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Hepatocurative and Histopathological Evaluations in Albino Rats Exposed to *Vitex Doniana* Alkaloids

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Abstract: The study investigated the effects of the alkaloid fraction of *Vitex doniana* on liver and kidney functions in albino rats. A solution of CCl_4 in paraffin (1:1) was used to induce liver damage in albino rats. The liver and kidney function tests were carried out by oral administration of varying doses (200, 400, and 600 mg/kg) of the bulk alkaloid to different groups of albino rats for 14 days. The rats were subsequently sacrificed, and the hepatocurative and histopathological assessment of the liver and kidney were carried out to determine the serum albumin, total protein, cholesterol (CHOL), triglycerides (TAG), low-density lipoproteins (LDLs), high-density lipoproteins (HDLs), blood urea nitrogen (BUN) and creatinine. The total protein, serum albumin, and HDLs were significantly ($p < 0.05$) increased by alkaloid fraction of *V. doniana* to values compared with the control. The results also showed that serum CHOL, TAG, LDLs, BUN, and creatinine were significantly reduced ($p < 0.05$) in rats treated with alkaloid fraction compared with untreated. The normal cellular architecture of the liver destroyed by the standard hepatotoxin was restored by the alkaloids. The alkaloids of *V. doniana* reverse the CCl_4-induced hepatocellular damage, supporting its use in managing liver-associated diseases.

Keywords: alkaloids; hepatocellular; hepatocurative; histology; liver; *Vitex doniana*

1. Introduction

The kidney and the liver are important organs involved in detoxifying the body [1]. The kidney, in particular, maintains the composition and level of the water as well as essential minerals in the body [2]. On the other hand, the liver controls protein synthesis, detoxification, regulation, and maintenance of homeostasis [3-5]. The imbalance between the formation of reactive oxygen species and the antioxidant action causes oxidative stress [6]. Medicinal plants can maintain balance because they contain antioxidants [7].

Biochemical parameters are used to monitor an individual's susceptibility to a toxicant [8]. Such biochemical parameters are total and peripheral proteins that are altered within the liver or released in response to cellular injury; serum albumin measures the extent of protein synthesis by the liver [9]. Lipid profiles are measured to study the effects of impaired liver function on the levels of these lipoproteins in the blood [10]. Blood urea nitrogen (BUN) and serum creatinine levels are estimated to test kidney function. The ratio of BUN to creatinine

provides accurate knowledge about kidney function and its underlying causes compared with the level of creatinine alone [11,12].

Vitex doniana (Family *Verbenaceae*) is widely used in folk medicine in tropical Africa [13]. The leaf extract is taken as a therapy for eye and liver problems, releases pain during childbirth, and enhances milk production in lactating mothers [14]. It serves as a supplement for deficiency of vitamins A and B, and also as a remedy for kidney diseases [15]. The leaves are used as vegetables in foods and as a sauce.

The authors' previous study on the *in-vitro* antioxidant and hepatoprotective effects of *V. doniana* showed good antioxidant activity of the plant. The study also identified, by HPLC-MS/MS, several potential and promising alkaloids from its active fractions that are suggested to cause a significant reduction in the concentrations of serum phosphatase and aminotransferases activities in tetrachloromethane (CCl_4)-intoxicated rats treated with the fraction of *V. doniana* [16]. It was, therefore, necessary to extract the bulk alkaloids and further determine their roles in oxidative stress management. In the further search for hepatocurative alkaloids from *V. doniana*, we evaluated the effects of the bulk alkaloid of *V. doniana* leaves on the histopathological and biochemical parameters of CCl_4- induced rats.

2. Materials and Methods

2.1. Preparation of extract.

Fresh, healthy leaves of *V. doniana* were collected from farmland in Obukpa, Nsukka LGA, Enugu State, Nigeria, in December 2019. Authentication was done by Prof. Charles N. Mba of the Department of Soil Science, School of Agriculture and Agricultural Technology of Federal University of Technology, Owerri. A voucher specimen (Number UUH 998) was subsequently kept at the herbarium of the Department.

The leaves were dried at room temperature for 8 days, ground into a fine powder, and extracted following the previously described method [16].

2.2. Extraction of bulk alkaloids.

A 50 g of the dried methanol extract was dissolved in 10 %v/v MeOH, then partitioned in an equal volume of dichloromethane and acidic water (0.1 M HCl) in a separatory funnel. The dichloromethane soluble (neutral and acidic alkaloid compounds) were collected and evaporated to separate the alkaloids. Thereafter, the aqueous layer was made basic by treating with 0.1 M NH_4OH and extracted again with dichloromethane. Again, alkaloids were collected in the dichloromethane layer as a free-base. Two drops of Dragendorff's reagent were added to the fractions, and the formation of orange-red precipitate showed the presence of alkaloids [17].

2.3. Experimental animals.

Thirty (30) male albino rats (weight 100. 71 ± 27. 13 g) were used for the hepatocurative assay. The rats were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and were kept in clean cages in a well-ventilated room under favorable conditions. The permission to use animals for this study was obtained from the University of Nigeria Ethics Committee (FPS/2020/TOA/VA/001) of 20th March 2020 following guidelines on the handling of laboratory animals "Principles of Laboratory Animal

Care" (NIH publication no. 85-23, revised 1985) and/or the declaration of Basel issued on 30th November 2010 as amended on January 2013.

2.4. Evaluation of hepatocurative activity.

Six groups (A-F) of male albino rats were kept in cages (five rats per each) and allowed free access to food and water. The rats were left to acclimatize for 8 days. A solution of CCl_4 in paraffin (1:1) was used to induce liver damage in rats on the 1st and 8th day. Group A (normal control) rats received 0.5 ml distilled water orally once daily. Group B (untreated) was given 0.5 ml of CCl_4 solution intraperitoneal (i.p.) on the 1st and 8th day. Group C (standard control) received 0.5 ml CCl_4 on the 1st and 8th day, i.p., and 200 mg/kg of butylated hydroxytoluene (BHT) orally, once daily for 14 days. Group D simultaneously received 0.5 ml of CCl_4 on the 1st and 8th day i.p., and 200 mg/kg of bulk alkaloid orally, once daily. Group E simultaneously received 0.5 ml of CCl_4 on the 1st and 8th day i.p., and 400 mg/kg of bulk alkaloid orally, once daily. Group F simultaneously received 0.5 ml of CCl_4 on the 1st and 8th day i.p., and 600 mg/kg of bulk alkaloid orally, once daily. Treatments with doses of alkaloid fractions and the standard drug lasted for 14 days [18].

2.5. Serum preparation.

After the last daily doses, the rats were fasted overnight and then sacrificed under chloroform anesthesia. They were made to bleed through the ocular puncture into centrifuge tubes. The blood sample was left to clot for 15 min and centrifuged at 3500 rpm at 4ºC for 10 min to get the sera. An assay of biochemical parameters was performed within 12 h of preparation of sera [18]

2.5.1. Determination of biochemical parameters.

Biochemical parameters (albumin and total protein, cholesterol, triglycerides (TAG), low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs), blood urea nitrogen (BUN), and creatinine were assayed following techniques described previously [19-21]

2.6. Histopathological study.

Liver sections from randomly selected rats in each group were collected for histological study. The samples were fixed for a minimum of 48 h in 10 % phosphate-buffered formalin. The 5µm thick sectioned tissues were stained with eosin and hematoxylin staining methods. They were observed using x4, x10, and x40 objective lenses with a Motic™ compound light microscope. The photomicrographs were taken randomly using a Motic™ 5.0 megapixels microscope camera at x160 and x200 magnifications [22].

2.7. Statistical analysis.

SPSS version 25.0 Windows was used for statistical analysis, while GraphPad Prism 8 was used for plotting the graphs. All the data were presented as mean ± standard deviation (SD) and analyzed using one-way ANOVA and Post-hoc Turkey test [23]. The difference between treatment groups was significant at $p < 0.05$ levels

3. Results and Discussion

3.1. Effects of alkaloid fraction on serum protein.

The effects of *V. doniana* alkaloids on the serum protein of rats are shown in Figure 1. There was no significant change ($p > 0.05$) in the serum albumin of rats treated with 200 mg/kg of the bulk alkaloids compared with the untreated group. In contrast, there was a significant increase ($p < 0.05$) in the serum albumin of rats treated with 400 and 600 mg/kg, and total protein of rats treated with 200-600 mg/kg of alkaloids compared with the untreated.

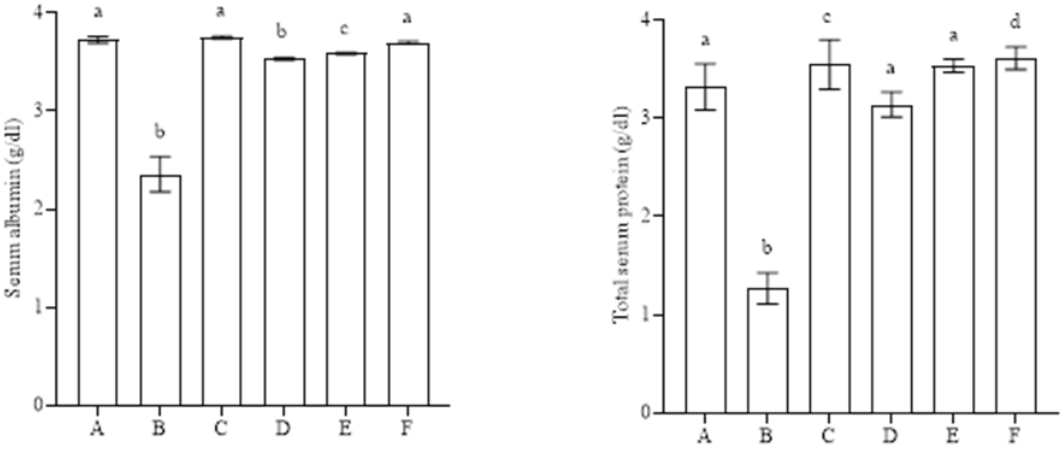

Figure 1. Serum albumin and total protein concentrations (g/dl) of CCl_4-intoxicated rats treated with alkaloid fractions of *V. doniana* leaves. Values are means ± SD, *n* = 5. [a-d]Bars with different superscripts with group B are significant at $p < 0.05$. A = control; B = untreated; C = BHT; D = 200 mg/kg; E = 400 mg/kg; F = 600 mg/kg.

Carbon tetrachloride (CCl_4) is a hepatotoxin that can quickly cross cell membranes and spread across tissues after being exposed to it. After that, it's bio-transformed into a trichloromethyl free radical, which is then converted into a peroxy radical [24]. The endoplasmic reticulum's cytochrome P450 (CYP2E1) facilitates this conversion in the liver. This radical causes cellular lipids and proteins to auto-oxidize, causing cell injury and the development of endogenous toxicants. This toxic agent causes liver cells' necrosis, apoptosis, fibrosis, and cirrhosis [25]. During liver injury due to CCl_4, the rate of hepatic synthesis of essential proteins in animals decreases, causing a drop in serum albumin and total protein concentrations [26]. This trend was reversed following the administration of *V. doniana* alkaloid in varying doses, suggesting their potential role in promoting protein synthesis and function of hepatocytes. Alkaloids reduce liver damage and inhibit the development of hepatic stellate cells in a dose- and time-dependent processes. The normalization of liver function could be linked with the maintenance of hepatocyte integrity potentials and stabilization of the membrane [27,28]. Several alkaloids have bitter flavors, interrupt protein function after absorption and biotransformation, and affect the central nervous system [29].

3.2. Effect of alkaloid fraction on lipid profile.

The effects of *V. doniana* alkaloids on the lipid profile of rats are shown in Table 1. All the treatment doses of alkaloids caused a significant change ($p < 0.05$) in rats' CHOL, TAG, HDLs, and LDLs compared with the untreated. Both 200 and 600 mg/kg of bulk alkaloid fraction significantly ($p < 0.05$) decreased the level of CHOL from 4.65 ± 0.06 to 3.09 ± 0.07 mMol/L and 4.65 ± 0.06 to 3.34 ± 0.07 mMol/L respectively. The serum TAG was also

significantly ($p < 0.05$) reduced when CCl_4-intoxicated rats were treated with 400 and 600 mg/kg bulk alkaloid. Treatment of CCl_4-intoxicated animals with 200 and 400 mg/kg of bulk alkaloid significantly ($p < 0.05$) reduced the level of serum LDLs from 1.10 ± 0.06 to 0.58 ± 0.04 and 1.10 ± 0.06 to 0.59 ± 0.04 mMol/L. Treatment with 400 and 600 mg/kg of bulk alkaloid fraction significantly ($p < 0.05$) increased HDLs level from 1.49 ± 0.01 to 1.78 ± 0.01 and 1.49 ± 0.01 to 1.80 ± 0.01 mMol/L respectively compared with the untreated.

Table 1. Effects of *V. doniana* alkaloids on lipid profiles of CCl_4-intoxicated rats.

Groups	CHOL (mMol/L)	TAG (mMol/L)	HDLs (mMol/L)	LDLs (mMol/L)
A	3.21 ± 0.06^{a}	1.79 ± 0.06^{a}	1.73 ± 0.07^{a}	0.66 ± 0.03^{a}
B	4.65 ± 0.06^{b}	2.43 ± 0.01^{b}	1.49 ± 0.01^{b}	1.10 ± 0.06^{b}
C	3.21 ± 0.06^{a}	1.84 ± 0.01^{c}	1.74 ± 0.03^{a}	0.69 ± 0.02^{a}
D	3.09 ± 0.07^{c}	1.77 ± 0.05^{a}	1.73 ± 0.04^{a}	0.58 ± 0.04^{c}
E	3.19 ± 0.05^{a}	1.84 ± 0.01^{d}	1.78 ± 0.01^{c}	0.59 ± 0.04^{d}
F	3.34 ± 0.07^{c}	1.86 ± 0.01^{e}	1.80 ± 0.01^{d}	0.71 ± 0.05^{a}

Values are mean ± SD ($n = 5$). Groups bearing different alphabet for each group are statistically significant ($p < 0.05$). A = control; B = untreated; C = BHT; D = 200 mg/kg; E = 400 mg/kg; F = 600 mg/kg.

The role of the liver as an important organ involved in lipid metabolism forms the basis for studying the effects of impaired liver function on the levels of lipoproteins in the blood. The amount of CHOL and fats called TAG were measured in the serum of rats to ascertain the effect of impaired liver function as a result of CCl_4-intoxication on the level of lipoproteins in the blood. A variety of partly disorder mechanisms causes changes in lipid metabolism and serum lipid profile. They are a common feature of the liver disorder [24]. These lipid abnormalities are temporary and can occur in the most common forms of liver diseases and severe and non-complicated cases. Intoxication with CCl_4 produces fatty liver and hepatic cirrhosis as well as an elevation in the total serum cholesterol level [24]. Antioxidants produce beneficial effects on serum lipids [30]. HDLs can transport excess LDLs deposited in walls of blood vessels back to the liver for catabolism. Liver disease resulting from exposure to CCl_4 reduces HDLs and affects the beneficial effects of HDLs [31]. Administration of different doses of the bulk alkaloid fraction on the rats was able to reduce the levels of cholesterol, LDLs, and TAG. Still, it increased the level of HDLs in the serum, indicating that they could alleviate dyslipidemia induced by CCl4. Alkaloids are a class of medicines that have played a significant role in human history. They are found in everyday foods, drinks, and supplements and are used to enhance immune function and physical health. Some alkaloids can play a more important role in plants as antioxidants assist in the detoxification of free radicals produced by various stresses [1, 32]. Although different doses of the bulk alkaloid fraction reversed these changes, the ameliorative effect of 400 mg/kg of the bulk alkaloid compared well with the BHT.

3.3. Effect of alkaloids on renal function parameters.

The effects of different doses of *V. doniana* alkaloids on the renal functions of rats are shown in Figure 2. There was a concentration-dependent decrease in all the renal function parameters, with the 200 mg/kg dose causing the highest decrease and the 600 mg/kg the least. Treatment of CCl_4-intoxicated rats with 200-600 mg/kg of alkaloids significantly ($p < 0.05$) decreased BUN and serum creatinine levels compared with untreated.

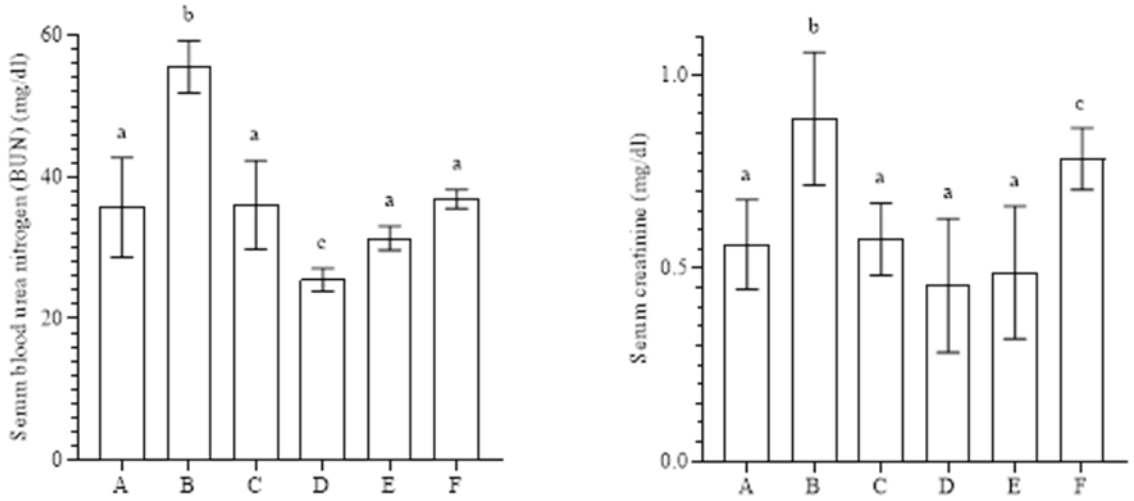

Figure 2. Effect of *V. doniana* alkaloids on some kidney function parameters of the rat. Values are mean ± SD (*n* = 5). Treatment groups with a different alphabet for each group are statistically significant ($p < 0.05$). A = control; B = untreated; C = BHT; D = 200 mg/kg; E = 400 mg/kg; F = 600 mg/kg.

The widespread toxic effect of CCl_4 makes it impossible to exonerate the involvement of other organ systems in metabolic abnormality [33,34]. Therefore, some renal function parameters such as urea (BUN) and creatinine were assayed on the sera specimens. Elevated serum creatinine level and the BUN signify kidney disease or impaired kidney function. Their elevation indicates poor kidney function since the kidney completely filters them from the blood. Their levels in the blood build-up due to poor clearance when the kidney becomes impaired for any reason [25,35]. Various doses of the bulk alkaloid fraction normalized the BUN and creatinine levels in the serum following CCl4-intoxication like BHT, indicating the curative effects of the studied bulk alkaloid fraction on the kidney. The ameliorative effect of 200 mg/kg of the bulk alkaloid fraction on the BUN was significant, and they compared well with BHT. Similar work by [2] reported that *V. doniana* decreased serum concentrations of BUN and creatinine in cadmium-induced toxicity in rats.

3.4. Effect of V. doniana alkaloid on histology of liver.

The histopathological effects of *V. doniana* alkaloids are shown in Figure 3. The histopathological studies of liver sections of the normal control (group A) showed normal hepatic histomorphology (slide 1). The tissue sections showed many normal hepatic lobules, consisting of normal hepatocytes arranged around the central veins (V) in radiating interconnecting cords. Normal-sized sinusoidal spaces separate the hepatic cords. Normal structures of the portal triads (PT) [bile ducts hepatic vein; and hepatic artery] were also observed. However, sections of untreated group B (slide 2) showed a marked cellular swelling of the hepatocytes in the periportal (P) and mid-zonal areas of the hepatic lobules. The hepatocytes around the central veins (V) are normal. The liver section of CCl_4-intoxicated rats administered with 200 mg/kg BHT (slide 3) showed a mild cellular swelling of the hepatocytes in the hepatic lobules' periportal (P) areas. There are normal hepatocytes within the central veins (V).

Meanwhile, sections of the liver of CCl_4-intoxicated rats treated with 200 and 400 mg/kg of alkaloid fraction (slides 4 and 5) showed the normal histo-architecture of the liver. However, sections of the liver of CCl_4-intoxicated rats administered with 600 mg/kg of alkaloid

fraction showed a mild cellular swelling of the hepatocytes in the periportal (P) and mid-zonal (MZ) areas of the hepatic lobules. The hepatocytes around the central veins (V) are normal.

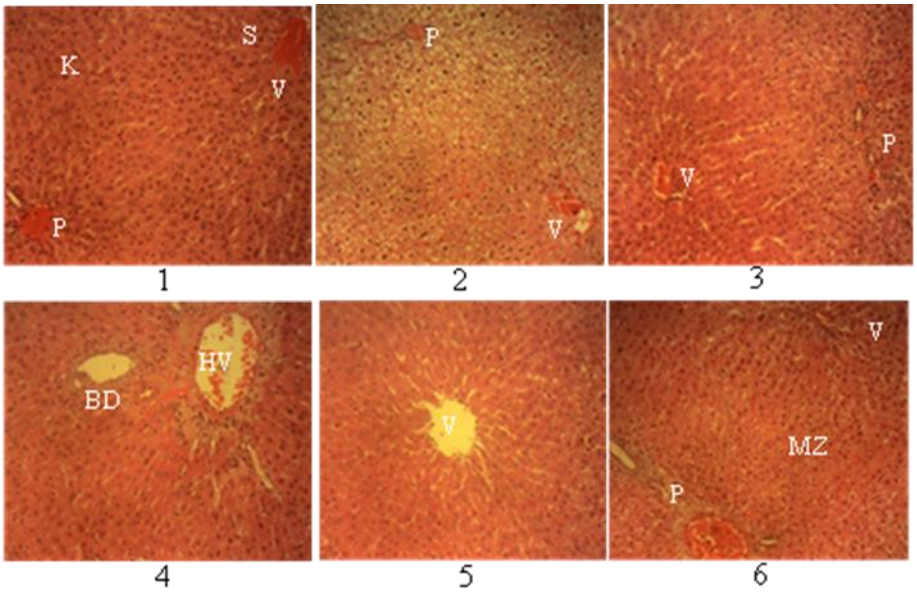

Figure 3. Liver sections of the rats administered with *V. doniana* alkaloids. Slide 1 (H & E x200) for group A, Slides 2-6 (H & E x160) for groups B to F; V = central veins; K = Kupffer cells; S = Sinusoid; HV = Hepatic vein; BD = Bile duct; MZ = Mid-zonal; P = Periportal.

Histopathological examinations further supported the hepatocurative activity of the alkaloids. According to Wei *et al*. [36], the CCl_4 intoxicated group had histological changes in the liver, which were manifested in fatty variations due to massive hepatocyte formation of fatty content. When compared to the control group, significant changes in histology caused by CCl4 resulted in increased coagulative necrosis, inflammation, and vacuolization [37,38]. There is an improvement in histo-architecture of the damaged liver cells of the treated groups compared with the untreated. Examination of the liver tissue of rats treated with 200 and 400 mg/kg of the bulk alkaloid fraction showed normal histo-architecture of the liver, which is evidence of its hepatocurative activity, while histology of the liver section of rats treated with 600 mg/kg of the bulk alkaloid fraction showed a mild cellular swelling of the hepatocytes in the periportal and mid-zonal areas of the hepatic lobules with normal hepatocytes around the central veins.

4. Conclusions

The study has demonstrated that tetrachloromethane-induced toxicity in the kidney and liver of albino rats can be ameliorated by the alkaloids of *V. doniana*. The presence of alkaloids in its fraction, which have earlier been shown to have antioxidant effects, could be responsible for the hepatocurative effect observed in the study. Alkaloids are a vast and complex group of compounds with a wide variety of biological functions in plants, animals, and humans and important medicinal properties. Its study can aid in developing new medicines by providing a deeper understanding of the diverse ecological functions of alkaloids. There was evidence that the *V. doniana* alkaloids exerted a significant curative effect against CCl_4-induced hepatotoxicity, which may be linked to the significant antioxidant activity of *V. doniana*. *V. doniana* alkaloids could alleviate dyslipidemia induced by CCl_4 and promote protein synthesis and function of hepatocytes. The results also showed significant restoration of the liver

architecture, which is evidence of its hepatocurative effects. There should be further research on the mechanisms of action of these bioactive alkaloids responsible for biological activities. Studies to isolate and characterize these bioactive alkaloids should be carried out. This additional research will provide an insight into the mechanisms that contribute to the alkaloid's unique bioactivities. There is ongoing research on the isolation of the specific alkaloids of *V. doniana* responsible for the observed effects.

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Conflicts of Interest

The authors declared no conflict of interest.

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