

Study of antimutagenic activity of medicinal plant infusions *Crataegus sanguinea* Pall. family Rosaceae in plant test systems

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Abstract. Medicinal plants, integral to traditional medicine systems, are rich sources of biologically active substances that benefit physiological and biochemical processes within living organisms. Amongst these activities, antimutagenic and genoprotective properties stand out, offering mitigation against genotoxic effects induced by adverse environmental factors on genetic material. This study delves into the mutagenic and antimutagenic capacities of aqueous and alcoholic infusions derived from *Crataegus sanguinea* Pall. (*Rosaceae* family), utilising *Hordeum vulgare* L. as a plant-based test subject. The assessment employed the metaphase chromosome analysis technique. Results indicate that these infusions exhibit no mutagenic activity, with the level of chromosomal aberrations in barley seeds treated with these infusions not exceeding the natural mutation rate in a statistically significant manner. When infusions were combined with Methyl methanesulfonate (positive control) exposure – irrespective of exposure sequence – a statistically significant attenuation in MMS-induced mutagenesis was observed ($p < 0.01$). A 56-60% reduction quantified the antimutagenic efficacy of *C.sanguinea* infusions. This metric underscores the infusions' capability to inhibit MMS-induced mutagenesis by 50–60%, positioning these water and alcohol-based extracts of common hawthorn as viable candidates for safeguarding against chemically induced mutagenic factors.

1 Introduction

Numerous toxic, mutagenic factors are diverse in the current living environment, primarily caused by intensive human economic activity. Recent observations have highlighted a concerning trend: an elevation in the naturally optimal mutation rates across various organisms, including humans. This shift is paralleled by a marked increase in cancer incidences and hereditary diseases, presenting a significant challenge to public health [1-3]. In addition, there is an increase in genetic load in populations of almost all species of living organisms. Global environmental pollution is already leading to a sharp decline in population numbers and even the extinction of entire species. The most common sources of pollution

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are exhaust gases, industrial waste, household waste, agriculture (pesticides, fertilisers), mining and processing industries, space industry, etc. Pollutants are present everywhere and contaminate the environment [4, 5]. Most of them have toxic, embryotoxic, teratogenic, mutagenic, genotoxic and carcinogenic effects [6]. They can activate free radicals, inhibit repair processes, or interact directly with DNA molecules, causing errors in their replication or directly damaging structures (single- or double-strand breaks).

Given the gravity of these developments, the quest to shield human genetic material from adverse factors has become critically important [7]. While the ideal preventative strategy against chemical mutagenesis involves removing mutation-inducing agents from the environment, practical limitations exist. Various sectors necessitate direct contact with mutagens, thereby prioritising the development of pharmacological interventions to protect genetic structures and mitigate mutagenic risks. Within this framework, antimutagens are identified as crucial entities, with studies highlighting their occurrence in amino acids, vitamins, polyamines, and intrinsic antioxidants [7-10]. The potential of medicinal plants as a rich reservoir of biologically active substances (BASs) with antioxidant and antimutagenic qualities is increasingly recognised. These naturally derived compounds, characterised by their extended utility and minimal side effects, confer a broad spectrum of health benefits while posing reduced allergenic threats compared to synthetic alternatives [10-14]. Against this backdrop, the current study aims to investigate the antimutagenic capabilities of *Crataegus sanguinea* Pall., a member of the *Rosaceae* family and a staple in traditional medicine through cytogenetic analysis on a botanical test subject. This research endeavours to elucidate the potential of standard hawthorn infusions in combating genetic alterations, thereby contributing to the broader discourse on genetic protection and public health resilience.

2 Materials and Methods

Antimutagenic activity was studied in aqueous infusion and alcohol tincture of common hawthorn *Crataegus sanguinea* Pall. of the *Rosaceae* family. The hawthorn's fruits, leaves, and flowers contain many biologically active substances with therapeutic and preventive effects. A cytogenetic assay, the metaphase chromosome analysis method, was employed to investigate the mutagenic and antimutagenic properties of various substances. This study focused on *Hordeum vulgare* L. [15], a barley cultivar known as Baisheshek, grown in Kazakhstan.

The negative control in our study was represented by the natural mutation rate observed in barley seeds germinated in distilled water. Conversely, the positive control was established by induction of structural mutations by applying methyl methanesulfonate (MMS, C₂H₆O₃S) at 10 mg/L. [16].

The initial phase sought to determine the mutagenic effects of hawthorn infusions on barley seeds, treated separately with the infusions and the mutagen. These infusions were prepared by the instructions on their pharmaceutical packaging, encompassing concentrated water-based formulations and alcoholic variants procured from pharmacy outlets. A sequential treatment regimen was implemented to evaluate the hawthorn infusions' potential antimutagenic effects: seeds were treated first with the infusion and then with MMS, or vice versa. Following treatment, the seeds were germinated in a regulated environment, maintained at a temperature of 25±1°C. Seeds demonstrating a primary root length of 0.5 cm were then submerged in a 0.01% colchicine solution for four hours, facilitating the accumulation of metaphase. Cytogenetic slides were prepared from the apical meristem cells of the barley roots using a fuchsin-sulfurous acid staining technique [17]. These slides were subsequently frozen on an FS-06 cooling table, set to -85°C, to produce permanent preparations. The infusions' mutagenic or antimutagenic capacity was assessed via the

metaphase chromosome analysis technique [17], applying Olympus BX 43F optical microscope to examine over 400 metaphase stages for each experiment option.

The evaluation of hawthorn infusions' ability to reduce the occurrence of chromosomal abnormalities induced by MMS was measured using the reduction factor (RF). The degree of antimutagenic impact was classified as moderate when inhibition rates ranged from 25% to 40%, significant for rates surpassing 40%, and minimal for rates below 25%.

The collected data was analysed using StatPlus software (AnalystSoft Inc., USA). We calculated the mean values together with their corresponding standard errors for every experiment option. In order to evaluate the significance of differences among the mean values of various treatments, we employed the independent Student's t-test, with differences considered statistically significant at a confidence level of 95% ($p < 0.05$).

3 Results and Discussion

The initial phase of our investigation focused on the mutagenic potential of hawthorn infusions, while the subsequent phase examined their DNA-protective capabilities against MMS-induced mutations in barley. We present the outcomes of cytogenetic analyses on barley root meristematic tissue, which was exposed to both aqueous and alcoholic hawthorn infusions, separately or in combination with MMS (table).

Treatment with methyl methanesulfonate markedly elevated the frequency of chromosomal aberrations in barley's root apical meristem cells, surpassing the control's established baseline. Notably, there was a 4.1-fold increase in aberrant cells ($p < 0.01$), and the occurrence of chromosomal aberrations per 100 metaphases increased by 4.8 times ($p < 0.01$), accompanied by a significant increase in both chromosome and chromatid type rearrangements.

Table. The frequency and spectrum of structural chromosome aberrations within barley seeds subjected to isolated and concurrent treatments with MMS and *Crataegus sanguinea* Pall. infusions.

| Experiment variant | Total metaphases studied | Aberrant cell frequency (M ± m%) | Number of chromosomal aberrations per 100 metaphase cells | | |
|------------------------|--------------------------|----------------------------------|---|---------------------|-------------------|
| | | | total aberrations | of chromosomal type | of chromatid type |
| Distilled water | 418 | 1,44 ± 0,58 | 1,44 ± 0,58 | 0,48 ± 0,34 | 0,96 ± 0,48 |
| MMS | 403 | 5,96 ± 1,18* | 6,95 ± 1,27* | 1,74 ± 0,65 | 5,21 ± 1,11** |
| Aqueous infusion | 432 | 1,16 ± 0,51 | 1,16 ± 0,51 | 0,69 ± 0,40 | 0,46 ± 0,33 |
| Alcohol infusion | 425 | 1,65 ± 0,62 | 1,65 ± 0,62 | 0,71 ± 0,41 | 0,94 ± 0,47 |
| Aqueous infusion + MMS | 431 | 2,32 ± 0,73* | 2,55 ± 0,76* | 0,93 ± 0,46 | 1,62 ± 0,61** |
| MMS + aqueous infusion | 410 | 2,44 ± 0,76* | 2,68 ± 0,80* | 0,49 ± 0,34 | 2,20 ± 0,72* |
| Alcohol infusion + MMS | 430 | 2,56 ± 0,76* | 3,02 ± 0,85* | 0,70 ± 0,40 | 2,33 ± 0,73* |
| MMS + alcohol infusion | 427 | 2,58 ± 0,77* | 2,81 ± 0,80* | 0,70 ± 0,40 | 2,11 ± 0,70* |

Note: * - $p < 0,01$; ** - $p < 0,001$ in comparison to the negative control (distilled water);
 • - $p < 0,05$; •• - $p < 0,01$ in comparison to the positive control (methyl methanesulfonate)

Conversely, treatment with hawthorn infusions, whether aqueous or alcoholic, did not escalate the mutation process within the meristematic zone of the primary roots' apical

section; both the aberrant cells and the structural mutations per 100 metaphases mirrored spontaneous mutation levels. These findings underscore the absence of mutagenic activity in the deployed hawthorn infusions.

Medicinal plants are rich in various BASs that enhance living organisms' physiological and biochemical processes, featuring antimutagenic and DNA-protective characteristics. These properties help counteract the genotoxic impacts primarily arising from environmental hazards with mutagenic potential. Consequently, the subsequent phase of our research involved examining the modifying influence of the studied infusions on MMS-induced mutagenesis.

The analytical results underscore a statistically significant diminution in the MMS-induced aberrant cells and structural chromosome rearrangements per 100 metaphases under the combined influence of the mutagen and both infusion variants. The combined treatment of water infusion and MMS resulted in a significant decline in the number of aberrant cells by 2.6-fold ($p < 0.05$), along with a decrease in structural rearrangements per 100 metaphases by 2.7-fold ($p < 0.05$). Furthermore, there was a significant decrease in the frequency of MMS-induced chromatid-type structural mutations, declining by a factor of 3.2 ($p < 0.01$). In the treatment variant where MMS was combined with water infusion, there was a statistically significant reduction in the numbers of aberrant cells ($p < 0.05$), structural rearrangements per 100 metaphases ($p < 0.05$), and in the frequency of chromatid-type aberrations ($p < 0.05$).

Cytogenetic examination of seeds in both the "alcoholic infusion+MMS" and "MMS+alcoholic infusion" treatments also revealed a statistically significant decrease in the level of chromosome aberrations induced by MMS. Preliminary exposure to the alcoholic infusion led to a 2.3-fold reduction in both the number of aberrant cells and chromosomal aberrations per 100 observed metaphases ($p < 0.05$), accompanied by a significant decrease in chromatid-type structural rearrangements ($p < 0.05$).

The spectrum of chromosomal abnormalities varied among all experimental setups, including unique centric rings, terminal deletions, and dot fragments for chromosomal types, as well as terminal fragments and acentric single rings for the chromatid type. The absence of abnormal anaphases in the control group is noteworthy, underscoring the specificity of the induced aberrations.

Through the chromosomal aberration assay on barley, this research evidences the capability of hawthorn infusions to mitigate the mutagenic impact of MMS, which is indicative of their antimutagenic properties. Notably, the study did not reveal significant differences in the reduction of mutagenic effects attributed to the sequence of treatments or the type of infusions used. The reduction factor, calculated to assess the antimutagenic action of the infusions, ranged between 56-60% for both hawthorn infusion types, showcasing their efficacy in inhibiting MMS-induced mutagenesis in barley seeds by an average of 60%.

It is known that DNA damage in the form of gene and chromosomal mutations contributes to the malignant transformation of cells and the further development of cancerous tumours. In addition, various gene, structural and genomic mutations lead to hereditary pathologies in the form of hereditary diseases. The increase in such pathologies among the population is due to the widespread pollution of the environment by agents with potential mutagenic activity. Therefore, the problem of protecting the genome from exogenous and endogenous mutagens is becoming increasingly relevant. In modern scientific research, the concept of antimutagenesis – the reduction or neutralisation of spontaneous or induced mutagenesis – has attracted considerable attention. This burgeoning interest stems from the potential of antimutagenic agents to offer promising avenues in preventive medicine and environmental protection. Recent scholarly efforts have increasingly focused on identifying substances endowed with antimutagenic properties, marking this field as a pivotal area of research [1].

According to the World Health Organization, over half of the global population depends on medicinal plants for their healthcare needs, utilising traditional and non-traditional

medicinal practices [18]. Consequently, pursuing medicinal plant-based inhibitors of induced mutagenesis, especially those plants integral to traditional medicinal systems, presents a highly promising research trajectory. The antimutagenic characteristics of plant-derived preparations are attributed to their abundance of biologically active compounds, including polyphenols, phenolic compounds, vitamins, and amino acids, among others [10].

In our investigation, we employed the chromosomal aberration assay to evaluate the antimutagenic potential of the hawthorn plant, *Crataegus sanguinea* Pall., belonging to the *Rosaceae* family. Recognised for its therapeutic and preventative properties, hawthorn's fruits, leaves, and flowers are replete with many biologically active substances. The infusions demonstrated antimutagenic efficacy through a statistically significant reduction in the frequency of chromosome aberrations triggered by methyl methanesulfonate. Interestingly, the research found no notable differences in the ability of both aqueous and alcoholic hawthorn infusions to modulate the mutagenic effects of MMS.

A substantial body of research highlights the presence of natural antioxidants, mainly phenolic compounds, within many medicinal plants. These phytochemicals, mainly phenolic and polyphenolic substances, are actively involved in redox reactions, neutralising reactive oxygen species and potentially exerting antimutagenic and anticarcinogenic effects [19, 20].

4 Conclusion

In our research, we utilised the chromosomal aberration assay to assess the antimutagenic efficacy of the hawthorn plant, *Crataegus sanguinea* Pall., a member of the *Rosaceae* family. Hawthorn is celebrated for its medicinal and prophylactic virtues, with its fruits, leaves, and flowers enriched with many biologically active compounds. Our cytogenetic study revealed a statistically significant reduction in the incidence of chromosome aberrations caused by methyl methanesulfonate when combined with infusions of *C.sanguinea*. Importantly, our results showed no significant difference in the efficacy of aqueous versus alcoholic hawthorn infusions in mitigating the mutagenic effects of MMS. These findings contribute to a better understanding of the mechanisms underlying the antimutagenic properties of hawthorn and suggest its potential application in mitigating the genotoxic effects of environmental mutagens.

Moreover, the comparable efficacy of aqueous and alcoholic hawthorn infusions indicates the versatility of hawthorn preparations in exerting antimutagenic effects, offering potential options for its practical use in various settings. This suggests that hawthorn could be a valuable natural agent for reducing the harmful chromosomal aberrations caused by mutagens. Further research and clinical trials are warranted to explore the potential use of hawthorn as a preventative or therapeutic agent in the context of genetic damage and mutagenesis.

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