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Phytochemical screening and hepatoprotective potential of *Niphidium albopunctatissimum* Lellinger on alcoholic hepatoxicity induced in albino rats

Tamizaje fitoquímico y potencial hepatoprotector de *Niphidium albopunctatissimum* Lellinger en la hepatoxicidad alcohólica inducida en ratas albinas

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ABSTRACT

Objectives: To identify the phytoconstituents and determine the effect of *Niphidium albopunctatissimum* Lellinger on alcoholic hepatoxicity induced in albino rats.

Methods: Phytochemical screening was performed using the drop Test. For hepatoxicity study, albino rats were randomized into 5 groups of 6 each. Healthy group: without hepatoxicity; Control group: hepatotoxicity; Curative group: hepatotoxicity plus fluid extract of *N. albopunctatissimum* Lellinger; Standard group: hepatotoxicity plus silymarin; Preventive group: Fluid extract of *N. albopunctatissimum* Lellinger for one week, then hepatotoxicity. Hepatotoxicity was induced with 56% (w / v) ethanol at doses of 7.6 mL / kg p.c every 12 hours for 7 days. Glutamic pyruvic transaminase (GPT) values were determined at baseline, 7 days after induction and 14 days after. A histopathological study of the livers was performed.

Results: Phytochemical screening revealed the presence of polyphenols, anthocyanidins, saponins, flavonoids and tannins. The preventive and standard groups showed a very significant decrease in serum GPT levels compared to control group ($p < 0.01$), and curative group did so significantly ($p < 0.05$). Histopathological analysis showed in curative group some degenerating hepatocytes, many with normal-looking cytoplasm; the preventive and standard groups presented hepatocytes with normal architecture and some in degeneration, unlike the evident degeneration and necrosis in control group.

Conclusion: *Niphidium albopunctatissimum* Lellinger may have hepatoprotective potential against alcoholic toxicity induced in albino rats.

Keywords: Phytochemicals; Liver; Plant extracts; Ethanol.

RESUMEN

Objetivos: Identificar los fitoconstituyentes y determinar el efecto de *Niphidium albopunctatissimum* Lellinger en la hepatoxicidad alcohólica inducida en ratas albinas.

Métodos: El tamizaje fitoquímico se realizó mediante la Prueba de la gota. Para el efecto en la hepatoxicidad, las ratas albinas fueron distribuidas al azar en 5 grupos de 6 cada uno, Grupo sano: Sin hepatoxicidad, Grupo Control: hepatotoxicidad, Grupo Curativo: hepatotoxicidad más extracto fluído de *N. albopunctatissimum* Lellinger, Grupo Patrón: hepatotoxicidad más silimarina y Grupo Preventivo: extracto fluido de *N. albopunctatissimum* Lellinger por una semana, luego hepatotoxicidad. La hepatotoxicidad se indujo con etanol 56% (p/v) en dosis de 7,6 mL/kg p.c cada 12 horas por 7 días. Se determinaron valores de Glutámico pirúvica transaminasa (GPT) al inicio, a los 7 días de inducción y a los 14 días. Se realizó el estudio histopatológico a los hígados.

Resultados: El tamizaje fitoquímico reveló presencia de polifenoles, antocianidinas, saponinas, flavonoides y taninos. Los grupos preventivo y patrón evidenciaron disminución muy significativa de niveles séricos de GPT en comparación con el grupo control ($p < 0,01$), y el grupo curativo lo hizo de manera significativa ($p<0,05$). El análisis histopatológico evidenció en el grupo curativo algunos hepatocitos en degeneración, muchos con citoplasma de aspecto normal; los grupos preventivo y patrón presentaron

hepatocitos con arquitectura normal y algunos en degeneración, a diferencia de la evidente degeneración y necrosis en el grupo control.

Conclusión: *Niphidium albopunctatissimum* Lellinger puede tener potencial hepatoprotector frente a la toxicidad alcohólica inducida en ratas albinas.

Palabras clave: Fitoquímicos; Hígado; Extractos vegetales; Etanol.

INTRODUCTION

The liver is one of the most important organs for the metabolic activity that the organism develops, synthesizes fatty acids, stores glycogen, forms lipoproteins, synthesizes plasma proteins, forms urea, synthesizes coagulation factors, catabolizes and excretes hormones, detoxifies endogenous and exogenous substances, forms bile, maintains the hydroelectrolytic balance, etc.[1]. Hepatocytes constitute 80% of the liver's cell population, giving the liver great regenerative capacity, but when it is constantly attacked as in alcohol intake, viral infections, it progresses to irreversible liver damage[2].

Alcohol is a direct liver toxin, intervening facilitating factors and comorbidities such as gender, inherited factors and immunity[3]. In Europe, liver diseases are a serious health problem, mainly cirrhosis and liver cancer[4], in the United States, deaths due to liver disease and liver cancer have increased[5], in Peru, liver diseases were one of the first ten causes of mortality in 2017[6].

In recent years, plants such as *Silybum marianum* "milk thistle", *Fumaria officinalis* L. "fumaria", *Peumus boldus* M. "boldo", among other plants, are used to treat liver diseases[7]. Among these herbal formulations, silymarin obtained from *Silybum marianum* is highly effective for liver disorders, increasing the survival of patients with alcohol-induced cirrhosis[8].

Niphidium albopunctatissimum Lellinger (Polypodiaceae) "calaguala" is a fern found in the Andes Mountains, from Colombia to southern Bolivia, at altitudes between 500 and 2720 m[9]. Although, popular uses of *N. albopunctatissimum* report that rhizomes are used for its anti-inflammatory, astringent, depurative, hepatic and emmenagogue properties[10], studies evidencing the mentioned properties are not found in the scientific literature.

Liver diseases are reaching increased prevalence rates over the years and alcohol consumption is one of the most important risk factors for these pathologies. In addition, many pharmacological treatments have side effects related to liver problems, so the use of alternative medicine with *N. albopunctatissimum* Lellinger could contribute to the prevention or treatment of liver disease. The objective of this study was to identify phytoconstituents and evaluate the effect of *Niphidium albopunctatissimum* Lellinger on alcoholic hepatoxicity induced in *Rattus norvegicus* var. albinus.

MATERIALS AND METHODS

Plant material

4 kg of *Niphidium albopunctatissimum* Lellinger rhizomes, collected in Queropuspo sector, Cospán district, Cajamarca Province, Peru, at an altitude of 3000 m was used. A sample was identified at *Truxillense Herbarium* (HUT) of the National University of Trujillo (UNT) and deposited with the code 58842.

Animal material

Thirty male *Rattus norvegicus* var. albinus 3-month-old, between 170 and 200 g of body weight, acquired at the Bioterium of the Faculty of Pharmacy and Biochemistry of the UNT were used. They were maintained at a temperature of 18 ± 22 ° C, with light / dark cycles of 12/12 h, fed with standard diet and water *ad libitum*.

METHODS

Sample and fluid extract preparation

The rhizomes of *Niphidium albopunctatissimum* Lellinger were mechanically ground until obtaining small particles which were sieved through sieve No. 0.10 (2 mm diameter), stored tightly in amber and wide-mouth glass jars after. 100 g of drug were moistened with 70 ° GL water and ethanol for half an hour, placed in the percolator, macerated by adding water for 48 hours. 75 mL of fluid extract was collected, which was stored in amber bottle. The second successive portion of 25 mL was transferred to another bottle, extracted until the drug was used up and 230 mL was collected, which was mixed with the volume of the second extraction and concentrated to 25 mL[11]. The final amount of fluid extract was 100 mL.

Phytochemical screening

A volume of the fluid extract was separated to evaporate the solvent, the residue was partitioned equally into two porcelain capsules and dissolved with 5 mL of ethanol and water to be subsequently treated according to the phytochemical march of the Drop Test. Chemical identification, coloring and/or precipitation reactions were performed to determine the presence of secondary metabolites: steroids and triterpenes (Liebermann-Burchard's test), flavonoids (Shinoda's test), phenolic compounds (Ferric Chloride Test), saponins (Foam test), tannins (Gelatin test), alkaloids (Dragendorff, Hager, Mayer and Wagner´s tests), anthocy-

anidins (Rosenheim´s test), anthraquinones and naphthoquinones (Borntrager's test)[11].

Experimental design

Induction of alcoholic hepatoxicity and study groups

12 hour fasted rats were randomly selected to conform 5 groups of 6 animals each. Four were administered 56% ethanol (w/v) at doses of 7.6 mL/kg bw every 12 hours for 7 days[12], except normal group.

Normal group (healthy rats): 7.5 mL/kg bw of boiled water every 12 h for fourteen days. Control group: with hepatoxicity and 7.5 mL/kg bw of boiled water every 12 hours for another seven days, completing fourteen days. Curative group: hepatotoxicity and fluid extract of *N. albopunctatissimum* Lellinger (500 mg/kg bw) every 12 hours for another seven days. Standard group: hepatotoxicity and silymarin (100 mg/kg bw)[13] every 12 hours for seven days. Preventive group: fluid extract of *N. albopunctatissimum* L. (500 mg/kg bw) for 7 days, and after from day 8 to 14, first the fluid extract and thirty minutes later the alcohol for induction. In all cases, the administration was orally by gavage.

Transaminases determination

In all groups, baseline levels of fasting GPT transaminase were measured, then at 7 and 14 days of the study, blood was drawn from the tail vein of albino rat by puncture. The enzymatic method of Wiener Lab for transaminases was used[14].

Histopathological study

At the end of the 14 days, the animals were sacrificed and the livers were removed for the respective studies. A portion of the liver of each albino rat was fixed in 10% formalin, infiltrated in paraffin, 6-micron thick blocks were cut and stained with hematoxylin and eosin for subsequent microscopic reading.

Ethical aspects

All procedures were performed in accordance with the protocols approved by the Ethics Committee for animal research of the UNT, (Res. Cons. Univ. No. 0247-2016 / UNT) and the Guide for the care and use of animal laboratory[15].

Statistical analysis

Averages and standard deviation for serum GPT transaminase levels were calculated. The comparative study was performed using two-way analysis of variance (ANOVA) followed by Tukey HSD Test using the statistical package SSPS v.22. P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of the rhizomes from *N. albopunctatissimum* Lellinger reports the presence of leucoanthocyanidins, flavonoids, tannins, polyphenols, saponins (Table 1), showing a greater presence of polyphenols.

Table 1. Phytoconstituents of the rhizomes from *Niphidium albopunctatissimum* L.

Tests	Secondary metabolites	Extract Et[a]	Extract A[b]
Dragendorff	Alkaloids	-	-
Mayer	Alkaloids	-	-
Wagner	Alkaloids	-	-
Hager	Alkaloids	-	-
Börntrager	Quinones	-	-
Liebermann-Bouchard	Triterpenes/ steroids	-	-
Rosenheim	Leucoanthocyanidins	-	++
Shinoda	Flavonoids	-	++
Foam	Saponins	+	-
Ferric Chloride	Polyphenols	+++	+++
Gelatin	Tannins	-	++

[a]Et: ethanolic, [b]A: aqueous. Presence: (+), Absence: (-). Intensity: low (+), moderate (++), high (+++)

Figure 1 shows that on day 14 of the study, the serum GPT transaminase values were significantly decreased in the preventive group and the standard group, compared to the control group ($p < 0.01$). It is also evident that the curative group has lower serum values than the control group ($p < 0.05$). The GPT enzyme is a transaminase and is located primarily at the cytological level in the hepatocyte, giving it greater specificity, and when the liver suffers an injury, GPT is released from the liver cells, increasing its serum levels. It serves as a fairly specific indicator of liver status[16]. The decrease in serum GPT values found in our study is consistent with those reported in other investigations with hepatoprotective medicinal plants[17,18], especially the fact that pretreatment with the extract may be effective in reducing serum GPT before drinking alcohol[17].

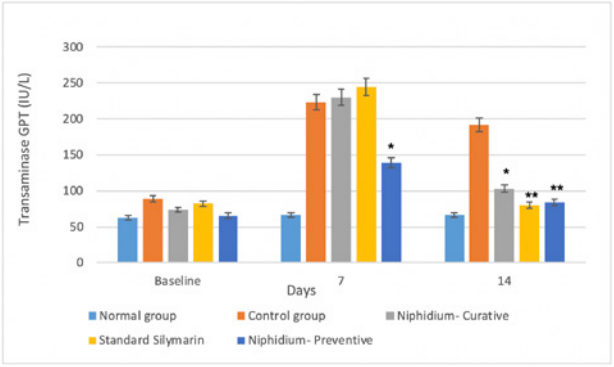

Figure 1. Averages of GPT (IU/L) transaminase concentrations at baseline, 7 days post hepatoxicity induction and 14 days post treatment in the study groups. The values in each group (n = 6) are expressed as the mean ± D.S.M. (Two-way ANOVA / Post Hoc HSD from Tukey); * p <0.05; ** p <0.01: expresses statistically significant difference.

The hepatotoxic action of ethanol in control group is evident, since the absorbed ethanol performs its biotransformation process mainly in the liver, at a rate of 10 mL hour[19]; its metabolism is mainly carried out by the enzymes alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and cytochrome P450 (CYP2E1)[20]. The first phase is performed by gastric ADH, then in the liver, ADH metabolizes 80% ethanol to acetaldehyde, which is a highly toxic metabolite. Simultaneously there is reduction of the nicotine-adenine-dinucleotide (NAD) cofactor to reduced nicotine-adenine-dinucleotide (NADH); Acetaldehyde is converted to acetate at the mitochondrial level by ALDH (3). The contrast between a healthy liver (Fig.2A) and another with alcoholic hepatoxicity (Fig.2B) is observed, with hepatocytes with cloudy cytoplasm and fat drops, several in degeneration and chromatin dissolution. ADH causes excess NADH, altering the redox balance, which favors the action of xanthine oxidase, which during the degradation of purines releases oxygen free radicals, the basis of ethanol-induced damage[3].

On the other hand, the hepatoprotective action of silymarin is shown in Fig. 1 and Fig. 3A, hepatocytes with normal contours, nucleus and cytoplasm are observed. Silymarin is a complex mixture of flavonolignans, which stabilizes cell membranes, stimulates detoxification pathways, stimulates of liver tissue regeneration[21], increases cell vitality, reduces lipid peroxidation and cell necrosis[22].

It is also observed (Fig. 3 B and C) that when *N. albopunctatissimum* is administered prior to alcohol induction, liver protection is greater than when administered as a curative treatment. The flavonoids, tannins, polyphenols and anthocyanidins that this plant contains could explain its hepatoprotective effect, since flavonoids inhibit enzymes that generate reactive oxygen species (ROS) such as microsomal monooxygenase, glutathione-S transferase, NADH transferase, etc.[23]; polyphenols decrease ROS levels by trapping and scavenging free electrons forming a phenoxy radical, less harmful to cells[24] and cyanidin 3- glycoside has been shown to be able to decrease the level of accumulated free radicals in the brain by effect of ethanol and decrease lipid peroxidation[25] protecting against alcoholic toxicity.

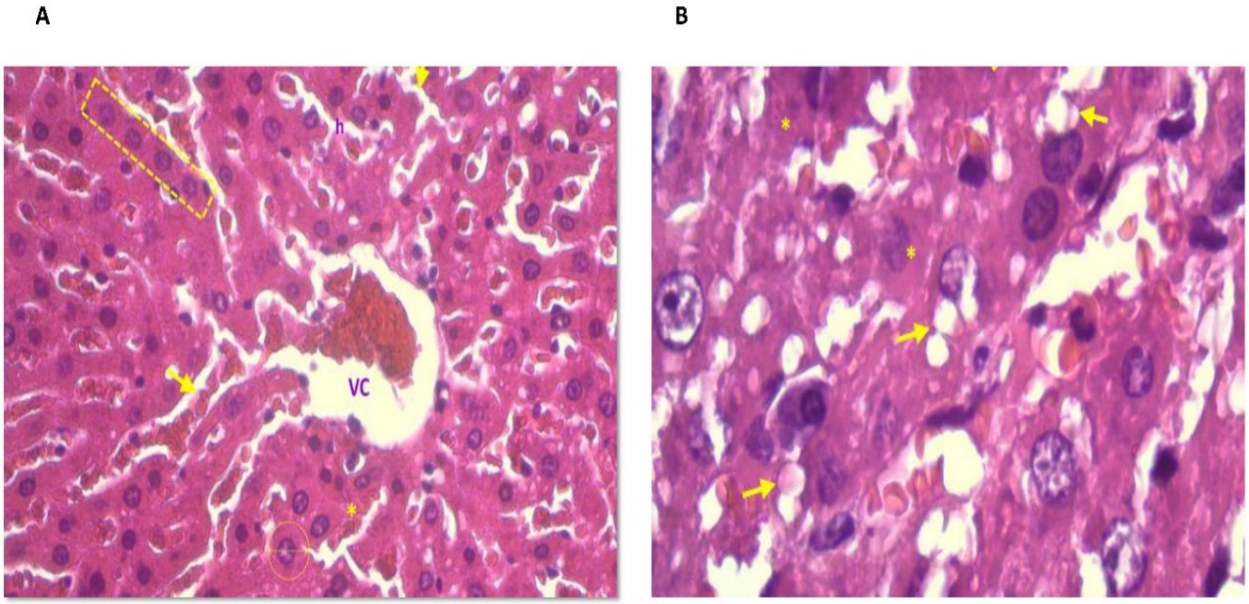

Figure 2. Rat liver. A) Normal group, healthy rats: radially to the central vein (cv) the hepatocyte (inset) and sinusoid plaques (arrows) flow. Hepatocyte nuclei and cytoplasm (circle) with normal staining and appearance. B) Control Group: hepatoxicity. Some hepatocytes with well-defined nucleus and nucleolus, others with cloudy cytoplasm and with fat drops (arrows), several in degeneration and dissolution of chromatin (*), necrosis. H&E.400x.

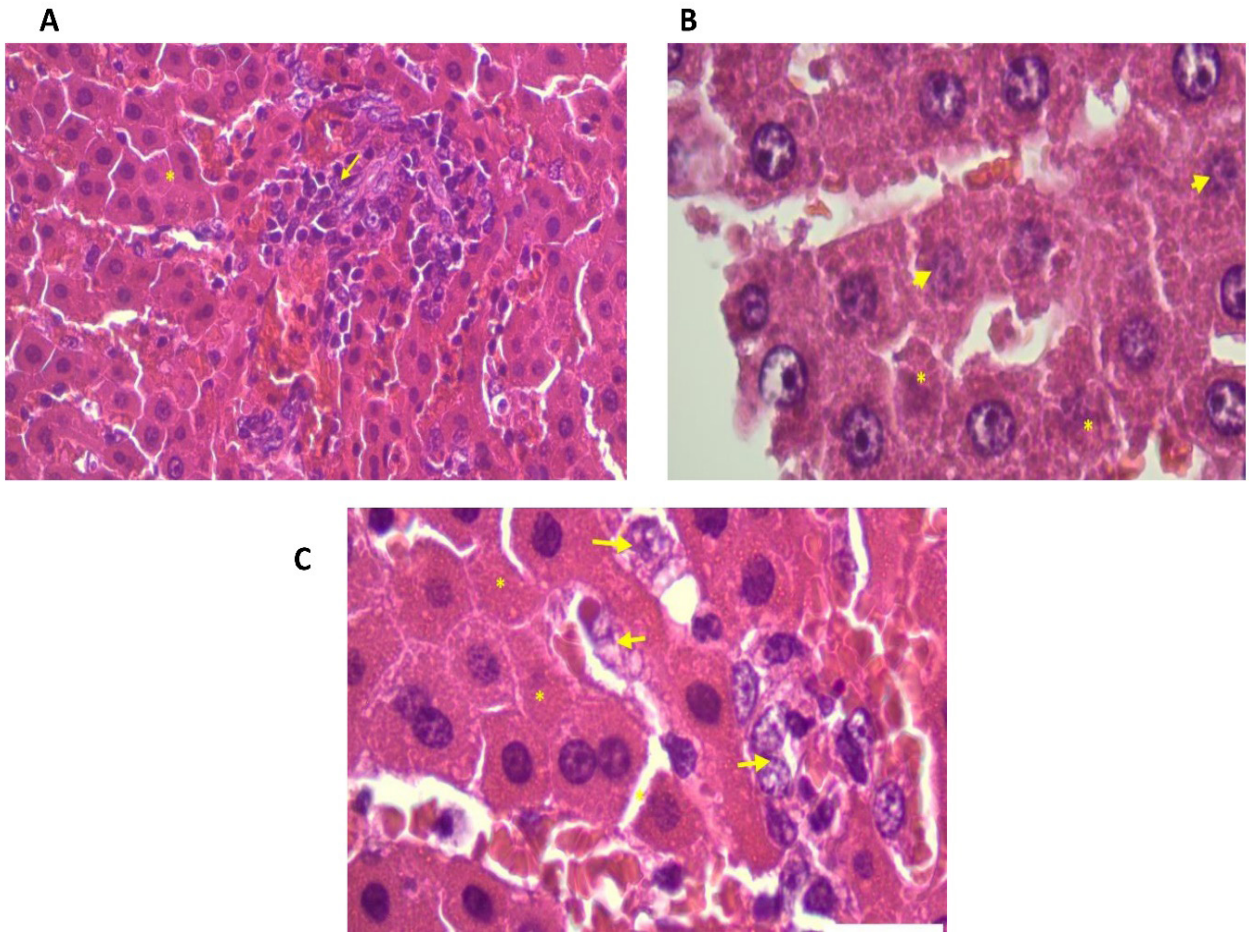

Figure 3. Rat liver. Treatments. A) Standard Group (silymarin). Hepatocytes with contours, nucleus and cytoplasm of normal appearance (*). Focus of inflammatory reaction (arrow). H&E 400x, **B)** Curative Group (*N. albopunctatissimum*). Some hepatocytes in degenerative process (arrow), many show normal-looking cytoplasm and others denote cell death (*). H&E 1000x, **C)** Preventive group (*N. albopunctatissimum*). Hepatocytes with nucleus and nucleolus with intense tintorial affinity, evidence of normality, few in degenerative process (arrow) and very few in degeneration (*). H&E 1000x.

CONCLUSION

The fluid extract of *Niphidium albopunctatissimum* Lellinger may have hepatoprotective potential by significantly lowering the plasma levels of GPT transaminase and preserving the hepatic architecture of albino rats against induced alcoholic toxicity. Further studies are planned to elucidate the specific chemical structures of polyphenols, tannins and flavonoids to identify the phytochemicals responsible for the hepatoprotective effect.

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