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Histopathological alterations in mice under sub-acute treatment with
Hintonia latiflora methanolic stem bark extract

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Short running title:

Histopathology in mice treated with Hintonia
Abstract

The indiscriminate use of herbal products is increasingly growing worldwide; nonetheless consumers are not warned about the potential health risks that these products may cause. *Hintonia latiflora (Hl)* is a tree native to the American continent belonging to the Rubiaceae family and its stem bark is empirically used mainly to treat diabetes and malaria; supplements containing *Hl* are sold in America and Europe without medical prescription, thus scientific information regarding its toxicity as a consequence of a regular consumption is needed. In the present study, the histopathological effect of 200 and 1000 mg/kg of *Hintonia latiflora* methanolic stem bark extract (*Hl*MeOHe) was evaluated in the small bowel, liver, pancreas, kidneys and brain of CD-1 male mice after oral sub-acute treatment for 28 days. No histopathological alterations were observed in the brain and small bowel of the treated animals; however, mice presented diarrhea from day 2 of treatment with both doses. No histological changes were observed in the tissues collected from the animals treated with 200 mg/kg, except for the liver that depicted periportal hepatitis.

Animals treated with the higher dose showed in the liver sections hydropic degeneration, hepatitis and necrosis, kidney sections depicted tubular necrosis and in pancreas sections, hydropic degeneration of the pancreatic islets was observed. In conclusion, *Hl*MeOHe damaged the liver with an oral dose of 200 mg/kg, and at 1000 mg/kg injured the kidneys and pancreas of the CD-1 male mice.

Key words: *Hintonia latiflora*, natural products, extracts, histopathology
Introduction

The use of herbal products has been increasing both in populations with a high socioeconomic level and in those living in areas of extreme poverty; the first ones, consume daily herbal supplements because of the common misconception that natural means harmless, while the second ones rely primarily on traditional medicines to solve their basic health problems because they cannot afford commercial drugs. The World Health Organization (WHO, 2011) estimates that 80% of the world's population depend on natural products to treat their diseases. The stem bark of *Hintonia latiflora* (Sessé & Moc. Ex. DC.) Bullock Rubiaceae commonly known as copalchi, is consumed based on traditional beliefs that it treats diabetes, malaria and gastrointestinal disorders. Currently several supplements containing micronized cortex of *Hl* can be purchased at electronic websites or drug stores without prescription. These supplements highlight its antioxidant and hypoglycemic properties because of a habitual consumption (Bruguera et al; 2007). The chemical composition focused on the antidiabetic effect of the stem bark of *Hl*, as well as its antimalarial efficacy, is being studied by several research groups (Argotte et al., 2006; Mata et al., 2013; Rivera et al., 2014). Although the WHO has encouraged studies with natural products as their use could confirm their effectiveness as drugs (WHO, 2011), the commercialization of any natural or synthetic drug requires a thorough investigation regarding its efficacy and toxicity before it can be released. Nowadays, it is known that some herbal medicines or herbal supplements could cause liver injury (Zheng and Navarro, 2016); nonetheless, there are very few reports regarding the damage to other tissues. Until now there is no available information concerning the histopathological effect of *Hl* stem bark extracts after regular consumption; therefore, in the present study the morphological changes in
different organs of CD-1 mice after a sub-acute (28 days) treatment with *Hl* methanolic stem bark extract were evaluated.

### Materials and methods

#### Animals

Forty CD-1 male mice weighing 28 g were used for the study and were obtained from the Faculty of Medicine of the National Autonomous University of Mexico vivarium. According to OECD guidelines, at least 10 animals should be used at each dose level, for a 28-day oral toxicity study in rodents (OECD, 2008). Mice were divided into 4 groups of ten mice each. Animal management was performed according to the Mexican Official Norm NOM-062-ZOO-1999 for the production, care, and use of laboratory animals in accordance with international guidelines and was approved by the Ethical and Research committee at the Faculty of Medicine, UNAM (project 095/2016).

#### Plant material

Stem bark of *H. latiflora* was collected, identified and prepared as previously described by Rivera et al (2014). A voucher sample (collect number 7772) of the plant was deposited at Dr. Salvador Nava y Esparza Herbarium (UAMIZ) collect number 83519 by Jhony Anacleto. The methanolic extraction was carried out as described by Rivera et al (2014). Methanol extract was used because it was reported previously that this extractant showed the higher biological efficacy and lower toxicity to the mice (Rivera et al., 2014).
Experiment

By oral gavage and for 28 days, 10 mice received daily 200 mg/kg of the extract, 10 more received 1000 mg/kg and 10 others received the vehicle (Tween 80%) at a concentration of 0.04%; ten mice remained as an untreated control group. Dose selection was made regarding the acute toxicity test reported by Rivera et al (2014), and by taking into consideration the doses recommended by the manufacturers of *H. I.* stem bark commercial products as CH-14 Copalchi (Bellsolá Laboratories), a herbal supplement containing *H. I.* micronized cortex in capsules of 400 mg each, recommended dose 4-6 capsules per day, and Sucontra® D capsules (Harras Pharma Curarina, Munich, Germany), a dry concentrate of *H. I.* stem bark extracted with ethanol in capsules of 100 mg each, recommendations, 2 capsules per day. Mice body weight was recorded daily, until the end of the experiment. At day 29, all animals were anesthetized with sodium pentobarbital (90 mg/kg by intraperitoneal route) and perfused via left ventricle with saline (0.9%) and then with 4% formaldehyde in 0.1 M phosphate buffer. Kidney, liver, small bowel, pancreas and brain were removed, weighed and placed in 10% formaldehyde for 24 hours, embedded in paraffin, cut into 5-mm sections, stained with hematoxylin and eosin and sudan III (oil red) for the pancreas sections and examined under light microscope. Histological examinations were performed via observations of not less than 10 fields per sample, images were obtained with Pro Image® software for Windows.

Statistical analysis

Obtained data were analyzed with one-way analysis of variance (ANOVA) and Tukey’s test using GraphPad Prism® software, version 7. All analyzed data had a normal distribution, and statistical significance was set at P < 0.05.
Results

No clinical signs or macroscopic lesions were observed in the animals from the untreated and tween 80 control groups. *H*/MeOHe treated animals showed hirsute hair and diarrhea from the second day of treatment, no statistically significant weight loss was observed. Four animals from the *H*/MeOHe 1000 mg/kg group presented abdominal distension from the 15th day of treatment and the body weight increased from then on; necropsy findings revealed hepatomegaly, with liver weight significantly greater than those from the untreated, tween 80 and 200 mg/kg groups (Fig. 1,2).

Please insert here figure 1 and 2.

Histopathology

No histological changes were observed in the organs obtained from the untreated and tween 80 treated mice (Fig. 3, 4, 5, 6 a,b). In the *H*/MeOHe 200 mg/kg treated group, only the liver showed microscopical alterations, as periportal hepatitis and multifocal eosinophilia (images not shown).

At 1000 mg/kg, brains from the treated mice appeared with normal histology in contrast with the duodenum small bowel sections that demonstrated dilated mucosal vessels suggesting lymphangiectasia (Fig. 6 c, d). Villi length of treated and non-treated groups were of 338.58 and 338.44 µm, respectively.

Liver sections from the animals treated with 1000 mg/kg showed diffuse inflammatory infiltrate (constituted by lymphocytes, plasma cells, neutrophils and few eosinophils) and hepatocyte necrosis (Fig. 7).

Please insert here figures 3-7.
Kidney histology of the *Hl*MeOHe 1000 mg/kg treated mice showed swollen glomeruli and a thickening of glomerular basement membrane in all the observed specimens. Congestion and tubular cell necrosis with loss of the epithelial cells was observed in some areas of the evaluated samples (Fig. 8).

Histology of the *Hl*MeOHe 1000 mg/kg treated mice pancreas showed multiple vacuoles compatible with hydropic degeneration in the pancreatic islets, (Fig. 9). Pancreas sections were sudan III (oil red) negative, which allowed us to discard lipid content in the vacuoles.

**Discussion**

The histopathologic changes on different organs of CD-1 male mice as a consequence of the *Hl*MeOHe sub-acute treatment were evaluated at two different doses. The selected organs for histology evaluation were small bowel, liver, pancreas, kidneys and brain. The small bowel was chosen as after oral dosing, molecules or compounds crossed enterocytes tight junctions or lipid cell membranes to cross luminal membrane, conditions that may alter the intestinal transit (El-Kattan A and Varma, 2012). The liver and kidneys were evaluated because these organs are frequently damaged since they must degrade and excrete numerous chemical compounds contained in herbal medicines. The Pancreas was selected because the antidiabetic properties of *Hl* stem bark have been studied (Korec, 2000; Guerrero et al., 2007; Mata et al., 2013) but nothing is known about its activity on this tissue, and finally, brain was
observed because it could be damaged mainly because of the alkaloids (Carrasco et al., 2017) contained in *Hl*, such as quinine and quinidine; quinoline antimalarials have been reported to cause neurotoxicity syndromes since the mid-1940s (Bañuelos et al., 2005; Nevin, 2014).

*Hl* treated animals presented diarrhea after the second day of treatment. Some alkaloids contained in the Rubiaceae family like quinine compounds, can cause diarrhea because of a local irritation of the intestinal tract (WHO, 1995). We have observed that *Hl*MeOHe increases the contraction of the smooth muscle in isolated mouse ileum assay (unpublished data), a condition than can increase the *in vivo* peristalsis and cause diarrhea. Also, the diarrhea could be due to the liver damage caused by the extract. It is difficult to assume the main cause of dilated mucosal vessels observed in the duodenum small bowel sections from the mice treated with 1000 mg/kg; Boyle et al (2012), observed lymphangiectasia in the small bowel of rats treated with indol-3-carbinol, a compound present in plants of the Brasica genus, and concluded that the damage could be done by an alteration in the lipid metabolism.

The implementation of extracts of some plants like nettle, sage, lemon balm and echinaceae in poultry and pig diet, result in the alteration of intestinal villi (Hanczakowska and Swiatkiewicz, 2012; García et al., 2007). Viveros et al (2011), described the alteration of the intestinal villi in poultry fed with diets rich in polyphenols; the stem bark of *Hl* contains a large quantity of polyphenols, although, more studies must be done in order to evaluate more precisely the effect of the plant on the intestinal tract.

The intake of natural products is linked with health risks, especially with those related to drug interactions or hepatotoxicity that may range from asymptomatic liver damage to massive hepatic necrosis (Bruguera et al., 2007). The cases of hepatotoxicity due to the consumption
of herbal products, are increasing in the United States of America and in Europe (Bailey et al., 2011; Navarro and Lucena, 2014; Navarro et al., 2017). Our results demonstrated that after an oral sub-acute daily dose of 200 mg/kg of Hl/MeOHe, mice livers showed periportal hepatitis and multifocal eosinophilia and with 1000 mg/kg even necrosis was observed. These results disagree with the ones obtained in previous studies, in which with a chronic toxicity test, mice, rabbits and rats treated with Sucontral® did not show histological damage after treatment (Garcia, 2010; Slijepcevic and Kraus, 1997; Korecova and Hladikova, 2014). In contrast, Bruguera et al (2007) described five clinical cases of liver damage as a consequence of the chronic daily consumption of 200 and 1000 mg of Hl stem bark. In this study, toxic hepatitis was established by blood high levels of hepatic enzymes; no histopathology was done. Another 6 clinical cases of hepatotoxicity associated with the consumption of Hl stem bark were described previously (Vial et al., 1998; Wurtz et al., 2002). In the present study, damage to the liver because of the treatment with Hl was dose-dependent, as more severe lesions were observed in the liver sections of the animals treated with the higher extract dose, in which generalized necrosis was reported. It is described that Hl stem bark contains alkaloids, tannins, steroids, terpenoids, flavonoids, phenolics, saponins and some compounds like 4-phenylcoumarins, cucurbitacins, coutareagenins, ursolic acid, chlorogenic, quinadine and quinine (García, 2010, Guerrero et al., 2007). Alkaloids contained in the Rubiaceae family are reported to be hepatotoxic, hepatic enzymes are described to increase after the chronic use of quinidine compounds and inflammation, necrosis and prominent eosinophils are observed in liver sections after its use (Deisseroth et al., 1972; Winkler, 1973; Handler et al., 1975). Quinine, the antimalric alkaloid has been reported to cause granulomatose hepatitis (Katz et al., 1983; Howard et al., 2003, Katsuma
et al., 2015). *Morinda citrifolia*, another plant of the Rubiaceae family commonly known as noni has been reported to cause liver damage after chronic consumption (Millonig et al., 2005; Stadlbauer et al., 2005, 2008; Yüce et al., 2006; López et al., 2007; Yu et al., 2011; Mrzljak et al., 2013). Noni extracts contain flavonoids, glycosides, anthraquinones and polyunsaturated fatty acids (Wang et al., 2002). It was suspected that the hepatotoxicity of noni, was due to the anthraquinones (Ueno et al., 1995). Natural phenols in some plants such as catechin in green tea are reported to produce necrosis and apoptosis in liver tissue in mice and humans (Pedrós et al., 2003; Lambert et al., 2010); a green tea ethanolic extract was retired from the French and Spanish market due to the hepatotoxicity reported in consumers after its use (Agencia Española de Medicamentos y Productos Sanitarios, 2003); thus, in addition to alkaloids, phenolics contained in *Hl* could be another cause of liver damage. The mechanisms and components by which *Hl* stem bark damages the liver remain unknown and are difficult to determine, as an extract is composed of numerous chemical compounds, resins, and oils. The damage could be mainly attributed to the alkaloids, as alkaloids have been described to produce cell damage by creating free radicals and leading to oxidative stress (Ueno et al, 1995; Mrzljak et al., 2013,) therefore, more studies about the pharmacokinetic and toxicity of the stem bark of *Hl* need to be done for the benefit of consumers.

In the scientific literature there is a lack of information regarding the damage to the pancreas due to the use of herbal medicines. In our research, we included the histopathological evaluation of this organ as it produces insulin and other hormones that play an important role in regulating blood glucose, likewise, the stem bark of *Hl* has been reported to exert
hypoglycemic effects in humans and in experimental animal models of type two diabetes (Guerrero et al., 2007; Mata et al., 2013). When cells are unable to preserve ionic and liquid homeostasis, intracellular vacuoles can be observed. This lesion is named hydropic degeneration and might cause cell lysis. In the pancreatic islets of 1000 mg/kg treated mice, hydropic degeneration was observed. This damage to the pancreatic islets can be due to the action of some components contained in the stem bark of *Hl* like alkaloids, glycosides or flavonoids, which could have altered the b-cells membrane, increasing the intracellular Ca++ levels and insulin release. Sulfonylureas induces initial improvement in beta cell function by reducing the burden of glucotoxicity, subsequently, by producing chronic stimulation of the cell, this drug accelerates the rate of beta mass loss (Aston, 2008).

Scientists and physicians have described that the use of herbal supplements can cause damage to several organs including kidneys (Jha and Chugh, 2003). Histological findings of acute kidney injury because of the intake of plant remedies in humans and experimental animals, include acute tubular necrosis, acute cortical necrosis and acute interstitial nephritis (Jha and Chugh, 2003); coincidentally, tubular necrosis was observed in mice after treatment with 1000 mg/kg of MeOH*Hl*e. Reports of kidney damage in humans and animals treated with some molecules and crude extracts obtained from the Rubiaceae plant family have been published. Iridoids, anthraquinones, triterpenes and alkaloids are considered chemotaxonomic markers of the Rubiaceae family (Cardoso et al., 2008); *Hl* belongs to this family, though, there are alkaloids in the plant (Bolzani et al., 2001). Alkaloids like quinine have been reported to cause acute interstitial nephritis in humans after a daily use of 2.5 g/kg for three weeks (Katsuma et al., 2015); quinine may also trigger a rare form of
hypersensitivity reaction in malaria patients termed blackwater fever, that results in massive hemolysis, hemoglobinemia, hemoglobinuria, and kidney failure (Rivera et al., 2013), as kidneys are very sensitive to the action of free radicals and hypoxia, factors that can be developed by plant alkaloids. The sub-acute administration of 2.5g/kg/day of an aqueous extract of *Nauclea latifolia* (Rubiaceae) leaves, produced acute tubular necrosis in rats (Usman et al., 2016). Noni juice obtained from *Morinda citrifolia* (Rubiaceae), can cause renal insufficiency which can develop to hyperkalemia in humans (Mueller et al., 2000). Mechanisms by which *Hl*MeOHe exerts kidney injury are not known, the damage could develop because of the production of free radicals or by an alteration of the blood flow rate due to the concentration of the multiple compounds found in the stem bark as kidneys are important organs for the excretion of toxic substances, this alteration can damage the endothelium and modify its hemodynamics. Adelekan et al (1999), reported the need for dialysis in patients with acute renal injury due to the use of herbal remedies. Direct damage to the liver by the multiple components contained in the herbal medicines, could cause kidney injury as well (Jha and Rathi; 2008).

It is difficult, if not impossible, to determine the pathophysiological mechanisms by which an extract or a natural product is exerting tissue injury, as many of its constituents could be responsible for the damage, even a synergic effect with other drugs, supplements or even food must be taken into consideration.

In conclusion, *Hl*MeOHe causes histology alterations in the liver, pancreas and kidneys of CD-1 treated male mice in a dose dependent manner, the liver and the kidney being the most affected organs. These results leave an open door to improve the regulation of the
indiscriminate use of natural products and to take into consideration that *Hintonia latiflora*
stem bark extracts or supplements may cause tissue damage.

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Conflict of interest

None declared
References


Agencia Española de Medicamentos y Productos Sanitarios. Suspensión de comercialización de la especialidad farmacéutica Exolise®: extracto etanólico de Camellia sinensis (té verde).


Bolzani V.S., Yong M.C.M., Furlan M., Cavalheiro A.J., Araujo A.R., Silva D.H.S. and
Lopes M.N. (2001). Secondary Metabolites from Brazilian Rubiaceae Plant Species:
Chemotaxonomical and Biological Significance. Recent. Res. Develop. Phytochem. 5, 19-
31.

Boyle M.C., Crabbs T.A., Wyde M.E., Painter J.T., Hill G.D., Malarkey D.E., Lieuallen
W.G. and Nyska A. (2012). Intestinal Lymphangiectasis and Lipidosis in Rats Following
Subchronic Exposure to Indole-3- Carbinol via Oral Gavage. Toxicol. Pathol. 40, 561-576

Acute hepatitis associated with Colpachi intake. A propose of 5 cases. Gastroenterol.
Hepatol. 30, 66-68.

Monoterpene Alkaloids from Chimarrhis turbinata DC Prodr.: A Contribution to the
Chemotaxonomic Studies of the Rubiaceae Family. Rev. Bras. Farmacogn. 18, 26-29.

Carrasco-Ramírez E., López-Camacho P.Y., Zepeda-Rodríguez A., Bizarro-Nevares P.,
Malagón-Gutierrez F., Basurto-Islas G. and Rivera-Fernández N. (2017). Stage-Specific
changes on Plasmodium yoelii yoelii following treatment with Hintonia latiflora stem bark
extract and phytochemical-antioxidant evaluation. Pharmacol. Pharm. 8,381-395

Intern. Med. 77, 595-597.

Pharmacology: Topics on Drug Metabolism. Paxton J. InTech. USA.


bark extract from *Hintonia latiflora* in a *Plasmodium yoelii yoelii* lethal murine malaria model. Parasitol. Res. 113, 1529-1536.


Fig. 1
Unpaired t test data of liver weights from CD-1 mice treated with *Hintonia latiflora* extract. There was a significant difference ($p<0.05$) between treated and untreated groups.

Fig. 2 a. CD-1 mouse treated with *Hl* extract (1000 mg/kg), increase in the liver size and rounded liver edges are observed. b CD-1 control mouse, normal-sized liver is observed.

Heart (H), Lungs (Lu), Liver (Li).

Fig. 3 Photomicrographs of sagittal sections of CD-1 control mice brains (a-c) stained with hematoxylin and eosin. a. Cerebral frontal cortex showing molecular layer (Mo) and granular layer (Gr) regions. b. Hippocampus: CA1–CA4 regions containing pyramidal neurons and dentate gyrus (DG) with dense granule. c. Choroid plexus (Cp) showing specialized secretory epithelial cells that extend into the ventricular cavities, cerebellum’s molecular layer (Mo), and granular layer (Gr) containing densely packed granule neurons are shown. Bars: a, 100 µm; b, 500 µm; c,100 µm.

Fig. 4 (a-d) Photomicrographs of pancreas and liver sections from CD-1 control mice stained with hematoxylin and eosin. a. Pancreas serous acini arranged in lobules (arrow) and pancreatic islet (asterisk) are observed. b. High magnification of pancreatic islet showing normal architecture, composed of cords of endocrine cells with pale acidophilic cytoplasm (asterisks). c. Liver section showing normal architecture with hepatocytes radiating from the central vein; sinusoidal space and portal triad are observed. d. Liver section with normal portal region containing the hepatic artery (A), bile duct (B), central vein (V) and several lymphatic vessels (L). Bars: a, 100 µm; b, 25 µm; c, 100 µm; d, 25 µm.

Fig. 5 (a-c) Photomicrographs of CD-1 control mice kidney section stained with hematoxylin and eosin. a. Renal cortex showing normal morphology in glomeruli (Gl) and tubules is appreciated. b. Proximal convoluted tubules (P) are shown, as well as distal convoluted tubules (D) and Bowman’s capsule (Arrow). c. High magnification image showing glomerulus (Gl), proximal convoluted tubules (P) and convoluted tubules brush border, distal convoluted tubules (D), Bowman’s capsule (Arrow) and urinary space (asterisk). Bars: a, 100 µm; b, 25 µm; c, 5 µm.

Fig 6 Photomicrographs of CD-1 small bowel (duodenum) section stained with hematoxylin and eosin. a. Control mice showing normal villi and duodenal glands (arrows). b. High magnification showing mucous cells (head arrows) and duodenal glands (arrow). c. Treated mice with *Hl* extract (1000 mg/kg) rather tall villi (arrows), dilatation of lymphatic vessels (black asterisks) and lymphocytic mild infiltrate (withe asterisks) are observed. d. High magnification of c showing tall villi (arrows) and dilatation of lymphatic vessels (black asterisks) Bar: a. 100µm; b, 25µm; c, 100µm; d, 25µm.
**Fig. 7** (a-c) Photomicrographs of CD-1 mice liver sections treated with *Hl* extract (1000 mg/kg) and stained with hematoxylin and eosin. **a, c.** Portal region showing periportal inflammatory cell infiltration (arrow) and necrosis, central vein (V). **b.** High magnification of a image showing inflammatory cell infiltrate composed of lymphocytes, neutrophils and eosinophils (head arrow), enlarged hepatocytes with clear cytoplasm (asterisk) and central vein (V). **d.** High magnification of c image showing necrosis (long arrow), lymphocytic and eosinophilic infiltration (head arrows) and karyolysis (asterisk). Bars: a, c, 100 µm; b, 5 µm; c, 25 µm.

**Fig. 8** (a-c) Photomicrographs of CD-1 mice kidney sections treated with *Hl* extract (1000 mg/kg) stained with hematoxylin and eosin. **a.** Renal cortex section glomeruli with thickening of the glomerular capillary wall (arrows) and tubule degeneration (head arrows). **b-c.** High magnification of glomeruli showing tubules with loss of epithelial integrity (head arrows), thickening of the glomerular capillary wall (arrows), increase of the urinary space (blue asterisks) and karyolysis (black asterisks). Bars: a, 100 µm; b, c, 5 µm.

**Fig. 9** (a-c) Photomicrographs of CD-1 mice pancreas sections treated with *Hl* extract (1000 mg/kg) stained with hematoxylin and eosin. **a.** Vacuolization of pancreatic islet cells (arrows). **b-c.** Pancreatic islets in high magnification showing vacuoles (arrows), swollen cells (asterisks) and mild plasma cell infiltration (white asterisk). Bars: 100 µm; b, c 25 µm.
Liver weight differences after *Hintonia latiflora* extract treatment in CD-1 mice

*P < 0.05 vs control group. HI\text{MeOHe}=Hintonia latiflora methanolic stem bark extract*