

*Full Length Research Paper*

# **The Mexican mistletoe *Struthanthus venetus* (HBK Blume) inhibits proliferation and synergizes antagonistic actions of Tamoxifen and Fulvestrant in breast cancer MCF-7 cells**

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Mistletoes are hemi-parasitic plants widely distributed around the world, used in folk medicine to treat many diseases including diabetes, hypertension, menopausal syndrome and as complementary or adjuvant treatment for cancer. The objective of the present work was the evaluation of oestrogenic activity of hydromethanolic extract of mistletoe *Struthanthus venetus* dried leaves (StvHME), to evaluate its potential benefits in menopause and breast cancer, which have not been reported so far. Uterotrophic activity was evaluated in immature female CD1 mice, administering StvHME (10, 100 and 500 mg/kg) for three consecutive days compared to the natural hormone 17 $\beta$ -oestradiol (E<sub>2</sub>, 10  $\mu$ g/kg). Comparison of the MCF-7 positive oestrogen receptor human breast adenocarcinoma cell proliferation in the presence of StvHME (0.5, 5 and 50  $\mu$ g/ml) or E<sub>2</sub> (10<sup>-12</sup>-10<sup>-10</sup>M) related to untreated control cells was assessed using MTT cell viability assay. StvHME, produced biphasic effects in mice uteri; low doses (10 mg/kg) decreased uterine weight (15-38%;  $p < 0.05$ ), while a higher dose (500 mg/kg) increased uterine weight (28-36%;  $p < 0.05$ ). StvHME concentrations tested inhibited MCF-7 cell proliferation, contrasting with E<sub>2</sub> which increased it. StvHME (50  $\mu$ g/ml) antagonized the proliferative response to E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) behaving as an anti-oestrogen. The antiproliferative response of StvHME (50  $\mu$ g/ml) showed synergism with the oestrogen antagonists Tamoxifen and Fulvestrant (ICI 182,780). Our results suggest the presence of oestrogenic and anti-oestrogenic components in the StvHME that could be acting through the oestrogen ER $\alpha$  and ER $\beta$  receptors. Therefore, StvHME has potential utility as a complementary therapy for breast cancer.

**Key words:** mistletoe, *Struthanthus venetus*, MCF-7 cells, oestrogenicity, anti-oestrogenic, anti-cancer, menopause.

## **INTRODUCTION**

Breast cancer is one of the leading causes of death among women in the world. Recently it has been

estimated that the incidence of all-cancer cases, including breast cancer, will almost double by 2030 (Bray

et al., 2012). Increased population aging due to the rise of life expectancy also increases the possibility of cancer incidence. As a result of a longer life span, the menopausal women population will also increase, coping with health disorders for a longer period (Angioli et al., 2018). During menopause, oestrogen deficiency induces vasomotor symptoms, hyperlipidemia, osteoporosis, and cardiovascular disease. Hormone replacement therapy (HRT) with exogenous hormones currently used to relieve menopausal symptoms, has proven to be effective in alleviating some of them such as hot flushes, night sweats, dyspareunia, sexual disorders, insomnia, and preventing osteoporosis (Rozenberg et al., 2013). Nonetheless, there is great controversy in recent menopausal population studies using HRT that have warned about the incidence of adverse effects, including increased risk of ischemic stroke, venous thromboembolism, and breast cancer. Because of the high risk among HRT users, the therapy is not allowed to be used for long periods, particularly in predisposed patients (NICE guideline NG23, 2015). Thus, the search for effective and safer treatments for menopause continues to be a priority.

Plants and herbs have reached an important approach as complementary or alternative treatment for many diseases and are considered to be important for the development of new strategies for both HRT and cancer. Numerous naturally occurring phytochemicals have recently gained interest as potential therapeutic breast cancer agents, which appear to directly affect oestrogen-dependent and oestrogen-independent breast cancer cell proliferation (Israel et al., 2018).

Mistletoes are widely used since ancient times in folk medicine of many cultures to treat diseases including diabetes, hypertension, cancer and to prevent menopausal syndrome (López-Martínez et al., 2013; Omeje et al., 2014). They are hemi-parasitic plants widely distributed around the world. Taxonomically, they belong to the families Misodendraceae, Loranthaceae, Santalaceae, Viscaceae among others, which are grouped in the order Santalales (Lim et al., 2016; Patel and Panda, 2014). The mistletoe *Struthanthus venetus*' taxonomic classification is: Phylum Plantae > Subphylum Magnoliophyta > Class Magnoliopsida > Order Santalales > Family Loranthaceae > Genus *Struthanthus* > Species *venetus*. This plant develops as an epiphyte hemi-parasite germinating in trunks, branches or stems of shrubs such as tulips, aralias, citrus fruits, walnuts, and trees like the casuarinas, among others (Plate 1).

The *S. venetus* is commonly known as "matapalo" as well as "injerto" (in Spanish) and is used in Mexican traditional medicine due to its anti-cough, sedative,

hypoglycaemic and antihypertensive properties (Andrade-Cetto and Heinrich, 2005; Gijón et al., 2010; Alvarez, 2003).

Recent studies have reported that long-term oral consumption of water extract from the Korean mistletoe *Viscum album* in ovariectomized rats (an oestrogen-deficient model of post-menopausal stage) alleviates menopausal symptoms and modulates glucose and lipid metabolism (Kim et al., 2015). The anti-osteoporosis effects of aqueous-methanol extracts of the Eastern Nigerian mistletoe *Lorantus micrants* have also been described (Omeje et al., 2014). Considering the oestrogens' protection mediation on bone health during women's reproductive life, these results may indicate the presence of phyto-oestrogens in the mistletoe extracts, however, it is unknown if these hemi-parasitic plants possess oestrogenic activity since this approach has not been explored.

On the other hand, anticancer activity of mistletoe extracts has been reported in human cancer cells and animal models (Varela et al., 2004, Alonso-Castro et al., 2012). The Korean mistletoe *Viscum album* is also used to treat hepatic, renal and uterine human tumour cells. In patients, an increase in health-related quality of life, remission rate, survival time, and alleviating adverse reactions to conventional breast cancer therapy has been previously described (Marvibaigi et al., 2014). The related mistletoe genera *Scurrula* and *Viscum* have also been described to possess anticancer, antioxidant and antihypertensive properties (Lim et al., 2016).

Since, there are no previous reports about oestrogenicity and anticancer activities of *S. venetus*, the objective of the present work was to explore them using two standardized protocols: the *in vivo* uterotrophic assay in immature female CD1 mice (Kleinstreuer et al., 2016) and the MCF-7 human breast oestrogen positive cancer cell proliferation known as the E-Screen method, considered the most sensitive assay to predict oestrogenic activity since their proliferation is oestrogen dependent (Soto et al., 1995; Körner et al., 1999).

## MATERIALS AND METHODS

### Reagents

17 $\beta$ -oestradiol (E<sub>2</sub>; 1,3,5(10)-estratrien-3,17 $\beta$  diol), Fulvestrant (ICI 182,780) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tamoxifen was a gift from ASOFARMA, (México). Cell culture reagents were obtained from Gibco (Invitrogen Corporation, Waltham, MA, USA).

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**Plate 1.** *Struthanthus venetus* adhered to the stem of a tulip.

#### **Collection and preparation of the *Struthanthus venetus* hydro-methanolic extract (StvHME)**

Leaves of *S. venetus* were collected during the spring in Oaxtepec, Morelos, México and registered with number 33,393 of the National Herbarium of the Biology Institute, UNAM. The material was air-dried in the shadow for 15 days, and then finely grounded with a Wiley mill (200 mesh) to powder. *S. venetus* hydro-methanolic extract was prepared from a 100 g sample of the plant, powder mixed with 200 ml of methanol-water (50:50 v/v) at room temperature (25°C) and allowed to stand for 24 h. Extractions were carried out over 5 days with brief daily manual shaking, the supernatant was removed and placed in a glass container for evaporation; the solid residue yielded 9.6 g/100 g of StvHME dry material.

#### **Solution used for the uterotrophic and MCF-7 cell proliferation assays**

A dry sample of 100 mg of StvHME was dissolved in 10 ml of 50:50 (v/v) ethanol-water solution and passed through a #1 filter paper (Whatman Inc., Hillsboro, OR, USA) to a final concentration of 10 mg of StvHME/ml (stock solution) which was stored at 4°C in amber glass vials until use. Previous to assays, the stock solution was diluted 1:10 in saline. Afterwards, work solutions were diluted with the solvent or culture medium at the appropriated concentrations.

#### **Preliminary phytochemical screening**

A sample of the stock solution (10 mg of StvHME/ml) was diluted 1:10 mg/ml with distilled water:ethanol (50:50) to get 1 mg/ml solution which was used for all phytochemical tests. To identify the

constituents present in StvHME standard methods were used (Zohra et al., 2012; Kuklinski, 2000). For each test 350 µL of StvHME sample was used, the following tests were carried out:

**Frothing test:** to the sample 10 ml of distilled water were added and shaken for 30 s. The mixture was then left for 30 min and observed. Formation of honeycomb froth indicates the presence of saponins.

**Lieberman-Burchard test:** the sample was treated with two drops of acetic anhydride, heated gently and let cool off. Then, concentrated sulphuric acid was carefully added through the walls of the test tube. The final reaction shows a brownish-red layer or ring above a greenish liquid, which indicates the presence of sterols and triterpenes.

**Mayer's test:** the sample was treated with 1 ml drop by drop of Mayer's reagent. The formation of a green-creamy precipitate indicates the presence of alkaloids.

**Dragendoff's test:** the sample was treated drop by drop with 1 ml of Dragendoff's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

**Folin's test:** to the sample five drops of Folin reagent and two drops of Na<sub>2</sub>CO<sub>3</sub> (7.5%) were added. A green colour indicates a few phenols, a light blue indicates moderate phenols and intense blue indicates abundance of phenols. **FeCl<sub>3</sub> test:** to the sample three drops of FeCl<sub>3</sub> solution (1%) were added; formation of a greenish-black colour indicates the presence of phenolic compounds.

**Shinoda's test:** to the sample some magnesium chips and 2 drops of diluted HCl (0.5 N) were added and heated gently. A pink or red

colour indicates the presence of flavonoids.

**Sodium hydroxide test:** to the sample 2 ml of NaOH (10%) solution were added. A yellow colour indicates the presence of flavonoids, which on adding diluted HCl (0.5 N) became colourless.

**Anthocyanidins:** the sample was treated with one drop of concentrated HCl and shaken. Then 0.5 ml of NaOH (20%) was added. A colourless solution indicates the presence of anthocyanidins.

**Non-reducing sugars:** the sample was treated with 1 ml of concentrated hydrochloric acid and gently heated. Then 1.5 mg of resorcinol were added and heated for 2 min. An orange or red colour indicates the presence of non-reducing sugars.

**Grignard's test:** in a glass tube with a lid, the sample was placed and 1 ml of distilled water with one drop of  $\text{CHCl}_3$  were added. A strip of filter paper, previously soaked with picric acid solution (0.5%) and  $\text{Na}_2\text{CO}_3$  (5%) was placed inside the tube and heated to 35°C for 24 h. A red or pink colour indicates the presence of cyanogenic glycosides.

**Borntrager's test:** to the sample, 5 ml of  $\text{CHCl}_3$  were added and shaken for 5 min, filtered and then 5 ml of an ammonia solution (10%) were added shaken again, a pink, red or violet colour in the aqueous layer after shaking indicates the presence of free anthraquinone.

**Filter paper strip:** the sample was placed in a glass tube with a lid; a strip of filter paper previously soaked in NaOH (5%) was placed inside the tube without touching the extract, closing the lid and then heated. The presence of fluorescent spots on the paper when observed with an UV light indicates the presence of coumarins.

**Ninhydrin test:** to the sample three drops of Ninhydrin reagent were added and boiled for few minutes. A blue colour indicates the presence of amino acids.

**Hydrolysable tannins:** to the sample 3 mg of  $\text{NaNO}_2$  and three drops of glacial acetic acid were added. A pink or brown colour indicates the presence of hydrolysable tannins.

**Condensed tannins:** to the sample 2 ml of butanol were added and shaken, then 0.5 ml HCl (0.5N) and heated. The blue colour when adding  $\text{NaHCO}_3$  indicates the presence of condensed tannins.

**Arnoff test:** 2 ml of HCl (0.5N), 2 ml of  $\text{NaNO}_2$  (10%) and three drops of NaOH (2N) were added to the sample. The pink, orange or purple colour when adding NaOH indicates the presence of phenylpropanoids.

**Carotenoids:** to the sample 0.5 ml of  $\text{CHCl}_3$  and 1 ml concentrated sulphuric acid were added and cooled. The appearance of red or blue ring at the contact zone of the two liquids indicates the presence of carotenoids.

**Lactones:** to the sample four drops of NaOH (10%) and two drops of concentrated sulphuric acid were added. A yellow colour indicates the presence of lactones.

## Animals

All experimental studies were conducted in accordance to the Mexican National Protection Laws on Animal Protection and the

General Health Law Related to Health Research (NOM-062-Z00-1999). The animals were obtained from the animal facilities of the Faculty of Medicine of the National University of México. Immature female CD1 mice (10-15 g, 21 days old) were used to evaluate oestrogenic activity by uterotrophic assay (Kleinstreuer et al., 2016). The animals were kept in a room at constant temperature (20-22°C) with 12-12 h light-dark cycle, food and water intake were monitored maintaining standard chow (Nutricubos, Purina) and water *ad libitum*.

## Evaluation of uterotrophic activity of the StvHME

The animals were weighted and distributed among groups according to a balanced Latin-square block design based on body weight (6-7 animals/group in each experiment) and randomly assigned to treatment groups. Different groups of animals were subcutaneously (s.c.) injected once a day for three consecutive days with the positive reference  $\text{E}_2$  (10  $\mu\text{g}/\text{kg}$ ), or StvHME (10, 100 and 500 mg/kg), the control group (C) only received the vehicle (V; 10 ml/kg). After 24 h from the last treatment, animals were weighted, and uteri were dissected, blotted, and weighted to obtain uterine wet weight. Then the uteri were dried at 37°C for 24 h, and weighted again to obtain uterine dry weight. Uterine weights of the treated and the control groups were expressed in mg.

## Cell proliferation experiments

### MCF-7 cell-line culture conditions

Human MCF-7 breast cancer oestrogen receptor-positive cells were obtained from the American Type Culture Collection (ATCC) (Manassas, VA, USA). MCF-7 cells were grown in phenol red-free Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Invitrogen Corporation, Waltham, MA, USA) high glucose, supplemented with 1% (v/v) of an antibiotic-antimycotic mix (penicillin G sodium, streptomycin sulphate and amphotericin B). For all experiments, 10% (v/v) of charcoal-dextran stripped foetal bovine serum (CDFBS) hormones free was used according to a reported method (Körner et al., 1999). The cell culture was maintained in a humidified atmosphere of 5% carbon dioxide in 95% air at 37°C. The cell stocks were sub-cultured weekly at 70% confluence over a maximum of 20 passages using 0.05% trypsin-0.02% EDTA (pH 7.3).  $\text{E}_2$  was prepared as stock solution in ethanol (0.1 M) and the StvHME stock solution (10 mg/ml) were freshly diluted in culture medium avoiding ethanol concentrations higher than 0.01% (v/v). To explore the mediation of the oestrogen receptors in the cell proliferation rate, they were treated with StvHME and co-incubated with the anti-oestrogens Tamoxifen ( $1 \times 10^{-6}$  M), or Fulvestrant (ICI 182,780; 10 nM).

### Experimental design of cell proliferation

In all the assays, 2500 cells contained in 150  $\mu\text{L}$  were placed into each of the 96 wells of plastic tissue culture plates (Falcon). The cells were left to attach for 24 h, afterwards the cell culture media was substituted for the treatment. In all assays each concentration was tested in 8 replicates including control cells.

## Experiments

1) A temporal course of proliferative response was explored comparing proliferation of MCF-7 cells in the presence of  $\text{E}_2$   $10^{-10}$  M or StvHME 50  $\mu\text{g}/\text{ml}$ , and control cells incubated only with CDFBS

**Table 1.** Uterotrophic activities of E<sub>2</sub> and the StvHME in immature CD1 mice.

Group	Dose	<b>a</b> Body weight (g)	Uterine wet weight (mg)	<b>b</b> Uww Δ%	Uterine dry weight (mg)	<b>c</b> Udw Δ%
V	10 ml/kg	9.3 ± 0.27	11.4 ± 0.85	100	3.40 ± 0.18	100
E <sub>2</sub>	10 µg/kg	9.4 ± 0.24	42.8 ± 2.34	275	9.37 ± 0.52	175
StvHME	10 mg/kg	9.4 ± 0.21	7.1 ± 0.50	-38*	2.88 ± 0.13	-15*
	100 mg/kg	9.3 ± 0.20	8.9 ± 0.51	-22	3.03 ± 0.19	-10
	500 mg/kg	9.4 ± 0.49	15.5 ± 1.35	+36**	4.35 ± 0.39	+28**

V = vehicle; E<sub>2</sub> = 17β-oestradiol; StvHME = hydromethanolic extract of the mistletoe *Struthanthus venetus*. n/group = 13.

**a** Body weight after treatment. Values are given in mean ± SEM.

**b** Uww = uterine wet weight. **c** Udw = uterine dry weight.

T test \*  $p < 0.001$  (Uww) and  $p = 0.027$  (Udw) vs V; \*\* $p = 0.017$  (Uww) and  $p = 0.044$  (Udw) vs V; Δ% = represent the uterotrophic effect calculated as a percentage difference related to the V) control group = [Uww or Udw of E<sub>2</sub> or StvHME × 100/Uww or UdwV] – 100.

media during 3 days.

2) The proliferative response to E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) compared with StvHME (0.5, 5 and 50 µg/ml); and their interaction of E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) + StvHME 50 µg/ml in a 200 µL medium and the control evaluated on the sixth day of culture.

3) Groups of cells were assigned to the treatment with E<sub>2</sub> 10<sup>-10</sup> M, StvHME 50 µg/ml, or StvHME 50 µg/ml co-incubated with the anti-oestrogens Tamoxifen (1x10<sup>-6</sup> M), or Fulvestrant (10nM) and the control.

The proliferative effect on MCF-7 cells was evaluated using the viability MTT assay (Mosmann, 1983). At the end of the experiment, 20 µL of MTT solution (5 mg/ml) in phosphate-buffered saline (PBS) were added to the wells' medium and maintained for 4 h at 37°C. The medium was removed and the formed formazan was dissolved in dimethyl sulfoxide (Merck, Kenilworth, NJ, USA). OD was read in an UV microtiter plate reader at 492-630 nm (Stat Fax 3200, Awareness Technology, Inc. Palm City, USA). The number of cells was obtained from a calibration curve of number of cells-OD and was expressed as a percentage related to the control cells.

### Statistical analysis

Numeric values presented for each experiment are the mean of at least two independent experiments. Data was analyzed with the Sigma Plot statistical package (version 2011, Jandel Corporation), using one-way analysis of variance (ANOVA) for comparisons between groups, and T student test or Dunn or Mann-Whitney tests as appropriate. Data are presented as ± standard error (SEM). *P* values below 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

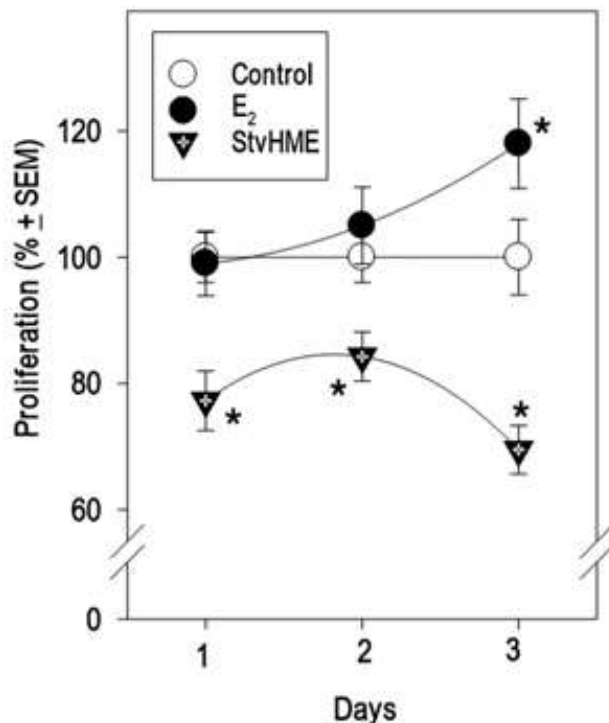
This study describes for the first time, the oestrogenic and anti-oestrogenic properties of the Mexican hemiparasitic mistletoe StvHME. The response elicited by StvHME in uteri of the immature female CD1 mice showed biphasic behaviour (Table 1). StvHME with the 10 mg/Kg dose decreased significantly uterine wet and dry weights (38%,  $p < 0.001$  and 15%,  $p = 0.027$ , respectively) related to the control group, inducing an anti-uterotrophic effect. The 100 mg/kg dose also

decreased uterine weight but the changes did not reach significance. In contrast, the 500 mg/kg dose increased significantly mice uterine wet (36%) and dry (28%) weights ( $p = 0.017$  and  $p = 0.044$  respectively) suggesting that high concentrations of StvHME are oestrogenic. The positive control E<sub>2</sub> (10 µg/kg) showed its classic significant uterotrophic effect increasing 274-275% of uterine wet and dry weights respectively ( $p < 0.001$ ).

Despite the lack of information about mistletoe oestrogenic activity, our results are in accordance with those reported by Pattanayak and Mazumder (2009) who also described weak oestrogenic activity of the hydroalcoholic extract of the large bushy parasitic plant *Dendrophthoe falcata*, (Loranthaceae family). Also, when the extract was administered with ethynyl oestradiol, it showed a low anti-oestrogenic activity in immature ovariectomized rats (Pattanayak and Mazumder, 2009). However, to confirm oestrogenic or anti-oestrogenic effects of StvHME in uteri, it is necessary to assess these effects using other *in vivo* models and longer treatments, particularly to have information about its potential utility in hipo-oestrogenic conditions like menopause.

On the other hand, StvHME displayed an anti-proliferative effect on breast cancer MCF-7 cells, behaving in an opposite way to E<sub>2</sub>. The temporal course effect of exposure to 10<sup>-10</sup> M of E<sub>2</sub> and 50 µg/ml of StvHME on MCF-7 cells is shown in Figure 1. The inhibitory proliferative effect of 50 µg/ml StvHME was detected after the first day and maintained in the same magnitude range along the treatment ( $p < 0.01$ ) without dose or time dependency (Figure 1). Meanwhile the E<sub>2</sub> positive (10<sup>-10</sup> M) control showed opposite proliferative effects with respect to StvHME after 3 days with high significance ( $p < 0.001$ ).

The effect of different concentrations of StvHME compared with E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) on MCF-7 cells is showed in Figure 2. E<sub>2</sub> displayed a dose dependent proliferative response mean while, StvHME in all the



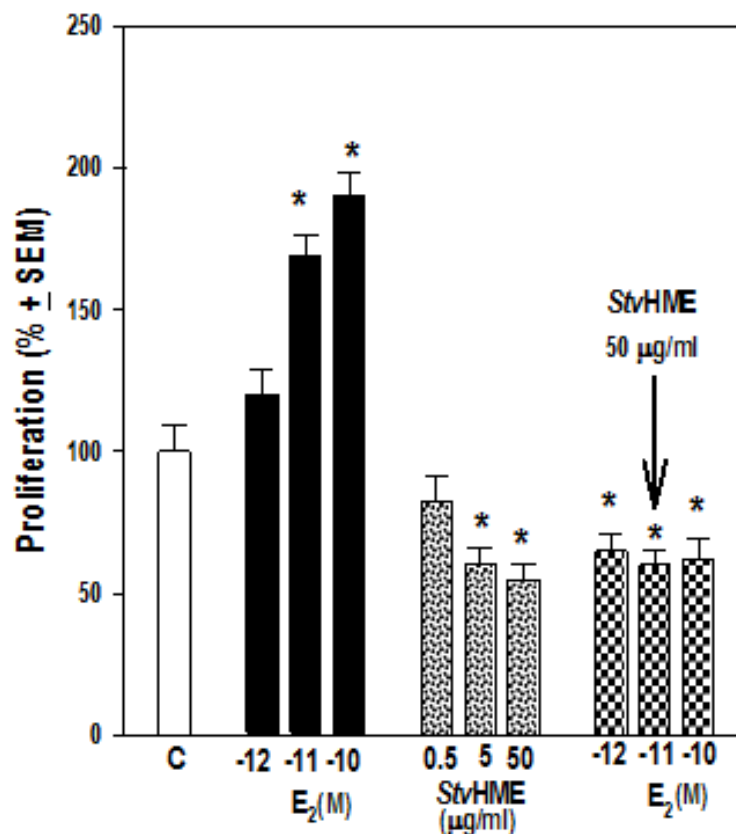
**Figure 1.** Temporal course effect of E<sub>2</sub> 10<sup>-10</sup> M and 50 µg/ml of StvHME on MCF-7 cells (StvHME vs control, \**p* < 0.01; StvHME vs E<sub>2</sub>, \**p* < 0.001).

evaluated concentrations (0.5, 5 and 50 µg/ml) significantly decreased MCF-7 cell proliferation (*p* < 0.05), and it is of note that the 5 and 50 µg/ml concentrations induced decreases of the same magnitude in relation to the control (*p* < 0.05, Dunn's method). Additional to the inhibitory effect of StvHME on MCF-7 cell proliferation, the extract (50 µg/ml of StvHM) was capable to abolish the proliferative response induced by E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) acting as anti-oestrogen.

Anti-cancer activity of the mistletoe genera *Scurrula* and *Viscum* has previously been described and attributed to the presence of antioxidant phytoconstituents such as quercetin, which confers protection against cancer and neurodegeneration (Lim et al., 2016). Diverse constituents have been described among other mistletoe species including: lectins and viscotoxins which exert anti-tumor effects by inducing cell cycle arrest, increasing apoptosis, inhibiting angiogenesis, and potentiating immune responses (Osadebe and Omeje, 2009). However, the phytochemistry of the genus *Struthanthus* has been scarcely studied. In the Brazilian hemi-parasite plants *Struthanthus marginatus* and *Struthanthus concinnus* (Loranthaceae) terpenoid compounds have been recently identified (Leitão et al., 2013). Preliminary phytochemical screening of the StvHME extract indicated that it contains saponins, phenols, flavonoids tannins, phenylpropanoids, and lactones (Table 2). In México,

reports about the effect of mistletoes in cancer are scant (Alonso-Castro et al., 2011; Rios et al., 2001; Rivero-Cruz et al., 2005; Waizel et al., 1994). *Phoradendron reichenbachianum* (Loranthaceae) from which moronic acid and a tetracyclic triterpene was isolated, has been described with cytotoxic activity (Rios et al., 2001) and also the Mexican hemiparasitic plant *Phoradendron robinsonii* (Loranthaceae) has been described to contain a flavonoid with antimycobacterial activity (Rivero-Cruz et al., 2005).

In order to know the possibility of oestrogenic agonistic properties of StvHME as indicated in the uterotrophic assay with 500 mg/kg dose, an experiment was performed comparing the effect of the oestrogen antagonists Tamoxifen and Fulvestrant on the response to StvHME 50 µg/ml in MCF-7 cells. Both antagonists increased significantly (*p* < 0.05) the inhibition of cell proliferation elicited by StvHME (Figure 3). Considering that breast cancer is the most common type of cancer among women worldwide with an increasing incidence and mortality during the last two decades (Kolak et al., 2017), and the fact that StvHME shows a noticeable anti-proliferative activity in the MCF-7 breast oestrogen positive cancer cells, such action is of great importance since it can be potentiated by oestrogen antagonists Tamoxifen and Fulvestrant which are used clinically. This Could indicate this extract may be useful as a

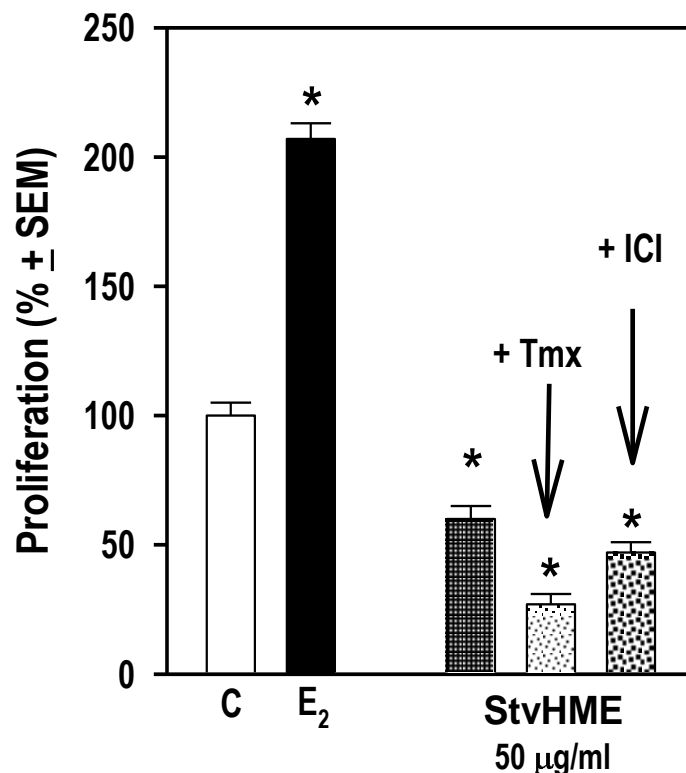


**Figure 2.** The effect of E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M), StvHME (0.5, 5 and 50 µg/ml) and the interaction of E<sub>2</sub> (10<sup>-12</sup> to 10<sup>-10</sup> M) + StvHME (50 µg/ml) on MCF-7 cells. \*  $p > 0.05$

**Table 2.** Screening of phytochemical components of StvHME.

Component	Result	Positive control/Standard
Saponins	++	n.d.
Terpenes/steroids	-	Dexamethasone
Alkaloids	-	Berberine
Phenols	+++	Green tea
Flavonoids	++	Quercetine
Anthocyanidins	-	Cyanidin-3-glucoside
Non-reducing sugars	-	Saccharose
Cyanogenic glycosides	-	n.d.
Anthraquinones	-	n.d.
Cumarins	-	4-hydroxycumarin
Aminoacids	-	L-arginine
Tannins condensed	-	Guazuma <i>sp</i> (proanthocyanidine b and c)
Tannins hydrolyzed	++	Green tea
Phenylpropanoids	++	Noradrenaline
Carotenoids	-	Lycopene
Fatty acids	-	Corn oil
Starches	-	Starch
Lactones	++	n.d.

Highly positive: +++; positive: ++; weakly positive: +; - negative. n.d. = not determined.



**Figure 3.** Effect of E<sub>2</sub> (10-10M) and StvHME (50 µg/ml) alone, and concomitantly with Tamoxifen (Tmx) or Fulvestrant (ICI 182,780 ; ICI). Control vs E<sub>2</sub>, \* $p < 0.01$ ; StvHME vs StvHME + Tmx, \* $p < 0.05$ ; StvHME vs StvHME + ICI, \* $p < 0.05$

complementary therapy in breast cancer patients treated with oestrogen antagonists, particularly in cases where tumour behaviour is aggressive, and there is intolerance to the side effects produced by toxicity of currently used drugs. Future studies in this area should focus on characterizing the effects of StvHME with more detail in long term *in vivo* studies, assessing possible toxicity, and determining selectivity in different cell lines.

In summary, our results show that StvHME in mice produced biphasic actions: at low doses an anti-oestrogenic effect, and at high doses, modest oestrogenic activity. More work is in progress to confirm possible oestrogenic effect of StvHME, to know its potential use during hypo-oestrogenic conditions. The remarkable fact is that StvHME on MCF-7 cells behaved only as an anti-oestrogen. The anti-proliferative properties of the StvHME against MCF-7 human adenocarcinoma breast cancer cells are clear and indicate that the extract could contain oestrogenic and anti-oestrogenic components, probably acting through the oestrogen ER $\alpha$  and ER $\beta$  receptors. These results provide the basis to encourage further pharmacological and chemical characterization of the mistletoe *S. venetus*, to support its use as phytotherapeutic agent as a promising source of novel bioactive compounds against breast oestrogen-positive

cancer.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## ABBREVIATIONS

**StvHME**, hydro-methanolic extract from the mistletoe *Struthanthus venetus* dried leaves; **E<sub>2</sub>**, 17 $\beta$ -oestradiol; **MCF-7**, Michigan Cancer Foundation-7; **MTT**, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **ER $\alpha$** , oestrogenic receptor alpha; **ER $\beta$** , oestrogenic receptor beta; **ATCC**; American Type Culture Collection; **DMEM**, phenol red-free Dulbecco's modified Eagle's medium; CDFBS, charcoal-dextran stripped foetal bovine



bovine serum; **EDTA**, ethylenediaminetetra acetic acid; **PBS**, phosphate-buffered saline; **OD**, optical density; **ANOVA**, analysis of variance.

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