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Publication Date

2010-02-02

Peer reviewed

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Introduction

Non-insulin-dependent diabetes mellitus (NIDDM) disproportionately affects Hispanics in the United States (1). Amid growing rates of U.S. immigration, patients bring health practices common to their land of origin such as the use of folk dietary therapies (2-4). Currently a total of 306 herbal species have records of popular use for NIDDM in México alone (5). Among the various herbs used in Mexican folk medicine exists several plants from the *Rubiaceae* family, known for their glycemic lowering properties (6). These include the *Hintonia latiflora (HLA)*, H*intonia standleyana (HSL), Exostema caribeaum (EC),* and the *Exostema mexicanum (EM)* species. They are collectively branded by consumers with synonyms such as copalchis, copalquin, jutetillo, or campanilla (7). Numerous pharmacological animal studies have reported antihyperglycemic properties of copalchi extracts, making them potential therapeutic agents for patients with NIDDM (8-18).

This paper discusses five recent animal studies evaluating the glycemic lowering effects of copalchis (mainly *HLA*) on streptozotocin (STZ)-induced diabetic rats (*Table. 1*). Four of these five studies have analyzed extracts to isolate and characterize the major active compounds (*Fig. 2*). Several toxicity studies have also assessed the safety profile of copalchi extracts in mice (*Table 2*). Studies evaluating therapeutic response in patients with NIDDM have been reported since 1953, this paper discusses one recent clinical trial (25).

Major Active Principles

Pharmacological studies suggest 4-phenylcoumarins glycosides, cucurbitacins glycosides, and coutareagenin as the major active markers derived from copalchi extracts (10,12,14,19). Chromatographic fractionation, spectrometric and NMR spectroscopic techniques have led to the isolation and characterization of eight 4-phenylcoumarin active compounds from the *HLA* species (compounds **1-8**, *Fig. 1*). In addition to 4-phenylcoumarins, *HLA* leaf extracts contain ursolic acid and desoxycordifolinic acid, two compounds recognized for stimulating glucose uptake in alloxan-induced diabetic rats and enhancing insulin receptor sensitivity through phosphorylation (20-21). Another substance isolated from *HLA* leaf extracts is chlorogenic acid, a compound shown to reduce plasma glucose peak on oral glucose tolerance tests (22). Chlorogenic acid in separate studies has also shown promise as a glycemic index lowering agent for lowering the risk of developing insulin dependent diabetes mellitus (23-24). The neoflavonoid coutareagenin is an additional active principle present in *HLA* bark stem extracts; when isolated and utilized to treat STZ-induced diabetic rats, coutareagenin produces marked reductions in blood sugar levels (14).

In contrast to *HLA*, experiments utilizing only *HSL* bark stem extracts indicate the active principles of copalchis to be not only 4-phenylcoumarins but also cucurbitacins (compound **9**, *Fig. 1*). The isolated cucurbitacin glycoside, when administered at doses of 10mg/kg or 30 mg/kg, significantly reduces blood glucose levels in STZ-induced diabetic rats. In addition to reports that have led to isolation and characterization of active compounds, the development of quality control procedures to monitor the presence of active compounds in copalchi products for potential regulatory measures has been undertaken (19).

Potentially Active Ingredients Isolated from H. latiflora and H. standleyana

1	R₁ = Ac	R₂ = -β-D-glucopyranosyl
1a	R₁ = Ac	R₂ = H
2	R₁ = H	R₂ = -β-D-glucopyranosyl

3	R₁ = Me	R₂ = -β-D-glucopyranosyl
4	R₁ = Me	R₂ = -β-D-galactopyranosyl
5	R₁ = H	R₂ = -β-D-glucopyranosyl
6	R₁ = Me	R₂ = -[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]
7	R₁ = Me	R₂ = -[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]

cucurbitacin glycoside

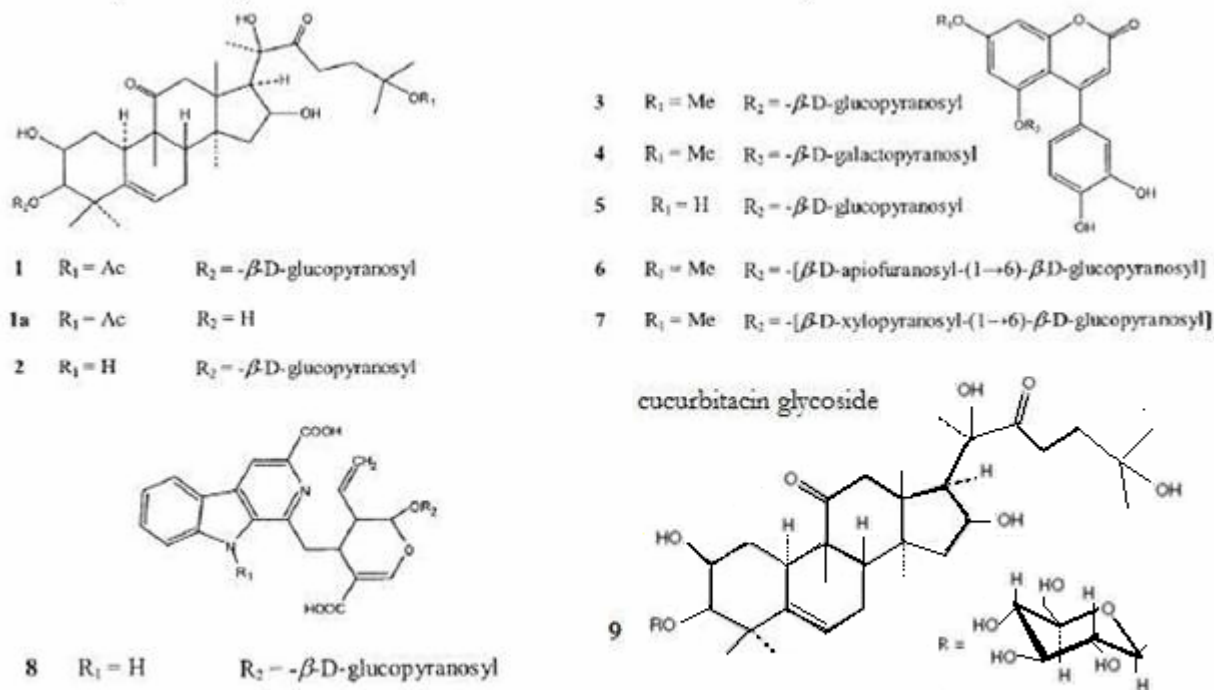|		
8	R₁ = H	R₂ = -β-D-glucopyranosyl

Figure 1. Represented above are eight 4-Phenylcoumarin compounds (1-8) isolated from HLA bark stem extracts in addition to a cucurbitacin glycoside isolated from HSL barks stem extracts. Adapted by permission from Elsevier: Phythochemistry (2007).

Animal Research

To evaluate antidiabetic properties of copalchis, all five animal studies employed an experimental design using a diabetes model with STZ-induced diabetic rats for comparison with normoglycemic rats. The STZ model functions by selectively destroying pancreatic β-cells while ensuring that some β-cells remain viable by administering only a low dose. To measure efficacy, an enzymatic glucose oxidase method determined plasma glucose after oral or intragastric administration of different extract doses over a period of several hours. To evaluate potential mechanisms of action, one study analyzed baseline and post-treatment plasma insulin levels and post-mortem hepatic glycogen content of rats (10). The control groups of the studies included saline-treated rats and two studies used a positive control using glibenclamide for hypoglycemic comparison. Four of the five studies assessed *HLA* extracts, two evaluated *HSL* extracts, and two studies also utilized the *EC* species (*Table.* 1).

Table 1. Summary of Animal (rat) Studies

Author/year	Copalchi Species	n	Mode of Admin.	Dose	Results (hypoglycemic effect on STZ-induced diabetic rats)
Cristians, et al (2009)	*H. latiflora*	36	intragastric	300mg/kg	- Crude extract produced -69% at 9h.
				100mg/kg	- Crude extract produced -62% at 9h.
	H. standleyana			300mg/kg	-Crude extract produced -32% at 7h.
				100mg/kg	-Crude extract produced -40% at 7h.
Guerrero-Analco, et al (2007).	*H. latiflora*	66		300mg/kg	-Crude extract produced -33% at 9h.
				100mg/kg	-Crude extract produced -21% at 9h
				30mg/kg	-Crude extract produced -16% at 9h.
				10mg/kg	-Crude extract produced -15% at 9h
	E. caribaeum	66	Intragastric	300mg/kg	-Crude extract produced -18% at 7h.
				100mg/kg	-Crude extract produced -16% at 7h
				30mg/kg	-Crude extract produced -11% at 5h.
				10mg/kg	- Crude extract produced -9% at 5h
	HLA and EC mix	66		300mg/kg	-Crude extract produced - 27% at 5h.
				100mg/kg	-Crude extract produced -17% at 9h
				30mg/kg	-Crude extract produced -13% at 7h.
				10mg/kg	- Crude extract produced -5% at 9h
Guerrero-Analco, et al (2005)	*H. standleyana*	90	intragastric	100 mg/kg	Crude extract produced -32% at 9h.
				10mg/kg	-Crude extract produced -17% at 9h.
Pinto, et al (1997)	Sucontral® (*H. latiflora* / *E. caribaeum* mix)	60	Oral	Altramin chow enriched with Copalchi dry substance at 0.028% m/m or at 0.28% m/m	20-25% decrease in blood glucose between 7th and 15th day of treatment with subsequent plateau.
Korec, et al. (2000)	*H. latiflora*	21	Intragastric	200 mg/kg	-crude extract produced -42% at 4h
				100mg/kg	-crude extract produced -36% at 4h
		13	Oral	100mg/kg	-solution produced -40% after 3 days

Guerrero-Analco investigated antihyperglycemic effects of *HLA* organic extracts on male rats. Low dose intraperitoneal streptozotocin (STZ, 50 mg/kg) was injected to simulate a non-insulin dependent diabetes state (glucose >250mg/dl) for comparison with normoglycemic rats (10). A vehicle-treated (saline) group was designed as a control, and a positive control group was also established by having rats treated with a known antidiabetic drug (glibenclamide, 10mg/kg). The *HLA* extract was given intragastrically twice a day, for a period of 30 days. In normoglycemic animals, *HLA* extract produced a -28% hypoglycemic effect at doses of 300mg/kg at 9h. In the diabetes model, the highest antihyperglycemic effect was observed at 9h with doses of 100mg/kg and 300mg/kg (-21.0% and -33.4%, respectively). The glycemic lowering effect of *HLA* extract was comparable to the glibenclamide group, which demonstrated a maximum effect (-32%) at 7 h in normoglycemic animals and at 5 h in STZ-induced diabetic rats (-35%).

To elucidate antihyperglycemic mechanisms of the active compounds, plasma insulin levels were measured among experimental and control groups. In comparison to plasma insulin levels of diabetic controls (1ng/ml), the *HLA* and *HSL* treated groups demonstrated insulin levels of 6.5ng/ml and 5.5ng/ml, respectively (*Fig. 2*). These results indicate *HLA* and *HSL* extracts increase plasma insulin in STZ-induced diabetic rats. Due to incomplete destruction of pancreatic beta cells in the STZ model, the presence of an insulin-release stimulatory effect on remaining β-cells is likely, or conversely, a recovery of *incompletely* damaged β-cells. However, authors speculate that an insulinomimetic effect may also be occurring. When hepatic glycogen content was measured post mortem, findings revealed improved hepatic glycogen content in STZ-diabetic rats (*Fig. 2*). When an *HLA* treated group (n=6) was analyzed for hepatic glycogen content, the treatment group possessed a mean of 42mg/g in wet tissue compared to 29mg/g in the diabetic control group (n=6). Authors propose that 4-phenylcoumarins alter glycogen breakdown by inhibiting the key enzyme catalyzing the gluconeogenesis and glycogenolysis reactions, glucose-6-phosphatase (G6Pase). They also propose an alternate mechanism whereby the active compounds trigger increased glucokinase activity thereby altering glucose metabolism.

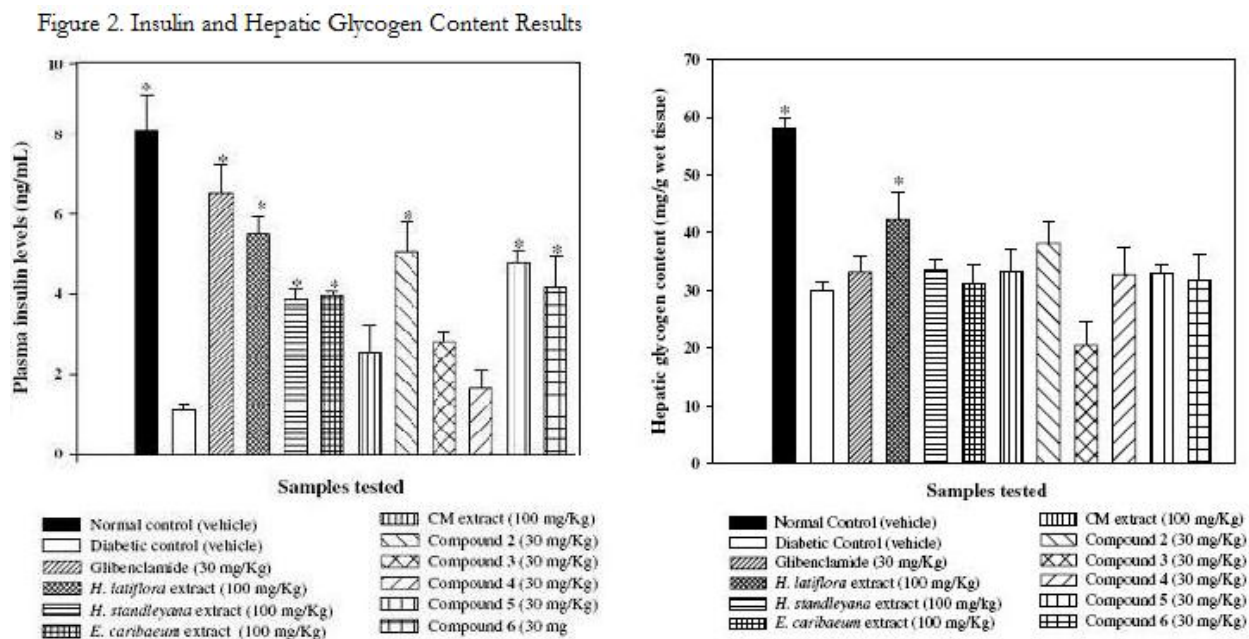Figure 2. Insulin and Hepatic Glycogen Content Results

Figure 2. The graph on left illustrates improved plasma insulin levels of HLA and HSL treated rats compared to diabetic, and glibenclamide treated controls. Right graph suggests enhanced glycogen content of HLA and HSL treated rats compared to controls. Adapted with permission from Elsevier, Copyright (2007).

In a different study, Guerrero-Analco reported decreased blood glucose levels from baseline in rats treated with *HSL* extracts (100mg/kg) using three different experimental conditions: normoglycemic, STZ-induced diabetes, and a "developing diabetes condition" (11). The latter two scenarios represented long term subacute experiments. The "developing diabetes condition" models the progressive hyperglycemia occurring in untreated diabetes where blood glucose values gradually increase over time. Results indicate that crude *HSL* extracts (100mg/kg) produce glycemic lowering activity in normoglycemic rats (-24% at 5h) and diabetic rats (-32% at 9h). From the active extracts, a cucurbitacin glycoside was isolated (compound **9**, *Fig. 1*), which at doses of 10mg/kg or 30 mg/kg, reduced blood glucose levels in STZ-induced diabetic

rats to near normal values. In the developing diabetes condition, daily administration of the cucurbitacin also prevented the increase of blood glucose levels in comparison to controls which showed a worsening diabetes state. The antihyperglycemic effect occurred in a dose-dependent manner similar to glibenclamide.

Due to commercial exploitation, copalchis have been placed in danger of extinction. This motivated study by Cristians to determine if the leaves and not solely the bark stem possess antidiabetic properties, providing an alternate formulation that can protect the plant (12). Different groups of normoglycemic and STZ-induced diabetic rats were administered extracts of *HSL* and *HLA* leaves according to the protocol that Guerrerro-Analco employed (13). Each crude leaf extracts of *HLA* and *HSL* (300mg/kg) produced antihyperglycemic effects in STZ-induced diabetic rats, quantified at 9h as -68% and -39%, respectively. From the leaf extracts of *HSL*, two new phenylcoumarins were isolated and characterized, which when administered alone, significantly decrease blood glucose levels in normoglycemic rats (-30.6% at 9h) and STZ diabetic rats (- 37% at 9h). Results indicate that both the bark stem and leaves possess antidiabetic properties.

Since 1914 a copalchi product (*HLA* and *EC* mixture) has been commercialized for human use under the trade name Sucontral® in Germany (30). Pinto evaluated the hypoglycemic effects of Sucontral® in adult Wistar rats in a 30-day randomized, placebo-controlled, parallel-comparative study where Sucontral® was compared to a standard diet, altromin animal feed (13). Results indicate hypoglycemic efficacy, where the onset of action was observed between treatment days 1 and 3, and a 25% reduction from baseline was achieved by the fifteenth day.

Korec reported glycemic lowering effects in STZ-induced diabetic rats after oral and intragastric administration of crude *HLA* barks stem extracts, and further confirmed that coutareagenin alone, a compound present in *HLA* extracts, also produces antihyperglycemic effects (14). Blood sugar levels were recorded at 2h and 4h post-treatment using glucose oxidase-peroxidase chromogen method. Results using intragastric *HLA* doses of 100mg/kg and 200mg/kg, showed decreased blood glucose values from baseline at 4h by -36% and -42%, respectively. The oral mode of administration similarly lowered sugar levels by -40% compared to baseline. Pure synthetic coutareagenin is insoluble in water; as a result, the compound was administered in a glycemic neutral solvent, propylene glycol (PRG) or macrogol (PEG). Oral coutareagenin-PRG mixture produced 27-29% glycemic reduction from baseline whereas the coutareagenin-PEG mixture caused 17-21% antihyperglycemic effect in diabetic rats.

In addition to the five pharmacological studies previously discussed, other studies conducted by German researchers have been carried out on rabbits and mice (15-18). In these experiments, all animal species demonstrated an antihyperglycemic response to treatment with *HLA* bark stem extracts. Analogous to the animal studies described in *Table 1* which demonstrated increased efficacy at higher doses, the results from these other experiments suggest a dose-effect relationship.

Research on Humans
The only clinical study reported in the *U.S. National Institutes of Health (NIH)* registry, a phase III interventional trial, suggests antidiabetic efficacy and good safety profile of *HLA* (Sucontral®, oral, drug-extract ratio 1:4.5, extraction solvent 32 % ethanol) in patients with NIDDM (25). However, the methodology raises major concerns, with the study characterized as single center, non-randomized, open label, uncontrolled, with single group assignment and a small sample size of 30 patients. The primary outcome measure for the study was HbA1c levels.

Authors reported that mean HbA1c decreased by 10.4% to 12.4% and remained stable for the duration of the 36-month study (25). Although this study appears to support the use of this Mexican plant for NIDDM, the efficacy of *HLA* lacks validation through randomized, double-blind and placebo-controlled human trials. There are additional published studies performed in Germany, not cited in the NIH registry, which have reported glycemic lowering effects of Sucontral® in patients with NIDDM (26-32).

Toxicity Survey
A total of 3 animal studies have examined toxicity in mice using standard procedures such as the Lorke Method, Ames test, and the brine shrimp lethality bioassay (*Table. 2*). Deciga-Campos reported an LD_{50} for the *HLA* stem bark (2.85 g/kg), and an *Artemia salina* lethality CL_{50} (719 µg/mL), suggesting that *HLA* is non-toxic in mice according to the Lorke method criteria (33). The brine shrimp test criteria suggest *HLA* extracts possess weak toxicity. Additionally, Ames tests confirmed *HLA* extracts as non-mutagenic in mice. Cristians reported an LD_{50} (1.67g/kg) for *HLA* leaf extracts with no evidence of behavioral alterations, wounds, or hemorrhage of internal tissues and organs in mice, suggesting non-toxicity according to Lorke criteria (12). In a separate study by Zhang, ursolic acid, a hypoglycemic compound identified in *HLA* leaf extracts, was determined to be non-toxic according to LD_{50} tests (21). Single-cell gel electrophoresis was also employed to assess DNA damage in ursolic acid treated mice; results indicate non-genotoxicity.

Table 2. Toxicity Survey

Author(s)	Species	LD50	Brine shrimp lethality bioassay, (CL50)	Geno-toxicity
Deciga-Campos, et al. (2007)	*HLA* stem bark HSL stem bark	2.85 g/kg; >5g/kg	719 µg/mL >1000 µg/mL	Non-mutagenic (Ames test)
Cristians, et al (2009)	*HLA* leaf extract HSL leaf extract	1.67 g/kg 5 g/kg	n/a	n/a
Zhang, et al (2006)	Ursolic acid, compound present in *HLA*	no toxic effect was found at up to 10X the effective dose	n/a	No DNA damage (Single-cell gel electrophoresis)

Additional toxicity studies performed by German researchers have been carried out on rabbits, mice, and rats (15,34-36). Result showed rabbits were devoid of discernable damage. The histological examination of organs in mice revealed no injury, and genotoxicity tests on primary hepatocytes of mice were negative. Chronic toxicity tests on rats revealed no histological damage to organs.

The human clinical trial evaluating Sucontral® reported no adverse drug reactions (ADR) on cardiac and liver functions tests and no adverse hypoglycemic reactions; however, since the trial was not designed as randomized, double blind and placebo-controlled, valid toxicity studies have yet to be performed on humans. In Spain, Bruguera published a case report describing five

cases of patients presenting with acute hepatitis during treatment with copalchis lasting several months at unknown doses, which resolved with discontinuation of the drug (37). A subsequent literature review by the same authors revealed six other reported cases of copalchi-induced hepatotoxicity in humans.

Conclusion
Active compounds in copalchis include 4-phenylcoumarins, cucurbitacins, coutareagenins, ursolic acid and chlorogenic acid. Five separate controlled studies employing the STZ-induced diabetes model have demonstrated the glycemic lowering efficacy of *HLA* and *HSL*. The antihyperglycemic effects are purported to be caused by improved pancreatic insulin secretions as confirmed by plasma insulin levels of diabetic rats. Hepatic glycogen content of treatment groups also demonstrated marked improvement, suggesting that copalchis may regulate glycogen metabolism. Results from studies utilizing the same animal species differed from one another possibly due to variations in age, sex and diet of the animals, but all five studies herein discussed reported antihyperglycemic effects of 5% to 69% from baseline at doses ranging from 10mg/kg to 300mg/kg.

With only one phase III clinical trial reported in the literature, evidence on the effectiveness of copalchis in providing therapeutic benefit is limited. The results of the trial demonstrate a therapeutic response as measured by decreased HbA1c values (-12.4%), but external validity remains to be addressed as the treatment consisted of Sucontral®, which differs from the common formulation of hot infusions. Furthermore, the trial was uncontrolled and lacked randomization. Toxicity studies involving mice, rats, and rabbits suggest a good safety profile, however, controlled toxicity studies in humans are lacking. While findings confirm the hypoglycemic effects of this Mexican plant, questions remain about the therapeutic index, optimal dosage, and potential adverse drug reactions in humans. Further research in these areas is advisable. In light of rising costs of prescription drugs, increasing commercialization of medicinal plants and the large percentage of patients seeking alternative therapies, it is suggested that healthcare professionals become aware of the consumption and the limited therapeutic evidence supporting copalchis in the treatment of NIDDM.

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