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Acute and Subchronic Toxicity Studies of Aqueous Extract of *Desmodium adscendens* (Sw) DC

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Abstract

Extracts of *Desmodium adscendens* (Sw) DC are used for the treatment of various diseases but limited toxicological evaluations have been done on the medicinal plant. This study investigates toxicity effects of the leave extract of *D adscendens*, and the possibility of drug-drug interaction of the plant extract when co-administered with other drugs. Oral administrations of leaf extract of *D adscendens* to white Wistar rats in an acute toxicity studies allowed the estimation of an LD_{50} (median lethal dose) value of 1122 mg/kg body weight. In a subchronic toxicity studies, the plant extract caused a decrease in zoxazolamine paralysis time and prevented thiopentone from causing sleep in test animals compared to controls. Overall, the results are consistent with the plant extract being safe at the doses administered in humans. However, the induction of the CYP enzymes is an indication of a possible drug interaction when the plant extract is co-administered with other drugs.

Keywords

acute toxicity, subchronic toxicity, drug metabolism, cytochrome P450 xenobiotic-metabolizing enzymes, *Desmodium adscendens* (Sw) DC

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Desmodium adscendens (Sw) DC is a medicinal plant found in tropical and subtropical parts of the world and its therapeutic importance have been extensively studied over the past few decades.[1-5] The plant has been found to be very useful in the treatment of constipation and other gastrointestinal ailments, bronchial asthma, inflammations, and coughs and colds.[6] Leaf extract of the plant is also applied externally for the treatment of snake bites and wounds in general.[5,7,8] High-performance liquid chromatography and spectroscopic analysis of biologically active fractions of *D adscendens* showed 2 pairs of flavonoid isomers (vitexin/vitexin 2″-*O*-xylodise and isovitexin/isovitexin 2″-*O*-xyloside) as the dominant constituents.[9] Active compounds that have also been isolated from *D adscendens* include phenylethylamines, indole-3-alkylamines, tetrahydroisoquinolones and triterpenoid saponins,[10] and tyramine and hordenin.[2] Soyasaponin I, soyasaponin III, and triterpenoid glycoside dehydrosoyasaponin I are also active components in *D adscendens* that stimulate calcium-dependent potassium ion channels involved in airway smooth muscle tone regulation.[2,11] Other compounds also identified were 3,4-dimethoxy-β-phenethylamine, salsoline-*N*,*N*-dimethyltryptamine, and other minor basic compounds.[2] In a recent study, high-performance liquid chromatography and high-resolution mass spectrometry permitted the identification of polyphenols that are relating to soyasaponins and alkaloids.[12] Currently, the leaf extract of *D adscendens* is administered at a Center for Plant Medicine in the Eastern Region of Ghana.

The extensive use of *D adscendens* extracts for its antiasthmatic and anti-inflammatory properties has been the major drive for various studies on the plant.[3,11] Extracts of the plant have also been shown to have effects on the central nervous system and induce hypothermia,[5] and as an analgesic, it suppresses the tonic

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phase of convulsion and mortality caused by pentylenetetrazole in mice.[5] An evaluation of a hydroalcoholic leaves extract of the medicinal plant by 2,2′-azino-bis(3-ethylbenzothiazoline 6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests suggests that *D adscendens* has a scavenging antioxidant activity.[13] Toxicity studies of the medicinal plant have however been limited, and in the minimal instance that it has been done, the studies have not comprehensively addressed the toxicological effects of *D adscendens*.

Elevated concentrations or activities of xenobiotic-metabolizing enzymes involved in the biotransformation of drugs, as well as liver and kidney function tests, have been used as toxicological determinants of medicinal plant preparations that are used therapeutically.[14-16] Medicinal plant extracts may contain only traces of the medicinal constituents, and thus, large doses of the preparations may have to be administered, as such, there could equally be high toxicity implications. Assessment of the potencies and toxicities of the plant preparations by indices such as the median effective dose (ED50) and the median lethal dose (LD_{50}) are therefore necessary for standardization and quality control.

In this study, the effect of *D adscendens* leaves extract on liver and kidney function in an acute and subchronic toxicity studies have been investigated using rats as experimental animals. The extract was also assessed for its potential for herb-drug interaction through CYP enzyme induction studies.

Materials and Methods

Materials and Reagents

Eight-week old white Wistar rats (180-200 g) were obtained from the Korle Bu Teaching Hospital. Grower mash (15%-16% protein, 3%-5% crude fiber, 1% calcium, 0.4% phosphorus, 0.65% lysine, 0.4% salt; with energy content of 2800 kcal/kg), which was used to feed the rats was from Kosher Feedmills, Accra, Ghana. Clean tap water was given as the source of drinking water, which was changed daily. The study was conducted in accordance with the guidelines provided by the Noguchi Memorial Institute for Medical Research of the University of Ghana, an internationally approved research facility in Ghana.

The following assay kits: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), total bilirubin, direct or conjugated bilirubin, total serum protein, serum creatinine, and blood urea nitrogen (BUN) were obtained from Randox Chemicals Co (Antrim, UK). Nicotinamide adenine dinucleotide phosphate (reduced) (NADPH), resorufin, 7-ethoxyresorufin, 7-pentoxyresorufin, zoxazolamine (2-amino-5-chlorobenzoxazole), bovine serum albumin (BSA), and perchloric acid were obtained from Sigma Chemical Co (St Louis, MO, USA). Sodium dithionite and dithiothreitol were from Fluka Garantie (St Loius, MO, USA), and *p*-nitrophenol was from BDH Laboratory (Radnor, PA, USA).

All other chemicals were obtained in their pure forms that were commercially available.

Preparation of Desmodium adscendens *Extract*

Fresh leaves of *D adscendens* collected from Mampong-Akwapim, in the Eastern Region of Ghana, were identified by a qualified botanist of the research facility. A voucher specimen (OQ001) from the location where the specimens used in this study was obtained has been deposited in the Ghana Herbarium, University of Ghana, Legon, Accra, and the herbarium of the Center for Plant Medicine Research, Mampong-Akwapim. The leaves were air-dried for 4 weeks at room temperature and boiled at 100°C in distilled water. The crude aqueous extract was subjected to rotary evaporation; 10 L of the aqueous extract yielded a powdered extract of 38.25 g after freeze-drying. The powdered extract was weighed (depending on the final concentration of the extract needed), dissolved in 0.15 M NaCl solution (0.88% standard saline solution), and the resulting solution administered was orally to test animals using a plastic syringe throughout the study.

Table 1. Effect of *Desmodium adscendens* Leaf Extract on Body and Organ Weights of Rats.[a]

Dose Groups of Animal	Weight of Excised Livers as % of Body Weight	Weight of Excised Kidney as % of Body Weight	% Body Weight Gain
Low dose	3.51 ± 0.52	0.53 ± 0.07	48.5
Medium dose	3.44 ± 0.72	0.55 ± 0.08	50.3
High dose	3.74 ± 0.63	0.58 ± 0.02	48.0
Control	3.26 ± 0.31	0.51 ± 0.05	51.7

[a]Body and organ weights of rats administered with *D adscendens* for 6 weeks. The percentage body weight gain was calculated as a percentage of the initial body weights, and the percentage liver and kidney weights were calculated as a percentage of the final body weights of the animals. Data are expresses as the mean ± SD.

Acute Toxicity Studies

Two preweighed rats (female and male) were orally administered with a dose of 10 g/kg body weight in a volume of 5 mL solution and the animals were observed for their physical behaviors, and days of deaths after the administration. Two rats (male and female), used as controls, were given 5 mL of standard saline solution. The dosage of the plant extract administered was reduced to 8, 5, 3, and 1 g/kg body weight, and the animals were observed for a maximum of 2 weeks after the administration. Based on the results obtained from the above studies, 6 different dose levels (0.5, 0.75, 1.00, 1.25, 1.50, and 1.75 g/kg body weights) were chosen, and the extracts were prepared and administered to 6 different groups of animals; each group consisting of 6 rats. Each dose was administered in a volume of 1 mL solution per animal, and the animals observed over a period of 2 weeks from the day of administration. The number of animals and days of deaths at the various dose levels were noted and recorded. The average of the doses that caused 50% lethality (LD_{50}) in the acute toxicity studies over the period of observation was calculated and used as the basis for determining the doses of the plant extract used in the subchronic toxicity studies. Animals that died during the study were dissected and their organs examined macroscopically.

Subchronic Toxicity and Biochemical Studies

Rats used in the subchronic toxicity studies were weighed on days 0, 1, and 2 of administration and then weekly for 6 weeks. Oral administrations of the extract, and standard saline solution in the case of the control group, were done daily over a period of 45 days. Based on the LD_{50} obtained from the acute toxicity studies, 3 dose levels (high dose of 337 mg/kg: 0.3X, medium dose of 112 mg/kg: 0.1X and low dose of

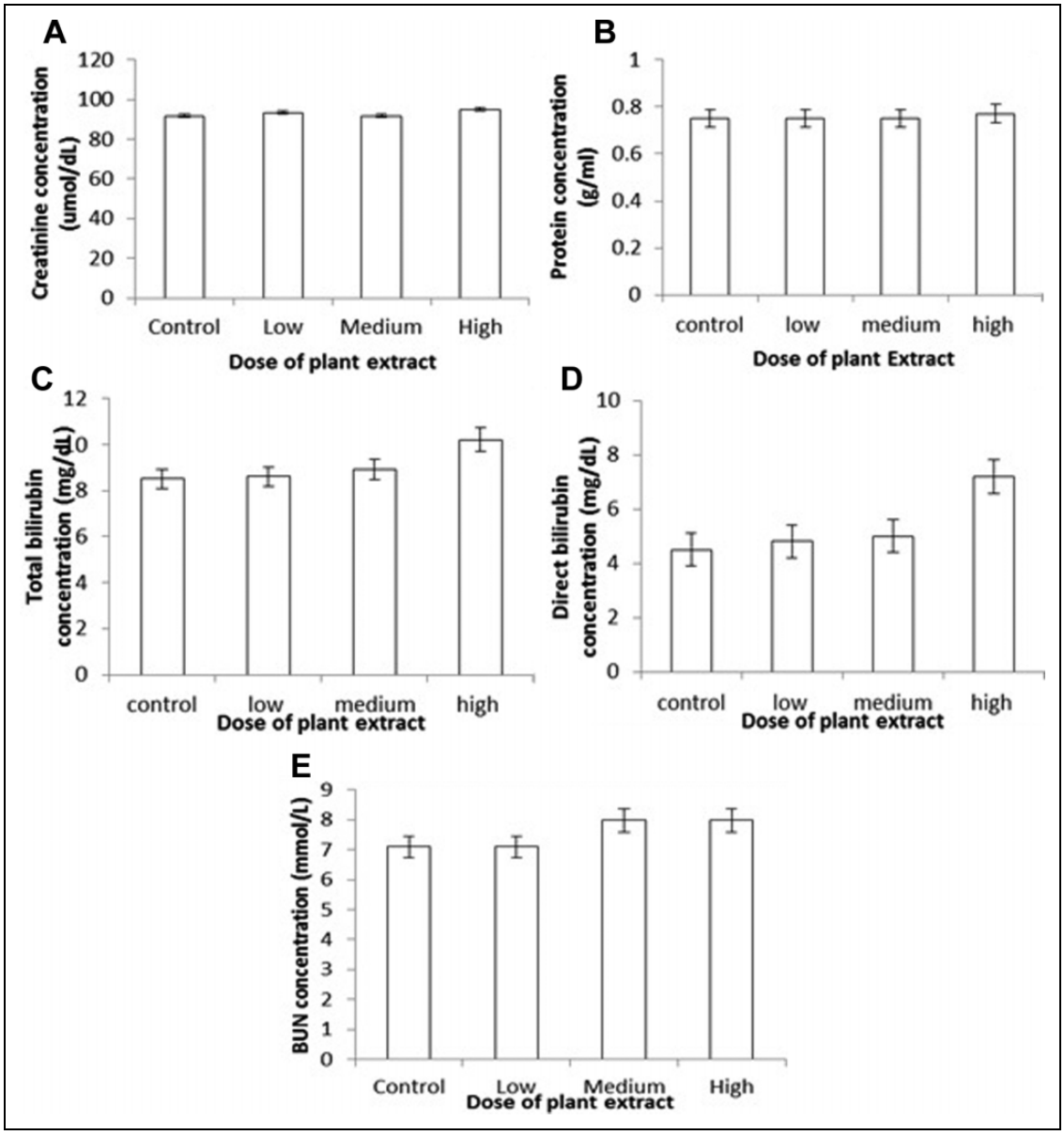

Figure 1. Serum biomarker levels following subchronic administration of *Desmodium adscendens*: (A) creatinine concentration, (B) protein concentration, (C) total bilirubin, (D) direct bilirubin, (E) blood urea nitrogen (BUN). All statistical significance was calculated using the 2-sided *t* test with unequal variance. $^{**}P < .001$.

34 mg/kg: 0.03X) were chosen and used in the subchronic toxicity studies, where X is the LD_{50}; the lethal dose that caused 50% mortality in the acute toxicity studies. The administrations were done in 0.5 mL solutions per animal.

The groups of animals in the subchronic toxicity studies were used to investigate the effects of the extract on liver and kidney function tests, as well as, cytochrome P450–dependent mono-oxygenase activities. For sera preparation, blood was drawn by cardiac puncture, allowed to clot and centrifuged at 2500 × *g* for 15 minutes at room temperature. The sera were stored at −40°C and used later to determine the levels of total serum protein, ALT, AST, GGT, total and conjugated bilirubin, BUN, and serum creatinine, in clinical chemistry tests for the determination of liver and kidney functions. The animals were dissected after the blood has been drawn and their livers and kidneys excised. The freshly excised organs were rinsed in prechilled KCl solution (rinsing buffer), blotted dry, and weighed.

Hepatic microsomes were prepared as previously described.[17,18] The microsomal pellets were resuspended in more storage buffer and stored at −80°C, and later used in biochemical assays to evaluate the presence of CYP isozymes by measuring ethoxyresorufin-*O*-deethylase, penthoxyresorufin-*O*-deethylase, and *p*-nitrophenol hydroxylation activities.

Herb-Drug Interaction Determination

The effect of the plant extract on the duration of sleep induced by thiopentone and paralysis induced by zoxazolamine was investigated following the procedures previously described.[16] Two groups of animals, one serving as a test group and the other as control, were used

to investigate thiopentone-sleeping time, and 2 other groups (test and control) were used to investigate zoxazoleamine paralysis time. Thiopentone was administered intraperitoneally at a dose of 100 mg/kg body weight and control animals were given standard saline solution. Zoxazolamine prepared in absolute dimethyl sulfoxide was administered intraperitoneally at a dose of 60 mg/kg body weight in a volume of 0.5 mL and the control group was given standard saline solution. The times it took for the animals to get back to normal posture and righting reflexes after the administrations were noted as paralysis and sleeping times for zoxazolamine and thiopentone, respectively.[16]

Data Analysis

Data for rat body and organ weights for the different dose and control groups were analyzed by determining the mean and standard deviation. Analysis of variance and Tukey's post hoc analysis were conducted to compare the serum biomarkers and enzyme activities for the different dose and control groups.

Results

Acute Toxicity Effect of Desmodium adscendens

Mortality of the animals increased with increasing doses of the plant extract and resulted in an estimated LD_{50} of 1122 mg/kg body weight. It was observed that higher doses of the extract caused rounding up, piloerection of both the fur and the whiskers of the rats, shiny eyes, agitation within the first 3 to 5 minutes with subsequent diarrhea. Compared with the control animals, the test rats that were administered with high doses of the plant extract had wrinkled lungs, darker coloured liver, and kidneys with numerous dark spots, on autopsy.

Subchronic Toxicity Studies of Desmodium adscendens

Results of change in body weight calculated as a percentage of the initial body weight, and organ (kidney and liver) weights expressed as percentage of the final body weights are shown in Table 1. There were no statistically significant differences in body weight changes and organ weights among the different treatment groups, and macroscopically, no significant differences were observed between organs from control and test rats for all the extract dose levels.

Effect of Desmodium adscendens *on Zoxazolamine Paralysis and Thiopentone Sleeping Times*

A dose of 60 mg/kg body weight of zoxazolamine dissolved in absolute dimethyl sulfoxide was administered to each treatment group. There was a significance difference in the zoxazolamine paralysis time between control (207.4 $\pm$ 21.6 minutes) and test (167 $\pm$ 8.1 minutes) animals with a P value of .01. For a dose of 100 mg/kg body weight of thiopentone administered, control animals slept for an average sleeping time of 9 minutes whereas test animals became very dizzy and wobbled when they attempted to move but did not sleep.

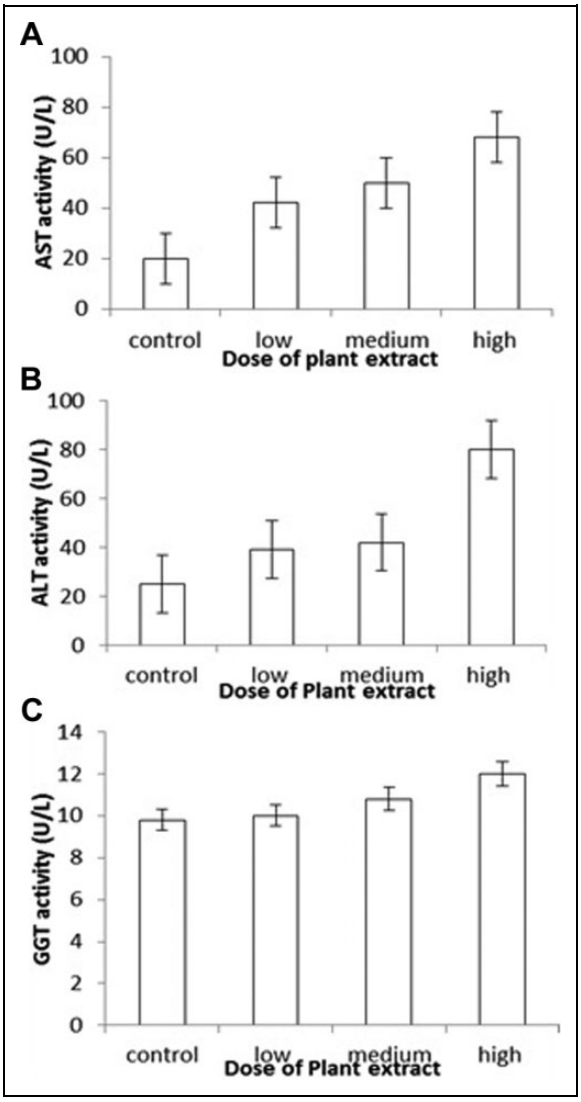

Figure 2. Activity of serum enzymes on the administration of *Desmodium adscendens* extract to rats for 6 weeks: (A) aspartate aminotransferase (AST), (B) alanine aminotransferase (ALT), (C) γ-glutamyl transferase (GGT). *$P < .01$.

Effect of Desmodium adscendens *on Liver and Kidney Function Tests (Clinical Chemistry)*

Figure 1A and B shows that increasing doses of the plant extract did not have any significant effect on creatinine and total serum proteins levels, and varying concentrations or doses of the plant extracts did not have statistically significant effects on other chemistry tests such as total bilirubin, GGT, and BUN (Figure 1C-E). Compared with controls, serum AST and ALT activities increased with increasing doses of the plant extract, and direct bilirubin level increased in the test group that was administered with the high dose of the extract ($P < .001$) (Figure 2A-C).

Biochemical Assays

The effect of *D adscendens* extract on cytochrome P450 enzymes, which are known to be involved in drug metabolism,

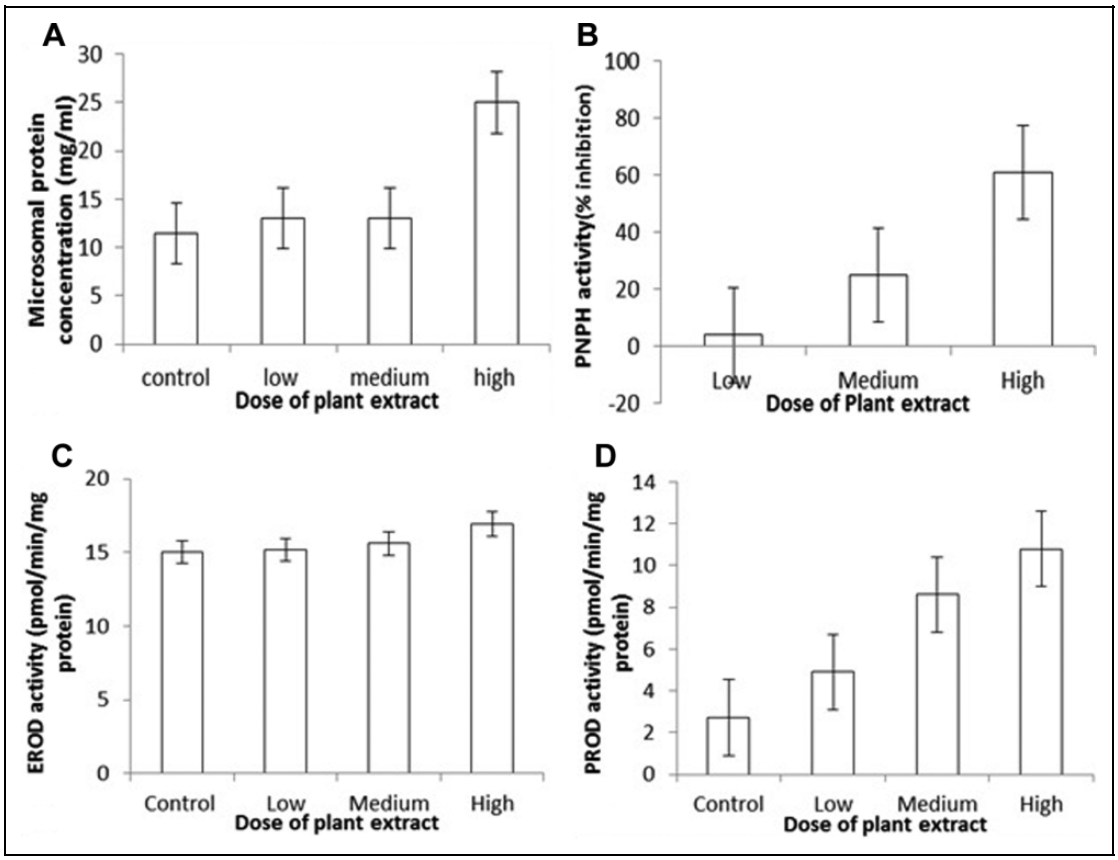

Figure 3. Liver microsomal content and CYP activities following subchronic administration of *Desmodium adscendens*: (A) microsomal protein concentration, (B) paranitrophenol activity for CYP2E, (C) ethoxyresorufin-*O*-deethylase (EROD) activity for CYP1A1/1A2, (D) pentoxyresorufin-O-deethylase (PROD) activity for CYP2B1/2B2. *$P < .01$; ** $P < .001$.

was analyzed. The average protein concentration of the microsomal preparations as estimated from a protein calibration curve (graph not shown) showed that the high dose of the extract increased microsomal protein concentrations significantly to about an average of 50% more than the medium and low doses, and 54% more than the control group (Figure 3A). The microsomal cytochrome P450 monooxygenase activity measured as *p*-nitrohydroxylation, and shown as a percentage inhibition of the control levels, decreased significantly with increasing extract dose levels (Figure 3B) ($P < .05$), whereas ethoxyresorufin-*O*-deethylase activity was not significantly altered (Figure 3C). However, all doses of the plant extracts (high, medium, and low) significantly raised the pentoxyresorufin-*O*-deethylase (PROD) activity compared with the control group (Figure 3D) ($P < .05$).

Discussion

Desmodium adscendens is a good source of traditional medicine for the treatment of disease conditions such as asthma, constipation, and dysentery.[6] It has also been exploited and used for its anti-inflammatory properties.[10,19,20] Ethanolic extract of the plant has been shown to have an effect on the central nervous system, leading to observable behavioral changes in mice at a dose range of 50 to 1000 mg/kg body weight for a test time between 30 minutes to 24 hours.[5] The studies described here investigated the acute and subchronic toxicity of the *D adscendens* extract.

The LD_{50} was estimated to be 1122 mg/kg body weight, which is about 450 times the prescribed dose dispensed at the Center for Plant Medicine Research (2.5 mg/kg body weight), making the leaf extract therapeutically safe enough for the treatment of diseased conditions. This is by no means an extrapolation of rat dose to human dose usage. A study, which set out to evaluate the safety and protective effect of *D adscendens* extract on liver and kidney cells in in vitro assays, revealed that 1 to 10 mg/mL of the extract protected against glucose-induced oxidative stress and suggestively safe, whereas 100 mg/mL of the extract decreased cell viability by 40%.[21] The behavioral changes or symptoms observed in the acute toxicity studies carried out in this study, such as piloerection, shiny eyes, agitation reduced activity, diarrhea, and dehydration leading eventually to death are consistent with findings by other investigators.[22-24] The observed darker liver and dark spots on the kidneys, relative to those of the control rats, suggest that these organs were adversely affected by higher doses of the plant extract.

Results from the subchronic toxicity studies, suggest that medium- to long-term administration of *D adscendens* had no

adverse effects on the rats. Administration of plant extract over the 6-week period neither produced abnormal physical behaviors nor altered liver, kidney, and body weights, or blood chemistries/biomarkers. Most medicinal plant preparations that are used for therapeutic purposes have been shown to be nontoxic at the doses at which they are used.[25-29]

The biomarkers were tested as indicators for damaged hepatic and/or renal tissues, and therefore suggest that low and medium doses of the *D adscendens* leaves extract did not have adverse effects on the liver and kidney. The extract however affected direct bilirubin concentration at a high dose, which may be due to induction of phase II xenobiotic-metabolizing enzymes (conjugating enzymes).[30-32] The high dose of the extract also resulted in elevated levels of ALT and AST activities suggesting extract-mediated tissue damage. This is consistent with the leaf extract of *D adscendens* being unsafe for use as a therapeutic plant at the high dose.

The administration of *D adscendens* extract decreased zoxazolamine-induced paralysis time and thiopentone-induced sleeping time. These indicated that *D adscendens* could cause herb-drug interactions when co-administered with certain drugs. The observed reduction in paralysis and sleeping times suggest induction of certain isozymes of cytochrome P450 mono-oxygenase that are capable of metabolizing zoxazolamine and thiopentone. These rapid biotransformations of drugs mediated by the responsible CYPs would thus render the drugs ineffective. This observation is consistent with the high microsomal protein concentration level obtained at the high dose of the extract (Figure 3A) and the enhanced pentoxyresorufin-*O*-deethylase activity; an indication of high CYP2B1/2B2 isozymes content/activity (Figure 3D). Both zozaxolamine and thiopentone are known to be metabolized by CYP2B1/2B2 isozymes, and therefore, *D adscendens* would interact with drugs that are metabolized by these isozymes. In contrast, the extract may also alter levels or activities of certain isozymes of CYPs as demonstrated by the inhibition of *p*-nitrophenol hydroxylation (Figure 3B), which is mediated by CYP2E, responsible for the metabolism of isoniazid and other drugs.[33-36] The absence of any significant effects of the plant extract on ethoxyresorufin-*O*-deethylase activity mediated by CYP1A/1A2 isozymes (Figure 3C) would suggest that the plant extract would not impair the clearance of drugs that are metabolized by CYP1A/1A2 isozymes.[14,15]

Conclusion

Acute administration of *D adscendens* leaf extract yielded an LD50 value of 1122 mg/kg body weight, and the extract was found to inhibit CYP2E subfamily of enzymes but had no effect on CYP1A1/1A2 isozymes. While chronic administration of the plant extract was nontoxic, *D adscendens* showed a potential to cause herb-drug interaction, which was demonstrated in the alteration of zoxazolamine-induced paralysis time, thiopentone-induced sleeping time, and liver CYP isozymes activities, when the medicinal plant is administered with other drugs.

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Authors Contributions

OQ designed the project, did the experiments, analyzed the data, and wrote sessions of the manuscript. PC did the experiments, analyzed the data, and wrote sessions of the manuscript. MO did the experiments, analyzed the data, and reviewed the manuscript. LKNO designed the project, analyzed the data, and reviewed the manuscript. AKN designed the project, analyzed the data, and reviewed the manuscript.

Declaration of Conflicting Interests

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Ethical Approval

Approval for the use of animals was obtained from the Animal Experimentation Unit of the Noguchi Memorial Institute for Medical Research.

References

1. Addy ME, Awumey EM. Effects of the extracts of *Desmodium adscendens* on anaphylaxis. *J Ethnopharmacol*. 1984;11:283-292.
2. Asante-Poku S, Sakakibara J, Addy ME. Alkaloids of *Desmodium adscendens*. *Ghana Med J*. 1988;22:111-115.
3. Barreto GS. Effect of butanolic fraction of *Desmodium adscendens* on the anococcygeus of the rat. *Braz J Biol*. 2002;62:223-230.
4. Magielse J, Arcoraci T, Breynaert A, et al. Antihepatotoxic activity of a quantified *Desmodium adscendens* decoction and D-pinitol against chemically-induced liver damage in rats. *J Ethnopharmacol*. 2013;146:250-256.
5. N'gouemo P, Baldy-Moulinier M, Nguemby-Bina C. Effects of an ethanolic extract of *Desmodium adscendens* on central nervous system in rodents. *J Ethnopharmacol*. 1996;52:77-83.
6. Rastogi S, Pandey MM, Rawat AK. An ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum* (L.) DC. and *Desmodium adscendens* (Sw.) DC. *J Ethnopharmacol*. 2011;136:283-296.
7. Coe FG, Anderson GJ. Snakebite ethnopharmacopoeia of eastern Nicaragua. *J Ethnopharmacol*. 2005;96:303-323.
8. Noumi E. Animal and plant poisons and their antidotes in Eseka and Mbalmayo regions, Centre Province, Cameroon. *J Ethnopharmacol*. 2004;93:231-241.
9. Zielińska-Pisklak MA, Kaliszewska D, Stolarczyk M, Kiss AK. Activity-guided isolation, identification and quantification of biologically active isomeric compounds from folk medicinal plant *Desmodium adscendens* using high performance liquid

chromatography with diode array detector, mass spectrometry and multidimensional nuclear magnetic resonance spectroscopy. *J Pharm Biomed Anal*. 2015;102:54-63.
10. Addy ME, Burka JF. Effect of *Desmodium adscendens* fraction 3 on contractions of respiratory smooth muscle. *J Ethnopharmacol*. 1990;29:325-335.
11. McManus OB, Harris GH, Giangiacomo KM, et al. An activator of calcium-dependent potassium channels isolated from a medicinal herb. *Biochemistry*. 1993;32:6128-6133.
12. Baiocchi C, Medana C, Giancotti V, et al. Qualitative characterization of *Desmodium adscendens* constituents by high-performance liquid chromatography-diode array ultraviolet-electrospray ionization multistage mass spectrometry. *Eur J Mass Spectrom (Chichester)*. 2013;19:1-15.
13. Muanda FN, Bouayed J, Djilani A, Yao C, Soulimani R, Dicko A. Chemical composition and, cellular evaluation of the antioxidant activity of *Desmodium adscendens* leaves. *Evid Based Complement Alternat Med*. 2011;2011:620862.
14. Burke MD, Thompson S, Elcombe CR, Halpert J, Haaparanta T, Mayer RT. Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem Pharmacol*. 1985;34:3337-3345.
15. Gokhale MS, Bunton TE, Zurlo J, Yager JD. Cytochrome P450 isoenzyme activities in cultured rat and mouse liver slices. *Xenobiotica*. 1997;27:341-355.
16. Nyarko AK, Ankrah NA, Ofosuhene M, Sittie AA. Acute and subchronic evaluation of *Indigofera arrecta*: absence of both toxicity and modulation of selected cytochrome P450 isozymes in ddY mice. *Phytother Res*. 1999;13:686-688.
17. Anjum F, Raman A, Shakoori AR, Gorrod JW. An assessment of cadmium toxicity on cytochrome P-450 and flavin monooxygenase-mediated metabolic pathways of dimethylaniline in male rabbits. *J Environ Pathol Toxicol Oncol*. 1992;11:191-195.
18. Nyarko AK, Okine LK, Wedzi RK, Addo PA, Ofosuhene M. Subchronic toxicity studies of the antidiabetic herbal preparation ADD-199 in the rat: absence of organ toxicity and modulation of cytochrome P450. *J Ethnopharmacol*. 2005;97:319-325.
19. Addy ME. Some secondary plant metabolites in *Desmodium adscendens* and their effects on arachidonic acid metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 1992;47:85-91.
20. Addy ME, Burka JF. Effect of *Desmodium adscendens* fractions on antigen- and arachidonic acid-induced contractions of guinea pig airways. *Can J Physiol Pharmacol*. 1988;66:820-825.
21. François C, Fares M, Baiocchi C, Maixent JM. Safety of *Desmodium adscendens* extract on hepatocytes and renal cells. *Protective effect against oxidative stress.* J Intercult Ethnopharmacol. 2015;4:1-5.
22. Bañuelos Pineda J, Nolasco Rodríguez G, Monteon JA, García López PM, Ruiz Lopez MA, García Estrada J. Histological evaluation of brain damage caused by crude quinolizidine alkaloid extracts from lupines. *Histol Histopathol*. 2005;20:1147-1153.
23. D'Angelo LC, Xavier HS, Torres LM, Lapa AJ, Souccar C. Pharmacology of *Piper marginatum* Jacq. a folk medicinal plant used as an analgesic, antiinflammatory and hemostatic. *Phytomedicine*. 1997;4:33-40.
24. Melita Rodríguez S, Castro O. Pharmacological and chemical evaluation of *Stachytarpheta jamaicensis* (Verbenaceae) [in Spanish]. *Rev Biol Trop*. 1996;44:353-359.
25. Anusuya N, Raju K, Manian S. Hepatoprotective and toxicological assessment of an ethnomedicinal plant *Euphorbia fusiformis* Buch.-Ham.ex D.Don. *J Ethnopharmacol*. 2010;127:463-477.
26. Modarresi M, Hosseinzadeh L, Nematy N, Siavash-Haghighi ZM, Ghanbari K. Acute and subchronic toxicological evaluation of *Stachys lavandulifolia* aqueous extract in Wistar rats. *Res Pharm Sci*. 2014;9:165-172.
27. Mohamed EA, Lim CP, Ebrika OS, Asmawi MZ, Sadikun A, Yam MF. Toxicity evaluation of a standardised 50% ethanol extract of *Orthosiphon stamineus*. *J Ethnopharmacol*. 2011;133:358-363.
28. Wongcome T, Panthong A, Jesadanont S, Kanjanapothi D, Taesotikul T, Lertprasertsuke N. Hypotensive effect and toxicology of the extract from *Coscinium fenestratum* (Gaertn.) Colebr. *J Ethnopharmacol*. 2007;111:468-475.
29. Ye BG, Feng Y, Wang S. Scientific evaluation of the acute toxicity and 13-week subchronic toxicity of *Rheum emodi* rhizome extracts in Sprague Dawley rats. *Food Chem Toxicol*. 2014;66:278-285.
30. Ahmad I, Shukla S, Kumar A, et al. Biochemical and molecular mechanisms of *N*-acetyl cysteine and silymarin-mediated protection against maneb- and paraquat-induced hepatotoxicity in rats. *Chem Biol Interact*. 2013;201:9-18.
31. Cheng YH, Huang SC, Lin CJ, Cheng LC, Li LA. Aryl hydrocarbon receptor protects lung adenocarcinoma cells against cigarette sidestream smoke particulates-induced oxidative stress. *Toxicol Appl Pharmacol*. 2012;259:293-301.
32. Ozawa S. Interindividual differences in efficacy and toxicity induced by therapeutic drugs and xenobiotics in relation to genetic polymorphisms in xenobiotic metabolizing enzymes [in Japanese]. *Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku*. 1998;116:69-81.
33. Mikstacka R, Gnojkowski J, Baer-Dubowska W. Effect of natural phenols on the catalytic activity of cytochrome P450 2E1. *Acta Biochim Pol*. 2002;49:917-925.
34. Pekthong D, Martin H, Abadie C, et al. Differential inhibition of rat and human hepatic cytochrome P450 by *Andrographis paniculata* extract and andrographolide. *J Ethnopharmacol*. 2008;115:432-440.
35. Zamaratskaia G, Zlabek V. Para-nitrophenol hydroxylation by fish liver microsomes: kinetics and effect of selective cytochrome P450 inhibitors. *Fish Physiol Biochem*. 2011;37:969-976.
36. Zerilli A, Ratanasavanh D, Lucas D, et al. Both cytochromes P450 2E1 and 3A are involved in the O-hydroxylation of *p*-nitrophenol, a catalytic activity known to be specific for P450 2E1. *Chem Res Toxicol*. 1997;10:1205-1212.