Mistletoe (viscum album L.) aqueous extract coronary vasodilation is mediated through nitric oxide/soluble guanylyl cyclase pathway

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Introduction: It has been estimated that 17 million people dies every year by a cardiovascular disease and 600 million people around the globe are affected by arterial hypertension, being this main risk factor for developing arterial coronary disease, heart failure, cerebral vascular disease and renal damage. Extracts from Viscum album L., commonly known as mistletoe, have been ethnomedically used as vasodilator. However, there are no reports regarding neither its action mechanism nor the chemical compound responsible of this pharmacological effect.

Materials and methods: The pharmacological effect of a mistletoe aqueous extract was evaluated on the Langendorff isolated and perfused heart model in male guinea pigs hearts, where coronary vascular resistance, left intraventricular pressure, nitric oxide release in the perfusion liquid and cyclic guanosine monophosphate production in ventricular tissue were recorded, in absence and presence of blockers and inhibitors such as 3 m M gadolinium (III) chloride (G3C), 100 m M NW-nitro-\(L\)-arginine methyl ester (\(L\)-NAME) and 10 m M 1\(H\)-[1,2,4]oxadiazolo[4,2-\(a\)]quinoxalin-1-one (ODQ).

Results: The aqueous mistletoe extract exerts a significant decrease in the coronary vascular resistance that ongoes with significant increases in nitric oxide and cyclic guanosine monophosphate release, the latter being both inhibited by the presence of blockers and inhibitors as well. In addition, this extract shows a positive inotropic effect, a pharmacological effect not previously reported in the literature, which is probably mediated by tyramine by means of a \(\beta 1\) adrenergic stimulation. In another group of animals, hypertension was induced by chronic blockade of nitric oxide synthesis by administrating NW-nitro-\(L\)-arginine methyl ester (70 mg/kg, i.p.). In these animals, Viscum album L. aqueous extract partially reverts hypertension induced by chronic inhibition of nitric oxide synthesis.

Conclusion: the coronary vasodilation showed by the extract is mediated by the nitric oxide/soluble guanylyl cyclase pathway, supporting the ethnomedical use given to this plant.

INTRODUCTION
It is estimated that 600 million people around the globe are affected by systemic arterial hypertension [1-3] which is associated with atherosclerosis, cardiovascular disease and deleterious effects on endothelial cells [4-6]. Current evidence about this pathology, shows that it increases oxidative stress, causes endothelial damage, and can also be related to disbalances in constriction/dilation, proliferative/antiproliferative, thrombosis/antithrombosis and fibrinolysis/antifibrinolysis mechanisms [5,6]. In addition, there are new evidences about systemic inflammation role in the pathogenesis of cardiovascular diseases where endothelium plays a key role on blood flow regulation, fibrinolysis and inflammation. By such, changes in the endothelial
function could partially explain the association between inflammation and the development of a cardiovascular disease [7-10].

*Viscum album* L. (Loranthaceae), commonly known as mistletoe, is an evergreen hemiparasitic plant widely distributed in the world. It has been reported that extracts from this plant possess a vast array of pharmacological activities (eg. hypotensive, vasodilator, cardiac depressive, sedative, antispasmodic, anticancer, antidiabetic and antioxidant [11-14]. Because it has been previously reported by our group [15] that *V. album* aqueous extracts showed a vasodilator activity on the Langendorff isolated and perfused heart model, mediated by increases in nitric oxide (NO) production, this study is an attempt to establish if coronary vasodilator effect elicited by such extracts is mediated through the nitric oxide/soluble guanylyl cyclase (NO/sGC) pathway.

**MATERIALS AND METHODS**

**Plant material**
The plant material was acquired, identified and authenticated as previously described [15].

**Preparation of the extract**
The fresh leaves were chopped into small pieces and heat-dried. Two hundred grams of the dried leaves were macerated with 80% aq. EtOH during 1 week, filtered and concentrated *in vacuo* (yield: 8.3%). Further on, the residue was diluted with water and filtered, at the moment of its use, by syringe filter (Membra-Fil Mixed Cellulose Ester, 0.22 mm, Whatman Ltd., London, England), as described [15].

**Animals**
Male guinea pigs (900-950 g), maintained at the Animal Facility of the National Institute of Cardiology "Ignacio Chávez" were used. They were housed under standard conditions of temperature and light (12-h light/dark cycle) and fed with standard diet (LabDiet 5026, PMI Nutrition International) and water *ad libitum*.

**Vasodilator activity of *V. album* aqueous extract**
Evaluation of the vasodilator activity of *Viscum album* aqueous extract was performed on the Langendorff isolated and perfused heart model, as previously described [16,17]. Under this model, we recorded the contraction force of the heart by inserting a latex balloon in the left ventricle, which was connected to a hydropneumatic transducer (Statham Instruments, Inc. 7320, Statham Instruments Inc., Hato Rey, Puerto Rico), and the coronary perfusion pressure with a pressure transductor (Gould P23ID, Gould Instruments, Cleveland, Ohio, USA). Coronary vascular resistance was calculated as stated [19]. Both variables were recorded by a polygraph (Grass 79-D, Grass Instruments Co., Quincy, Massachusetts, USA). Cardiac rate was kept constant to 1 Hz by stimulating with a ventricular epicardic pacemaker (Grass-SIU5, Grass Instruments Co., Quincy, Massachusetts, USA). The extract and drugs were continuously infused at a rate of 0.3 mL/min by means of an infusion pump (SP200i, World Precision Instruments, Inc., Sarasota, Florida, USA) connected into a dosificator (Hamilton, Hamilton Company, Reno, Nevada, USA) adjacent to the perfusion cannula.

**Biochemical estimations**
In order to evaluate if coronary vascular activity showed by *V. album* extracts could be mediated by the NO/sGC pathway, various groups of isolated and perfused guinea pig hearts were set. In one group, *V. album* aqueous extract, at a dose of 450 mg, was continuously infused alone. In other groups of animals, the extract was simultaneously with inhibitors or blockers such as: (1) a blocker of stretch-activated ion channels and calcium recapture [18], 6 mM gadolinium (III) chloride (Sigma Chemical Co., St. Louis, MO, USA), an inhibitor of NOS activity [19], 100 mM Nω-nitro-L-arginine methyl ester (L-NAME) (Sigma Chemical Co., St. Louis, MO, USA) and a specific inhibitor of the NO/sGC pathway [20], 10 mM 1H-[1,2,4]oxadiazolo[4,2-a]quinoxalin-1-one (ODQ) (Sigma Chemical Co., St. Louis, MO, USA). Under these conditions, we estimated: (1) NO release in the perfusion liquid by the method of Kelm and Schrader [21], (2) cGMP release in
ventricular tissue simples under the method of Hagen et al. [22], and (3) NOS-2 and NOS-3 expression in ventricular tissue simples by the methods of Shah [23] and Laemmli [24].

**Statistical analysis**
The results are expressed as mean ± S.E.M. Statistical analysis of difference between groups was evaluated by one-way ANOVA followed by Student's t test [25].

**RESULTS AND DISCUSSION**
Continuous infusion of *V. album* aqueous extract, at a dose of 450 mg, significantly decreases coronary vascular resistance (CVR), when compared to control group (36 ± 2 vs. 15.8 ± 1.96 dyn s cm⁻⁵) (Fig. 1). This decrease in CVR is reverted when blockers and inhibitors such as 6 m M gadolinium (III) chloride, 100 m M *L*-NAME and 10 m M ODQ are simultaneously infused with mistletoe aqueous extract, observing a greater vasoconstrictor effect with ODQ (141 ± 1.86 vs. 15.8 ± 1.96 dyn s cm⁻⁵), confirming the fact that the coronary vasodilator effect showed by this extract is mediated by increases in NO production, with a significant role of the NO/sGC pathway. These findings are consisting with increases in NO and cGMP production (Figs. 2 and 3). Infusion of *V. album* aqueous extract increases NO production, when compared with control group, from 6.2368 ± 2.4927 to 147.9522 ± 2.7901 pmol, an effect clearly abolished by the simultaneous administration of these noxious agents. Because NO can act as an activator of the sGC, increases of NO, if the vasodilator effect of this extract could be mediated by the NO/sGC pathway, cGMP production might increase (Fig. 3). Again, mistletoe aqueous extract significantly increases cGMP production, when compared to control group (43.94 ± 2 vs. 74.81 ± 1.96 pmol/mg of tissue), production that is significantly decreased by the blocking and inhibiting effect of 6 m M gadolinium (III) chloride, 100 m M *L*-NAME and 10 m M ODQ (9.81 ± 2; 21.04 ± 2.24; 8.92 ± 1.86 pmol/mg of tissue, respectively). These findings are well correlated with previously published reports regarding vasorelaxation properties showed by *Rubus idaeus* L. [26], green tea [27], epigallocatechin-3-gallate [28], where an endothelial-dependent vasorelaxation mechanism was clearly evident. These increases in NO production are in concordance with NOS-2 and NOS-3 overexpression (Fig. 4). *Viscum album* aqueous extract causes increases in NOS-2 and NOS-3 expression (4.6487 and 7.8914, respectively), an increase that is not observed when 100 m M *L*-NAME, 6 m M GdCl 3 and 10 m M ODQ are simultaneously infused with mistletoe extract. This overexpression could be induced mainly by flavonoids such as quercetin, rutin, luteolin and cyranoside, which can act as NOS-2 and NOS-3 inducers [29,30].
Figure 1: *Viscum album* aqueous extract effect on coronary vascular resistance (CVR) in isolated and perfused guinea pig hearts according to Langendorff. *P<0.05* vs. control group; value represents mean ± MES; N=10, *P<0.05*, one-way ANOVA followed by Student's *t*-test.

Figure 2: *Viscum album* aqueous extract effect on nitric oxide production in isolated and perfused guinea pig hearts according to Langendorff. *P<0.05* vs. control group; value represents mean ± MES; N=10, *P<0.05*, one-way ANOVA followed by Student's *t*-test.

Figure 3: *Viscum album* aqueous extract effect on cGMP production in isolated and perfused guinea pig hearts according to Langendorff. *P<0.05* vs. control group; value represents mean ± MES; N=10, *P<0.05*, one-way ANOVA followed by Student's *t*-test.
CONCLUSIONS
From the above results, we can confirm that the coronary vasodilator effect elicited by V. album aqueous extract is mediated by the NO/sGC pathway, supporting the ethnomedical use given to this plant as a vasodilator. However, future research needs to be focused on the isolation, purification, structural elucidation and pharmacological reevaluation in order to find the chemical compound responsible of such pharmacological effect.

BIBLIOGRAPHY