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PREDICTIVE EVALUATION OF THE TOXICITY AND ADVERSE EFFECTS OF PLANTANONES A-D

AVALIAÇÃO PREDITIVA DA TOXICIDADE E EFEITOS ADVERSOS DAS PLANTANONAS A-D

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Abstract

Chronic prostatitis, a common condition in urology, is responsible for inflammation of the prostate tissues and can be aggravated by factors such as oxidative stress and inflammatory responses. Conventional treatments, such as anti-inflammatories, hormone therapies, and surgery, have shown limited efficacy, so new treatment methods are needed. Components of the herb *Hosta plantaginea* may offer new therapeutic approaches due to their anti-inflammatory, antibacterial, and

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antioxidant properties. Studies on obtaining new pharmacological compounds derived from *H. plantaginea* have obtained plantanones A-D, which belong to the flavonoid class and have anti-inflammatory activities. In this sense, the study aims to predictively evaluate the toxicological safety of plantanones in rats, to understand their adverse effects as drugs. The analysis was based on the GUSAR© and ProTox© tools to assess toxicity (LD50) through the routes of administration (intraperitoneal, intravenous, oral, and subcutaneous) and the toxicological endpoints in rats. GraphPad Prism® software was also used for statistical validation using the one-way ANOVA and normality tests. The results indicate that plantanones are possibly dangerous if ingested, that they can generate toxic effects via the IP and IV routes, and that plantanones A-C cause immunotoxicity. Further studies evaluating the toxicity of plantanones in organs and *in vivo* and *in vitro* tests could be carried out to corroborate the results presented.
Keywords: *Hosta plantaginea*; Anti-inflammatories; Dosage; Rats.

Resumo

A prostatite crônica, uma condição comum na urologia, é responsável pela inflamação dos tecidos da próstata e pode ser agravada por fatores como o estresse oxidativo e respostas inflamatórias. Os tratamentos convencionais, como anti-inflamatórios, terapias hormonais e cirurgia, têm mostrado eficácia limitada, logo, novos métodos de tratamento são necessários. Componentes da erva *Hosta plantaginea* podem oferecer novas abordagens terapêuticas devido às suas propriedades anti-inflamatórias, antibacterianas e antioxidantes. Estudos sobre a obtenção de novos compostos farmacológicos derivados da *H. plantaginea* obtiveram as plantanonas A-D, que pertencem à classe dos flavonóides e apresentam atividades anti-inflamatórias. Nesse sentido, o estudo visa avaliar preditivamente a segurança toxicológica das plantanonas em ratos, para a compreensão dos seus efeitos adversos como medicamentos. A análise foi baseada nas ferramentas GUSAR© e ProTox©, para avaliar a toxicidade (LD50) através das vias de administração (intraperitonial, intravenosa, oral e subcutânea) e os pontos finais toxicológicos em ratos. Também, foi utilizado o software GraphPad Prism® para validação estatística por meio dos testes de one way anova e normalidade. Os resultados indicaram que as plantanonas são possivelmente perigosas por ingestão, podem gerar efeitos tóxicos pelas vias IP e IV, além das plantanonas A-C acarretarem imunotoxicidade. Dessa forma, podem ser realizados estudos complementares que avaliem a toxicidade das plantanonas em órgãos, e ensaios *in vivo* e *in vitro* para corroborar com os resultados apresentados.
Palavras-chave: *Hosta plantaginea*; Anti-inflamatórios; Dosagem; Ratos.

1. Introduction

Chronic prostatitis or chronic pelvic pain syndrome is a very common autoimmune disease in urology that affects patients by inflaming the tissues of the

prostate. The syndrome occurs due to the relationship between immune, psychological, neurological, and endocrine dysfunctions. Recent studies indicate that the main factors in the pathogenesis and worsening of prostatitis are inflammatory responses and oxidative stress (WANG *et al.*, 2023).

Treatment has consisted of the use of anti-inflammatory drugs, hormonal therapies, α-blockers, and surgery, however, these methods have proved to be ineffective, and new means are needed for the therapy of this disease. Considerably, components of the perennial herb *Hosta plantaginea* (Lam.) may contribute to the treatment of chronic prostatitis.

H. plantaginea, popularly known as Yu zan, belongs to the genus *Hosta* of the *Liliaceae* family and is widely used in folk medicine on the continents of America, Europe, and especially in Asia. Some countries, such as Mongolia, use the species in procedures to treat sore throats and dumbness, but also pulmonary and toxic heat. Because of the many structurally different and biologically important compounds that make up its structure, *H. plantaginea* has anti-inflammatory, antibacterial, and antioxidant properties, among others (YANG; HE, 2019).

Given this, studies have been carried out using the constituents extracted from *H. plantaginea* to create new compounds with pharmacological activity. These include plantanones A-D, which were obtained using chromatography techniques to separate and characterize the structures. Plantanones (Plt) belong to the flavonoid class and have anti-inflammatory (Plt A-D) and antioxidant (Plt D) biological activities (Plt D) (YANG *et al.*, 2024, 2021).

Thus, the study aims to predictively evaluate the toxicity and toxicological endpoints of plantanones A-D in rats, making it the precursor to analyzing the toxic effects of plantanones as anti-inflammatory drugs.

2. Literature review

2.1 Flavonoids

Flavonoids are molecules derived from secondary metabolites found in plants, with polyphenolic structures characterized by having a C6-C3-C6 skeleton, formed by phenolic rings (A and B) connected to an oxygenated ring with

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heteroatoms (C). These compounds can be divided based on their oxidation and substitutions in the C ring, among these classifications are flavonols, flavanols, flavones, flavanones, isoflavones, and anthocyanins (LI *et al.*, 2020).

The flavonoid group can be found in vegetables, fruits, and plants, as well as in drinks, due to their ability to modulate enzymatic activity, causing changes in the action of cellular systems and exerting beneficial effects on the body. In addition, other pros of these compounds are their anti-inflammatory, antiviral, antitumor, antioxidant biological activities, cardioprotective effects, and immunomodulatory activity (BADSHAH *et al.*, 2021; JUCÁ *et al.*, 2020).

3. Methodology

3.1 (GUSAR©) General Unrestricted Structure-Activity Relationships

The GUSAR© platform performs acute toxicity prediction analyses using median lethal doses (LD50, mg/kg) in rats. The predictions are based on the Organization for Economic Co-operation and Development (OECD) labeling of compounds and are provided for intraperitoneal (IP), intravenous (IV), oral, and subcutaneous (SC) routes of administration (MADDULURI; SAH, 2020).

The classification method is divided into classes that indicate the dangerousness of the substance when ingested. The classes are, class I (LD50 ≤ 5), deadly; class II (5 < LD50 ≤ 50), fatal; class III (50 < LD50 ≤ 300), toxic; class IV (300 < LD50 ≤ 2000), harmful; class V (2000 < LD50 ≤ 5000), possibly dangerous; class VI (LD50 > 5000), non-toxic (USHARANI; PANNEERSELVAM; FLORA, 2023).

3.2 ProTox©

The ProTox© online server predicts oral toxicity and adverse effects in rodents, using Python programming models and machine learning such as scikit-learn and RDKit (ARULANANDAM *et al.*, 2022).

The deleterious effects are classified into acute toxicity, organ toxicity, endpoints, toxicological pathways, molecular initiation events, metabolism, and toxicity targets. The probability index of these parameters is also added to the

prediction, ranging from 0 (least likely) to 1 (likely) (BANERJEE *et al.*, 2024).

3.3 Statistical assessment

GraphPad Prism® is a statistical analysis software that was used to apply the normality test (Shapiro-Wilk) to the LD50 values of the administration routes. To check that the results followed a Gaussian distribution, the P value had to be ≥ 0.05.

The one-way ANOVA analysis was then applied using the Turkey multiple comparisons test to identify significant differences (P value < 0.05) between the routes evaluated.

4. Results and Discussion

4.1 Toxicity by routes of administration

Mice have been the most widely used animals in biomedical research since the 1900s. The reason for this is their rapid reproduction, sociable behavior, omnivorousness, adaptability, small size, and, above all, their 97% genetic similarity to humans. These factors make these rodents fundamental in drug discovery and in assessing toxic effects on people (GOYAL; BANDARI, 2023).

Considering the importance of rats in the analysis of drug toxicity, Table 1 was drawn up with the LD50 values of plantanones A-D, administered by IP, IV, SC, and oral routes.

Table 1. Toxicity in rats by routes of administration

Compounds	LD_{50} (mg/kg)				
	IP	IV	Oral	Toxicity class	SC
Plt A	178.7	2635	4410	5	6970
Plt B	56.55	2197	3429	5	5903
Plt C	48.85	2034	4468	5	5467
Plt D	1045	1517	2036	5	3520

Source: Authors

Notes: DL50 - Lethal dose 50%; IP – Intraperitoneal; IV – Intravenous; SC – Subcutaneous.

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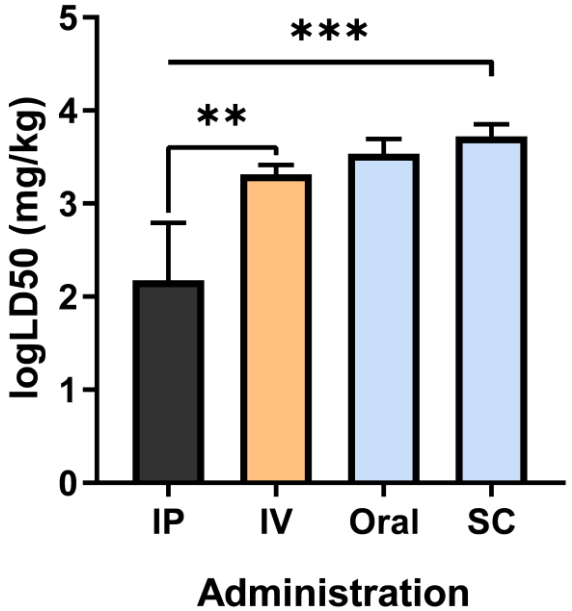
When viewing the doses in Table 1, it can be seen that the IP values are the lowest among the routes, with Plt C having the lowest LD50 (48.85 mg/kg) and Plt D the highest (1045 mg/kg) in the IP route. The low values may indicate possible toxic effects in rats since IP administration has rapid absorption and high bioavailability. In the IV route, the LD50 ranged from 1517 (Plt D) to 2635 mg/kg (Plt A). The drug enters this route via systemic circulation, where it is taken to the liver and metabolized there, generating possible toxic metabolites (KJÆRGAARD *et al.*, 2020). Continuing with the analysis, SC administration showed the highest doses, ranging from 3520 (Plt D) to 6970 mg/kg (Plt A). Finally, the oral route showed an LD50 oscillation between 2036 (Plt D) and 4468 mg/kg (Plt C), doses that fall into the toxic class of possibly dangerous compounds. In addition, plantanones may be more harmful to the health of the organism, given that they have undergone first-pass metabolism and may have their structures altered as they are degraded by the gastric fluid, affecting their concentrations and bioavailability (SETYAWATI *et al.*, 2023).

From the information presented, it can be seen that depending on the route, the toxicological response to the drug is altered. Therefore, a one-way ANOVA analysis was carried out to see if the routes can also interfere with the LD50 of plantanones.

Figure 1. Graph of the difference in LD50 between the routes of administration



Source: Authors

Note: * - Significant difference between routes.

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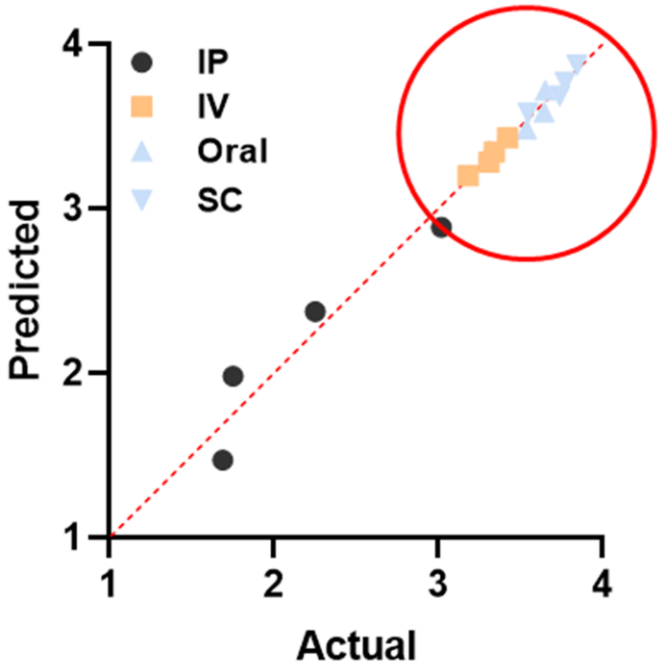
Figure 1 shows that the IP route has the lowest mean logLD50 and, when compared with the other routes (IV, oral, and SC), shows a significant difference. This contrast between IP and the other routes is evidenced by the P-value, which was < 0.0001. Therefore, it is understood that the LD50 can be influenced when plantanones are administered via the IP route, while the other routes do not show significant interference in the dose values.

To check whether the logLD50 results of the routes of administration follow a normal distribution, the QQ Plot graph in Figure 2 was drawn up, which, through the straight line, indicates the normality of the data.

Figure 2. Gaussian distribution test for logLD50



Source: Authors

The graph shows the residues of the routes close to the straight line, revealing that the dose values follow a Gaussian distribution. This distribution is also indicated by the P-value of the routes, which varied between 0.1846 and 0.8469. Another point to note is how the data is distributed along a straight line, with the IP residues at the bottom, while those of the other routes are grouped at the top. This is due to the significant difference between the logLD50 values, pointed out earlier in the one-way ANOVA analysis.

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4.2 Endpoints

The improper use of drugs can lead to adverse effects on the body when they are not administered following the prescriptions for the use of the drug in terms of route of administration, dosage, and treatment time (CRACOWSKI *et al.*, 2022). Given this, Table 2 was constructed to evaluate the toxicity endpoints (Carcinogenesis, Immunotoxicity, Mutagenicity, and Cytotoxicity) in rats.

Table 2. Endpoints generated by the inappropriate use of plantanones

Compouds	Carcino.	Immuno.	Mutagen.	Cyto.
Plt A	Inactive-0.9	Active-0.95	Inactive-0.69	Inactive-0.55
Plt B	Inactive-0.9	Active-0.99	Inactive-0.75	Inactive-0.64
Plt C	Inactive-0.9	Active-0.99	Inactive-0.75	Inactive-0.64
Plt D	Inactive-0.72	**Inactive-0.84**	Inactive-0.66	Inactive-0.97

Source: Authors

Notes: Carcino. - Carcinogenicity; Immuno. – Immunotoxicity; Mutagen. – Mutagenicity; Cyto. – Cytotoxicity.

Looking at the carcinogenicity in Table 2, it can be seen that the plantanones had a probability of 0.72 and 0.9 of being inactive. In immunotoxicity, only Plt D showed inactivity (0.84), while the other molecules proved to be active (0.95 and 0.99). Subsequently, all the compounds showed inactivity for mutagenicity, with scores ranging from 0.66 to 0.75. Finally, the cytotoxicity test indicated that all the drugs were inactive, with a possibility of 0.55 to 0.97.

5. Conclusion

The toxicological evaluation indicated that plantanones are drugs that can be dangerous to the body when ingested. In addition, administration by other routes, such as IP and IV, can generate toxic effects due to bioavailability and first-pass metabolism, generating more dangerous secondary metabolites. The study also showed that, depending on the dosage and treatment time, plantanones A-C generated immunotoxicity. Therefore, further in vivo and in vitro studies are needed to ratify the results, as well as to assess the organ toxicity of these drugs.

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