of scopolamine from *H. reticulatus*

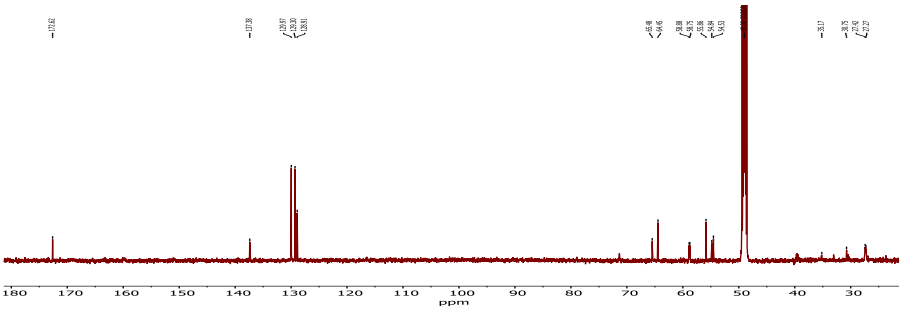

Figure 8. ^{13}C NMR spectrum of scopolamine from *H. reticulatus*

The ^{1}H NMR spectrum of anisodamine showed analogous resonances with hyoscyamine however instead of the methylene group in position 6 a methine resonance at δ 4.58 ppm (dd, J = 7.9 Hz, 2.9 Hz, 1H, H-6) appeared. In the ^{13}C NMR spectrum a new resonance

appeared at δ 71.6 ppm (C-6) indicating the presence of a hydroxy group connected to the tropane ring at position 6. In order to distinguish the two possible diastereomeric forms (3*S*,6*S*,2'*S* and the 3*R*,6*R*,2'*S* configurations i.e. the 6-OH vs. the 7-OH derivatives) the ^{1}H NMR chemical shifts of the free base form had to be recorded. The remarkable ^{1}H NMR chemical shift differences of the H-6 resonance of the diastereomers, we could unequivocally assign compound as (3S,6S,2'S)-6β-hydroxyhyoscyamine.

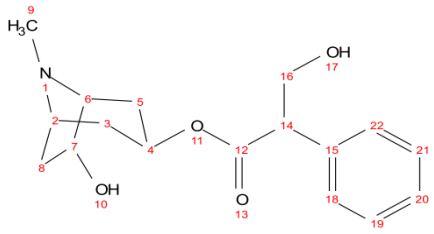

Figure 9. Structure of 6β-hydroxyhyoscyamine based on ^{1}H and ^{13}C NMR spectra

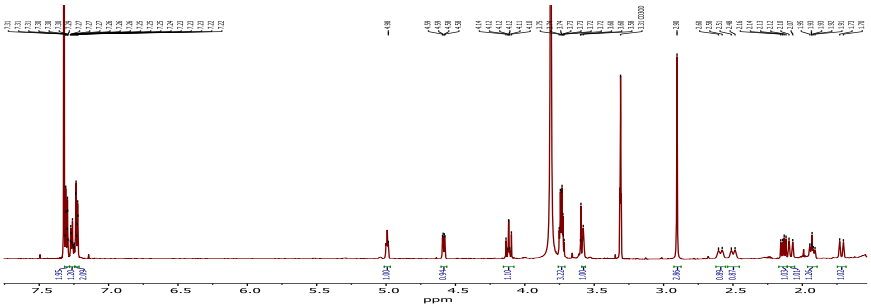

Figure 10. ^{1}H NMR spectrum of 6β-hydroxyhyoscyamine

In case of of *N-trans*-feruloyl tyramine ^{1}H NMR spectrum showed resonances at δ 7.05 ppm (d, J = 8.5 Hz, 2H, H-14, H-18), 6.71 ppm (d, J = 8.5 Hz, 2H, H-15, H-17) which indicated the presence of a para-substituted aromatic ring while the three aromatic resonances at δ 7.12 ppm (d, 4JH,H = 1.9 Hz, 1H, H-2), 7.02 ppm (dd, 3JH,H = 8.1 Hz, 4JH,H = 1.9 Hz, 1H, H-6) and 6.80 ppm (d, 3JH,H = 8.1 Hz,

1H, H-5) along with their coupling constants indicated a *1,2,4*-trisubstitued aromatic moiety. The resonance at δ 3.89 ppm (s, 3H, H-19) suggested a methoxy group at position 19. The broad resonance between δ 8.14-8.35 ppm (br, 1H, H-10) with no ^{13}C HSQC correlation confirmed the amide structure. The resonances at δ 3.47 ppm (*m*, 2H, H-11) and 2.76 ppm (m, 2H, H-12) revealed the presence of two methylene units. Furthermore, two olefinic ^{1}H resonances appeared at δ 7.43 ppm (d, J = 15.7 Hz, 1H, H-7) and 6.40 ppm (d, J = 15.7 Hz, 1H, H-8), their coupling constant suggested *trans* configuration of the double bond. The presence of two extra olefinic ^{1}H resonances with much smaller relative intensities at δ 6.61 ppm (d, J = 7.4 Hz, H-7) and 5.81 ppm (d, J = 7.4 Hz, H-8) suggested the presence of the *cis* isomer as well.

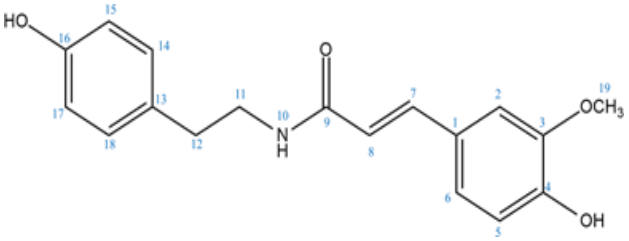

Figure 11. Structure of *N-trans*-feruloyltyramine based on ^{1}H and ^{13}C NMR data

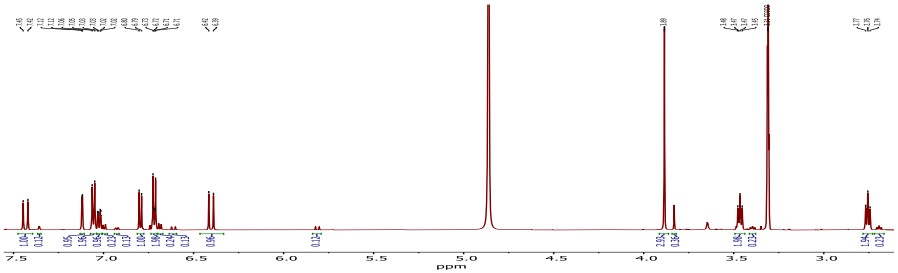

Figure 12. ^{1}H NMR spectrum of *N-trans*-feruloyltyramine

The alkaloids were extracted from the plant samples using the acid-base extraction method and used for quantitative determination by applying the HLPC-DAD method. Scopolamine was detected in

higher quantities in *D. innoxia* herb (62,31% in alkaloid-rich extract), while atropine was found in maximal amounts in *A. caucasica* (5,755 mg/g*)* and *D. stramonium* (4,423 mg/g). A statistical analysis of the obtained results was performed, and significant differences in the quantities of atropine and scopolamine across various samples were identified ($p<0.05$).

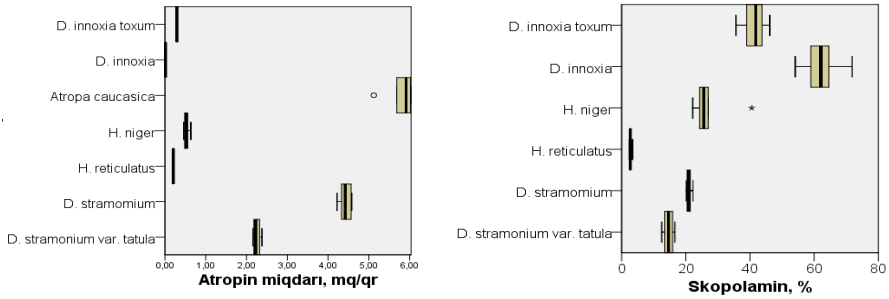

Figure 13. Comparative graphical describtion of some statistical parameters obtained based on the quantitative determination results of atropine and scopolamine

Alkaloids, as well as flavonoids, spermidines, and tyramine derivatives, were detected using the LC-MS method in methanolic extracts obtained from *D. stramonium*, *D. stramonium* var. *tatula,* and *H. niger.*

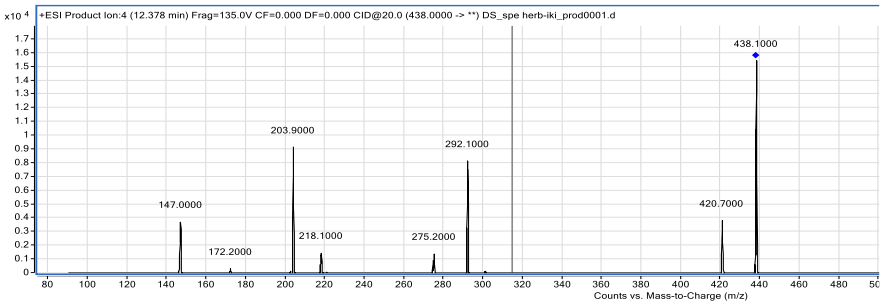

Figure 14. LC-MS fragments of the *N,N'*-Dicoumaroyl spermidine (*cis/cis*) molecule detected in *D. stramonium*

Ursolic and oleanolic acids individually extracted from *D. innoxia* seeds were identified using the TLC method, compared against standards. Compounds were derivatized on Silufol plates with iodine solution and analyzed using a mobile phase of hexane - ethyl acetate - methanol (82:18:5 v/v). A 20% sulfuric acid solution was used as the identification reagent. The isomers have been differentiated based on their R_f values (ursolic acid - R_f 0.45, oleanolic acid - R_f 0.58). The chemical structure of ursolic acid was identified based on ^{1}H and ^{13}C NMR spectroscopic data.

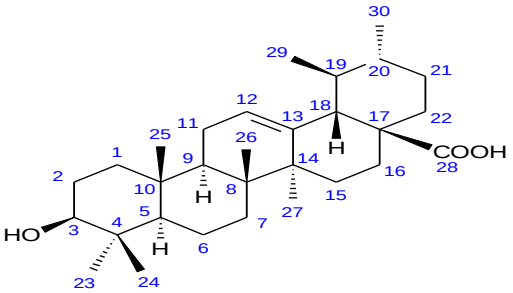

Figure 15. Structural elucidation of ursolic acid based on ^{1}H and ^{13}C NMR spectroscopic data

The quercetin and kaempferol glycosides, also quinic acid derivatives, in the fruit and leaves of *H. reticulatus* have been identified based on characteristic LC-MS fragments and UV spectra, through a comparative analysis with literature sources.

The total phenolic content in the extract of *H. niger* (0.0702 mg/g) and the total flavonoid content in *D. innoxia* (0.0107 mg/g) were higher compared to other samples.

The natural polyamines *N-cis*- and *N-trans*-feruloyl tyramine, grossamide and pellitorine in the *H. reticulatus*, as well as *N-cis*- and *N-trans*-feruloyl tyramine and methyl tropate in *A. caucasica*, were identified using the LC-MS method. Tyramine derivatives were individually isolated from the alkaloid-rich extract of *H. reticulatus*, while methyl tropate was isolated from *A. caucasica.* Their chemical structures were determined based on NMR spectroscopy data.

Higher quantities of the unsaturated fatty acids linoleic acid (55-79%) and oleic acid (11-26%) were identified in the plant seed oil samples. In the samples, the main constituents of saturated fatty acids were palmitic acid (4-12%) and stearic acid (2-3%).

β-sitosterol, campesterol and stigmasterol were identified in the investigated seed oil samples.

In the essential oil samples of *D. innoxia*, mono-, di-, and sesquiterpenes, phenolic compounds, ketones, aldehydes, alcohols, aromatic and aliphatic hydrocarbons, volatile esters of fatty acids, and sterols were identified.

In the leaves of *D. innoxia*, the quantities of 18 amino acids were determined comparatively with standard samples, revealing that 22.0% of the total amino acids were identified in the sample, with 7.94% being essential ones.

Compared to other macroelements, calcium (Ca) and potassium (K) have been identified in higher quantities in plant organs. It has been determined that the amounts of heavy metals in the samples, which could potentially have toxic effects, are within the limits established by WHO and US Pharmacopeia standards.

Ethanolic extracts from *D. innoxia* stems and leaves exhibited higher antimicrobial activity against *Bacillus anthracoides*, leaf extract against *Staphylococcus aureus*, and fruit and seed extracts against *Candida albicans.*

Ethanolic, ethyl acetate, and butanolic extracts prepared from the leaves, fruits, stems, seeds, and roots of *Hyoscyamus, Datura,* and *Atropa* species were tested for their antimicrobial activity against *S. aureus, Bacillus cereus, Salmonella enterica, Listeria monocytogenes* and *Escherichia coli* organisms. The extracts from *D. innoxia* leaves exhibited higher antimicrobial activity against *B. cereus* and *S. aureus.* The hexanoic phase of the ethanolic extract from *H. niger* fruits showed the highest activity against *L. monocytogenes* culture.

Using the DPPH reagent, the determination of antioxidant activity in *Datura* and *Hyoscyamus* species revealed higher activity in samples of *H. reticulatus* and *D. innoxia* compared to other samples.

Investigation of the chronic toxicity of the alkaloid-rich extract of *D. innoxia* demonstrated that there were slight changes in the

activity of liver enzymes (ALT, AST, ALP) following exposure to the extract, suggesting a weak hepatotoxic effect of the extract on the liver. In the experimental groups, there has been a decrease in the quantity of urea and creatinine in blood. Under the influence of the extract compared to the control group, an increase in urine excretion and a decrease in fecal mass were observed. Results show that, the alkaloidal extract indicates a certain degree of positive effect on renal function. Statistical analysis of the research revealed significant differences between groups in indicators such as ALT and ALP enzyme activity, as well as in the total amounts of lymphocytes, leukocytes and neutrophils ($p<0.05$).

In parallel with the determination of chronic toxicity of *D. innoxia* on white rats, the analysis of metabolites formed in the urine, feces, and blood plasma of animals has been conducted. Scopolamine residue, aposcopolamine, and 3-tigloyloxy-6-acetoxy tropane fragments have been identified in the samples as metabolites.

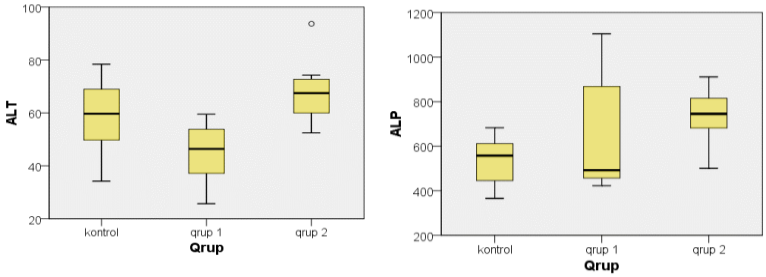

Figure 16. Graphical representation of statistically significant differences in ALT and ALP enzyme activity between groups based on certain indicators

Result of microscopic investigation. The results revealed increased vascular permeability due to damage to the endothelial cells of central veins and sinusoids. Edema formation was observed in the periendothelial and perivascular spaces. Stagnation in the sinusoidal lumen and the presence of bridge-like connections among the majority of sinusoids were identified. Necrosis was observed in the perivascular

spaces of veins. The membranes of hepatocytes, which constitute the parenchyma of the liver, were damaged, and cytoplasmic organelles migrated to the intercellular and Disse spaces. Glycogen in the cytoplasm of hepatocytes has transformed into an amorphous form, with certain nuclei of hepatocytes experiencing dystrophy, the tight junctions of the bile canaliculus have been disrupted, and sometimes has not been visible. These pathological changes indicate that the utilization of the alkaloid-rich extract of *D. innoxia* seeds, at a dose of 5 mg/kg during 30 days results in toxic effects on the animals.

The impact of the alkaloid-rich extract of *D. innoxia* seeds on behaviours and adaptation reactions was studied on white laboratory rats. The extract was dissolved in isotonic solution and administered intraperitoneally at doses of 5, 15, 30, and 50 mg/kg. The study was conducted under conditions of "Open field" and "Light/dark box". At doses of 30 and 50 mg/kg, a decrease in locomotor activity and food-seeking adaptation was observed. As the dose increased, the duration of staying in the light area decreased in the "Light/dark box" model of animals, however, this effect was observed to be opposite at the dose of 15 mg/kg. The dose of 15 mg/kg can be considered as an activating dose corresponding to the observed effect. Statistical analysis was applied on the results obtained in the study of behavioural changes, revealing significant differences depending on the administered dose in terms of mice's locomotor activity and behaviour under the "Light/dark box" model conditions ($p<0.05$).

Between 2010 and 2021, cases of poisoning involving 46 instances of *Datura*, 3 instances of *Atropa*, and 1 instance of *Hyoscyamus* plants have been encountered in the Toxicology Department of № 1 CMC. Because of the high incidence of poisonings involving *Datura* species, we conducted a retrospective study focusing on the comparative analysis of patients' clinical symptoms, blood analyses, liver enzyme activities, recovery periods, and other relevant indicators. The most frequently observed pathological changes in patients, locations with higher incidence of poisonings, age groups more commonly affected by poisonings, and similar factors have been identified.

CONCLUSIONS

1. Atropine, scopolamine, 6*β*-hydroxyhyoscyamine, apoatropine, *N-cis*- and *N-trans* feruloyl tyramine from *H. reticulatus*, atropine, apoatropine, methylhyoscyamine, and methyl tropate from *A. caucasica*, scopolamine and ursolic acid from *D. innoxia* were individually isolated and identified based on UV, MS, and NMR spectroscopic data. 118 alkaloids have been identified using the GC-MS method, comprising 75 tropanes, 5 nortropanes, and 38 pyrrole and pyrrolidine derivatives in the different extracts of plants [13; 24; 28; 29].
2. An efficient method has been developed and optimized for the extraction of scopolamine from the seeds of *D. innoxia*, a species from the flora of Azerbaijan.
3. The quantification of atropine and scopolamine in plant extracts was performed using the HPLC-UV method. A higher amount of scopolamine (62.31% of total alkaloid content) was found in the aerial parts of *D. innoxia*, while the maximum quantity of atropine was detected in samples of *A. caucasica* (5.755 mg/g) and *D. stramonium* (4.423 mg/g) [24; 29].
4. In *A. caucasica*, tyramine derivatives and organic acids; in *H. reticulatus*, phenolics, tyramine derivatives, polyamines and organic acids; in *H. niger*, phenolics and tyramine derivatives; in *D. innoxia*, triterpenoids, essential oils, amino acids, and organic acids; in *D. stramonium*, tyramine derivatives, organic acids, phenolics, and polyamines; in *D. stramonium* var. *tatula*, phenolics and polyamines were determined. In the seed oils of *Datura* and *Hyoscyamus* species, as well as in *A. caucasica*, higher concentrations of unsaturated fatty acids – linoleic acid (55-79%) and oleic acid (11-26%) have been detected. Campesterol, stigmasterol, and *β*-sitosterol were identified in all samples. In the aerial parts of *Datura* and *Hyoscyamus* species, the total phenolics and the total flavonoids were determined using spectrophotometric methods. Phenolics were found in higher quantities in *H. niger* (0.0702 mg/g), while flavonoids were identified in higher amounts in *D. innoxia* (0.0107 mg/g). The elemental composition of the plants has been investigated, revealing a

predominance of calcium (Ca) and potassium (K) in the samples. The levels of toxic microelements were within the limits established by the US Pharmacopeia and WHO [1; 10; 12; 16; 21; 23; 24; 29].

5. The antimicrobial activity of plant extracts was tested on Gram-negative and Gram-positive bacteria, as well as on *C. albicans*. The extracts of *D. innoxia* exhibited higher antimicrobial activity against *S. aureus* and *B. cereus*, while the hexane extract of *H. niger* demonstrated higher antimicrobial activity against *L. monocytogenes*. The antioxidant activity of the ethanolic extracts of aerial parts of *Datura* and *Hyoscyamus* species was determined using the DPPH reagent. The maximum DPPH activity was observed in the extract of *H. reticulatus* (0.0042 mg/g). The chronic toxicity of the alkaloid mixture from *D. innoxia* seeds was evaluated in white laboratory rats, revealing a moderate toxic effect on the liver and cells of the immune system. As a result of microscopic examination, pathological changes were identified in the liver due to toxicity. Metabolites, including scopolamine residue, aposcopolamine, and 3-tigloyloxy-6-acetoxy tropane, were identified in the blood, urine, and feces samples of the rats. The effects of the alkaloid mixture on the behavioural responses of white laboratory mice were investigated, and dose-dependent changes in their behaviour were observed [12; 14; 15; 19; 26; 27; 29; 30; 31].

PRACTICAL RECOMMENDATIONS

1. The efficient extraction method for scopolamine from *D. innoxia* seeds could be applied in future research and it can be applied to obtain the pure substance for industrial production.
2. Based on the obtained results, *A. caucasica* and *D. stramonium* may be considered more prospective as a natural sources for atropine, while *D. innoxia* may be regarded as more beneficial as a natural source for scopolamine.
3. The compositions of plant oils identified in the seeds of the studied species (17-35%), along with their predominant compounds of unsaturated fatty acids (83-91%), can be considered useful for the preparation of various formulations in the pharmaceutical, cosmetic and food industries.

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7. Garayev E. A, Valiyeva A. K, Thin layer chromatography of alkaloids from *Hyoscyamus niger* leaves, XIII International Symposium on the Chemistry of Natural Compounds, October 16-19, 2019, Shanghai, China, p. 97.

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LIST OF ABBREVIATIONS AND SYMBOLS

ALP	– Alkaline Phosphatase
ALT	– Alanine aminotransferase
ANAS	– Azerbaijan National Academy of Sciences
AST	– Aspartate Aminotransferase
br.	– broadening
CMC	– Clinical Medical Center
CNS	– Central Nervous System
d.	– doublet
DAD	– Diode Array Detector
DPPH	– 2,2-diphenyl-1-picrylhydrazyl
FID	– Flame Ionization Detector
GC-MS	– Gas chromatography/mass spectrometry
ICP-MS	– Inductively coupled plasma mass spectrometry
LC-MS	– Liquid chromatography-mass Spectrometry
m.	– multiplet
m/z	– fragment ions
max.	– maximum
MHz.	– Mega Hertz
min.	– minimum
NMR	– Nuclear Magnetic Resonance
ppm	– parts per million
s.	– singlet
t.	– triplet
UHPLC/Q-TOF-MS	– Ultra-high-performance liquid chromatography-quadrupole time of-flight mass spectrometry system
UV	– Ultraviolet
WHO	– World Health Organization

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