

Uterus-Relaxing Study of a Sudanese Herb (El-Hazha)

¹Aimun Abdelgaffar Elhassan Ahmed, ¹Robert Gaspar, ¹Arpad Marki, ³Andrea Vasas,

²Mahmoud Mudawi Eltahir Mudawi, ³Judit Hohmann and ¹George Falkay

¹Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy,
University of Szeged, H-6720 Szeged, Eotvos u. 6, Hungary

²Department of Therapeutics, Faculty of Pharmacy, Omdurman Islamic University, Sudan

³Department of Pharmacognosy, Faculty of Pharmacy,
University of Szeged, H-6720 Szeged, Eotvos u. 6, Hungary

Abstract: Problem statement: The aim of this study is to investigate the pharmacological effects of the Methanolic-extract (AH2) of El-Hazha and its sub-fractions. **Approach:** These investigations were carried out on *in vitro* isolated uterus preparations from Non-Pregnant (NP) and Late-Pregnant rats (LP). In parallel displacement radio-ligand binding assay was performed for β -Adrenergic Receptors (β -ADR). **Results:** Showed that the herb and its different fractions produced dose-dependent relaxant effect ($p < 0.05$, t-test, $n = 6$) on uterine contraction elicited by 25 mM KCl in both NP and LP uteri that affected significantly by fractionation, however, the effect of the most active fraction (AH2-11) was reversed by prior addition of propranolol (non-specific β -antagonist), but not affected by progesterone pre treatment of the LP rats. In addition, AH2 only in high concentration displaced isotopes from β -ADR, this affinity changed markedly by fractionation. **Conclusion:** We validate the fractionation effect on its relaxant activity and found partial role for β -ADR on mediating this activity. Future study was recommended to isolate and investigate its active components to enhance this activity or to discover a new novel natural therapeutic agent(s).

Key words: *Haplophyllum tuberculatum*, radio-ligand binding assay, β -Adrenergic Receptors (β -ADR), Non-Pregnant (NP), Late-Pregnant rats (LP), Sudanese plant, Charles-River laboratories, medicinal and aromatic plants, tocolytic agent, pharmacological effects, progesterone treatment, uterus-relaxing study

INTRODUCTION

Despite extensive efforts, the incidence of preterm birth has not decreased recent several decades (Monga and Creasy, 1995; Hannah, 2000). Preterm labor is still a health challenge, because there are as yet no effective primary means of its prevention (Koucky *et al.*, 2009).

Tocolytic agents are drugs designed to inhibit the contractions of myometrial smooth muscle cells. The aim of tocolysis is not only to stop uterine contractions and to prevent preterm delivery, but also to decrease the prenatal morbidity and mortality associated with preterm birth (Tsatsaris *et al.*, 2004). The main drugs used as tocolytics are indomethacin and other prostaglandin inhibitors (Vermillion and Landen, 2001), calcium channel blockers such as nifedipine (Pryde *et al.*, 2001; Giles and Bisits, 2007), β -adrenergic agonists and oxytocin receptors antagonist,

while the Medical prevention consists of antibiotic or progesterone administration (Tara and Thornton, 2004).

One of the most well-known mechanism of action through which a tocolytic agent acts was to relax the uterus, so generally any agent has ability to relax the uterus can be considered as a tocolytic agent (Clouse *et al.*, 2007) demonstrates that relaxation of rat myometrium is mediated by β_2 -adrenoceptors. also α_1/β -adrenoceptor ratio determines not only the spontaneous motor activity of the rat uterus, but also the potency of the agents with tocolytic effect (Zupko *et al.*, 1998).

However, the efficacy and safety of tocolytics are not adequate, new agents are therefore required including substances from natural sources. Many plants have been recently investigated world-wide in the search for tocolytic or uterus-relaxing agent such as *Curcuma aeruginosa* Roxb. Rhizome (Thaina, 2009), *Scutellaria baicalensis* root (Shih *et al.*, 2009) and *Ficus capensis* Thunb (Owolab *et al.*, 2009).

Corresponding Author: George Falkay, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, H-6720 Szeged, Eotvos u. 6, Hungary Tel: +36 62 341974 Fax: +36 62 545567

El-Hazha (*Haplophyllum tuberculatum*) (Forssk.) A. Juss. (*Rutaceae*); is an herb indigenous to the northern part of Sudan, North Africa and other areas of the Middle East (Boulus, 1983). Named locally in Sudan as “a plant of all disease”, it is to be found in every Sudanese home as an emergency drug that used extensively by old Sudanese in the rural areas. The herb is utilized in Sudan as an antispasmodic, as an antifatulant, to relieve toothache and to treat allergic rhinitis (Mohamed AH *et al.*, 1996), malaria, gynaecological disorders, asthma, inspiration difficulties, renal disorders and others.

This plant is also well known among herbalists and widely used traditionally in other counties such as Saudi Arabia (Mohammed *et al.*, 1991) and Oman (Mossa *et al.*, 1987).

Its essential oils were investigated for antimicrobial activity by (Al-Burtamani *et al.*, 2005) and were found to cause partial inhibition of the growth of *Escherichia coli*, *Salmonella choleraesuis* and *Bacillus subtilis* to the same extent as gentamycin sulfate.

Its cardiovascular effect were studied by (Mohamed AH *et al.*, 1996), who reported that its aqueous extract significantly decreased the contractility and the heart rate, but did not affect the flow rate of the isolated perfused rabbit heart. This action was not blocked by atropine, but the muscarinic antagonist blocked the fall in blood pressure seen when the extract was administered to anaesthetized cats. The extract also stimulated rabbit aortic strip, rat vas deferens and rat *anococcygeus* muscles. These adrenergic effects were largely reduced by phentolamine.

Its hepatoprotective activity was investigated on the liver damage induced by paracetamol in mice by (Ali *et al.*, 2001) and proved to be relatively ineffective protecting only 16% of the animals against the lethal effect of paracetamol (1 g kg⁻¹) in comparable to that of the standard hepatoprotective agent silymarin.

When its cytotoxic activity was checked against 11 tumor cell lines, where strong cytotoxic activity was observed (Varamini *et al.*, 1992).

On the other hands and from phytochemical's point of view, some compounds of this Sudanese plant were isolated by (Khalid and Waterman, 1981).

Both its uses to relax the uterus and to treat asthma and inspiration difficulties catalyzed us to carry out this study to evaluate their effects in order to find a new therapeutic agent(s) to aid in solving of two major medical challenges (preterm labor inhibition and asthma control).

Literature survey revealed that the aqueous extract of this plant obviously possess contracting activity, while traditional uses suggested contradictory

applications such as muscle relaxant and contracting effect in the same time.

This study was an attempt to proof and evaluate some of its traditional uses in gynaecological area as uterus-relaxing agent and to see the influence of fractionation on its activity.

MATERIALS AND METHODS

Plant material:

Crude plant collection and identification: The aerial parts of *Haplophyllum tuberculatum* (El-Hazha) were collected freshly during the 1st week of November 2008 from their natural habitats in the North part of Sudan (Abu-hamad, Nahr El-Neel State). The voucher specimens (No. M23/08) were identified by Dr. Wai'l E. Abdalla and Yahia S. Mohamed of Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, where the specimens were also deposited for future references.

Extraction, fractionation and preparation of the plant material for pharmacological tests:

The air-dried powdered aerial parts of *Haplophyllum tuberculatum* (AH) (2 kg) were extracted with Methanol for 1h. The MeOH extract (AH2) was concentrated and completely dried under vacuum to yields 315 g of the crude extract which reconstituted in distilled water to get the desired concentration and tested pharmacologically (Ganguly *et al.*, 2007), then fractionated and tested. The most active fraction in each step was selected based on comparing the *in vitro* pharmacological results, then fractionated and tested. The most active fractions are (AH2-11) 88.31 g, (AH2-11-4) 4.31 g and (AH2-11-4-6) 161mg (Fig. 1).

Pharmacological studies:

Ethical considerations for housing and handling the animals:

The animals were treated in accordance with the European Communities Council Directives (86/609/ECC) and the Hungarian Act for the Protection of Animals in Research (XXVIII.tv.32. §). All experiments involving animal subjects were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/1758-2/2008). Sprague-Dawley rats (Charles-River Laboratories, Hungary) were kept at 22 ± 3°C, the relative humidity was 30-70% and the light/dark cycle was 12/12 h. They were maintained on a standard rodent pellet diet (Charles-River Laboratories, Hungary) with tap water available *ad libitum*. The animals were sacrificed by CO₂ inhalation.

Mating of the animals: Mature female (180-200 g) and male (240-260 g) rats were mated in a special mating

cage. A metal door, which was movable by a small electric engine, separated the rooms for the male and female animals. A timer controlled the function of the engine. Since rats are usually active at night, the separating door was opened before dawn. Within 4-5 h after the possibility of mating, vaginal smears were taken from the female rats and a sperm search was performed under a microscope at a magnification of 1200 times. If the search proved positive, or if smear taking was impossible because of an existing vaginal sperm plug, the female rats were separated and were regarded as first day- pregnant animals.

Isolated organ bath studies: Uteri were removed from non-pregnant (180-200 g), 22-day-pregnant (270-350 g) rats. Muscle rings 5 mm long were sliced from the uterine horns and mounted in an organ bath (8 parallels) containing 10 ml de Jongh solution (in mM: 137 NaCl, 3 KCl, 1 CaCl₂, 1 MgCl₂, 12 NaHCO₃, 4 NaH₂PO₄, 6 glucose, pH 7.4). The organ bath was maintained at 37 °C and carbogen (95% O₂+5% CO₂) was bubbled through it. After mounting, the rings were equilibrated for about 1 h before experiments were undertaken; with a solution change every 15 min. The initial tension was set to about 1.5 g, which was relaxed to about 0.5 g at the end of equilibration. The tension of the myometrial rings was measured and recorded with a gauge transducer and an S.P.E.L. Advanced ISOSYS Data Acquisition System (Experimetria Ltd., Hungary), respectively. Contractions were elicited with KCl (25 mM) and cumulative dose-response curves were constructed in each experiment by addition of plant extract or its fraction(s) in different concentrations (range from 10⁻⁵-100 µg ml⁻¹) alone or in the presence of propranolol (β-blocker) (10⁻⁶ mM). Recording was performed for 5 minutes. Dose-response curves were fitted from areas under curves (AUCs) using Prism 4.0 computer program. E_{max} and EC₅₀ values were calculated (E_{max}: the maximum relaxant effect of extract or its fractions, EC₅₀: the concentration of extract or its fraction (s) alone which elicits half of the maximum relaxant effect of extract or its fraction (s) or one of its fraction (s)).

Progesterone treatment of pregnant rats: The progesterone treatment of the pregnant rats was started on day 15 of pregnancy. Progesterone was dissolved in corn oil and injected subcutaneously every day up to day 21 at a concentration of 0.5 mg 0.1⁻¹ ml kg⁻¹. On day 22, the uteri were collected and the organ bath studies were performed as described above. The experimental data on the non-treated and the progesterone-treated animals were analyzed statistically.

Radio-ligand binding assay:

Membrane preparation: The selected tissue of abundant receptors of interest was rat brain membrane.

Animals were sacrificed by rapid cervical dislocation, both side of the skull were cut from back to forward. The intact brain was expose and removed carefully using forceps. The brain were freed from other tissues and homogenized in ice-cold homogenizing buffer (20 mM NaHCO₃) in a ratio of 1:5. The homogenate was centrifuged at 15500 rpm speed for 40 min, the re-suspended pellets were centrifuged under the same conditions. Finally the pellet were re-suspended in binding buffer (50 mM tris + 0.5 mM EDTA with pH = 7.5) and divided into small stock aliquots 2.6 mL each and frozen at -70°C which diluted and used in radio-ligand binding displacement assays.

Displacement assay: The affinities of the tested extract and its fractions for β-adrenergic receptors were measured on above membrane preparation using [3H] Dihydroalprenolol (DHA) (β-adrenergic antagonist) as radioligand (~1.5 nM).Radioligand were purchased from Amersham International plc (UK). Under standard assay conditions, the final incubation system volume was 300 µl consisted of diluted membrane preparation (protein content approximately 0.5-1 mg ml⁻¹), radio-ligand and incubation buffer or with the tested extract or its fractions (its concentration ranging from 10⁻⁷-10⁴ µg mL⁻¹), following the incubation period, the membranes were collected on a Whatman GF/C filter, using a Brandel M24 Cell Harvester. Filters were collected in liquid scintillation vials and the radioactivity was measured with LKB Wallac liquid scintillation counter. The experiment were performed at 25°C for 45 min, the nonspecific binding were determined using 10⁻⁵M Alprenolol. Displacement experiments were analyzed individually with the computer program Prism 4.0 to determine the inhibition constants (K_i) of the investigated agents.

Statistical analysis: The statistics was done by using Prism 4.0 (GraphPad Software, USA) computer program. For the statistical evaluations, data were collected from at least 6 animals and analyzed by performing two-tailed unpaired t-test to compare the significance mean differences for various results. The differences were considered to be significant at levels of p≤0.05.

RESULTS

Phytochemical extraction and fractionation: The Methanolic-maceration of the plant produced a yield's percentage of (5.5%), while the steps of fractionations were highlighted in a summary diagram (Fig.1) emphasizing on that mentioned as most active pharmacologically and used throughout the study.

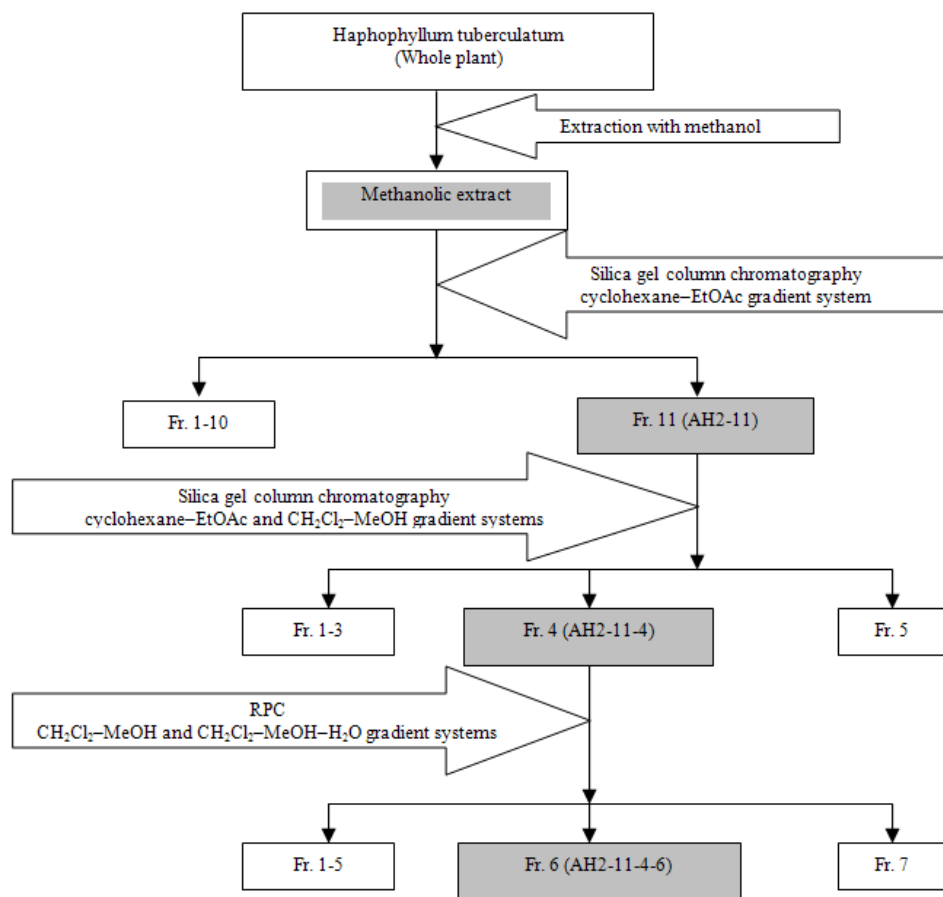


Fig. 1: Diagram illustrated the main steps of Bioactivity-guided fractionation of the Methanolic-extract of El-Hazha emphasized on the most active fractions resulted and used throughout the study.

Table1: The basic effects of the Methanolic-extract on non-pregnant and late-pregnant rat uterus *in vitro* results, general pilot screening for the direct plant relaxant activity

Gestation period	$E_{max} \pm SEM \%$	$EC_{50} \pm SEM (\mu g mL^{-1})$
Non-pregnant	25.7 ± 3.6	$(2.2 \pm 0.6) \times 10^{-3}$
Late-pregnant	39.9 ± 5.8	$(0.4 \pm 0.3) \times 10^{-3}$
P-value	0.094	0.027 *

*: $p < 0.05$; E_{max} : The maximal relaxing effect of the Methanolic-extract against (KCl-induced contraction); EC_{50} : The concentration of the Methanolic-extract producing 50% of their maximal relaxing effect of Methanolic-extract against (KCl-induced contraction) in the system

***In vitro* pharmacological studies:**

Rat uterus results:

Effect of the Methanolic-extract on rat uteri: Basically, the extract showed relaxant activity in NP and LP rat uterus without significant difference in E_{max} ($p = 0.094$), but with significant difference in EC_{50} ($p = 0.027$), Table 1.

Effect of the different AH2 fractions after the 2nd and 3rd fractionation on rat uteri: AH2 and its fractions showed different activity level on both uteri type (data not shown), while the most active fraction was selected for the next step.

Effect of the most active fractions of AH2 on rat uteri: In non-pregnant (Fig. 2A and Table 2), the basic AH2 relaxant activity was increased significantly after fractionation while it decreased by further fractionation.

In late-pregnant unlike non-pregnant results, (Fig. 2B and Table 2), the extract activity was not significantly affected by different fractionations steps.

After this step the fraction AH2-11 was considered as the most active one and selected for the advanced β -adrenergic study using propranolol as standard blocker (Fig. 3 and Table 3) and progesterone treatment (Fig. 4).

Table 2: Relaxant effect of the Methanolic-extract AH2 and its most active fractions on non-pregnant and late-pregnant rat uterus *in vitro* results

Fraction	Non-pregnant		Pregnant D 22	
	$E_{max} \pm SEM$ (%)	$EC_{50} \pm SEM$ ($\mu g mL^{-1}$)	$E_{max} \pm SEM$ (%)	$EC_{50} \pm SEM$ ($\mu g mL^{-1}$)
AH2	25.6 \pm 2.6	$(2.2 \pm 0.6) \times 10^{-3}$	31.7 \pm 5.4	$(0.4 \pm 0.3) \times 10^{-3}$
AH2-11	81.0 \pm 12.6 *	1.3 \pm 0.6	33.4 \pm 4.5	$(4.5 \pm 3.8) \times 10^{-2}$
AH2-11-4	58.2 \pm 5.0	$(7.8 \pm 5.9) \times 10^{-3}$	27.9 \pm 5.2	$(4.4 \pm 2.1) \times 10^{-5}$
AH2-11-4-6	38.8 \pm 6.3	$(6.8 \pm 3.2) \times 10^{-3}$	34.9 \pm 3.5	$(1.7 \pm 0.7) \times 10^{-4}$

* $p < 0.05$, AH2 was used as control for its different fractions, E_{max} : the maximal relaxing effect of the Methanolic-extract AH2 or its sub fractions (AH2-11, AH2-11-4 and AH2-11-4-6) against KCl-induced contraction; EC_{50} : the concentration of the Methanolic-extract AH2 or its sub fractions (AH2-11, AH2-11-4 and AH2-11-4-6) producing 50% of their maximal relaxing effect of Methanolic-extract against KCl-induced contraction in the system

Table 3: Propranolol antagonistic effect on the relaxant effect of the most active fraction AH2-11 on isolated non-pregnant and late-pregnant rat uterus *in vitro*

Most active Fraction AH2-11	Non-pregnant		Pregnant D 22	
	$E_{max} \pm SEM$ (%)	$EC_{50} \pm SEM$ ($\mu g mL^{-1}$)	$E_{max} \pm SEM$ (%)	$EC_{50} \pm SEM$ ($\mu g mL^{-1}$)
Alone	81.0 \pm 12.6	1.3 \pm 0.6	49.5 \pm 3.9	$(3.9 \pm 3.2) \times 10^{-2}$
With Propranolol [10^{-6} M]	124.1 \pm 16.9	$(34.6 \pm 8.8) \times 10^{-2}$	29.5 \pm 3.4 **	$(0.5 \pm 0.4) \times 10^{-3}$

*: $p < 0.05$, E_{max} : the maximal relaxing effect of the most active fraction AH2-11 against KCl-induced contraction; EC_{50} : The concentration of the most active fraction AH2-11 producing 50% of their maximal relaxing effect against KCl-induced contraction in the system

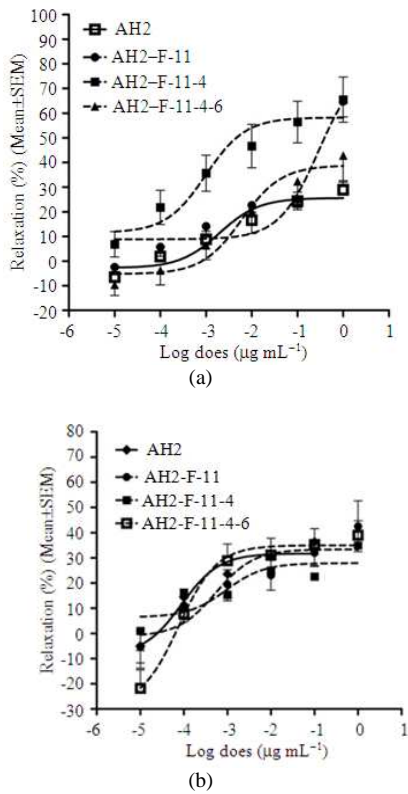


Fig. 2: Dose-response curves of the relaxing effect of the Methanolic-extract (AH2) (continuous-line) and its most active fractions (dotted-lines) on non-pregnant (a) and late-pregnant (b) rat uterus *in vitro* against KCl-induced control contractions. Values presented are means of 6-8 observations, while vertical bars denote Standard Errors of the Mean (SEM)

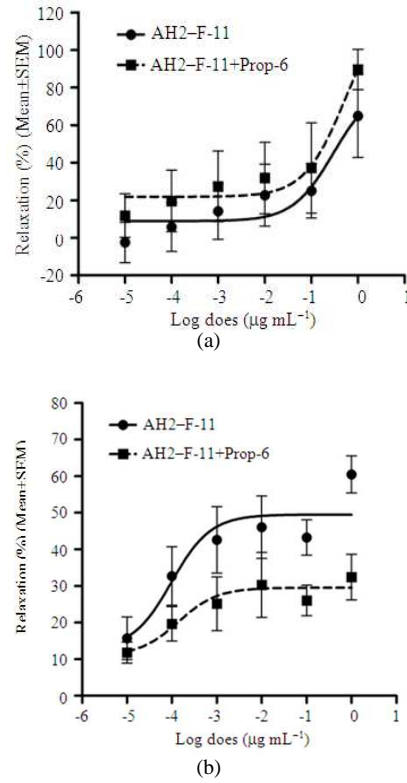


Fig. 3: Dose-response curves of the most active fraction AH2-11 alone (continuous-lines) and with propranolol 10^{-6} (dotted-line) on non-pregnant (a) and late-pregnant (b) on isolated rat uterus *in vitro* against KCl-induced control contractions. Values presented are means of 6-8 observations, while vertical bars denote Standard Errors of the Mean (SEM)

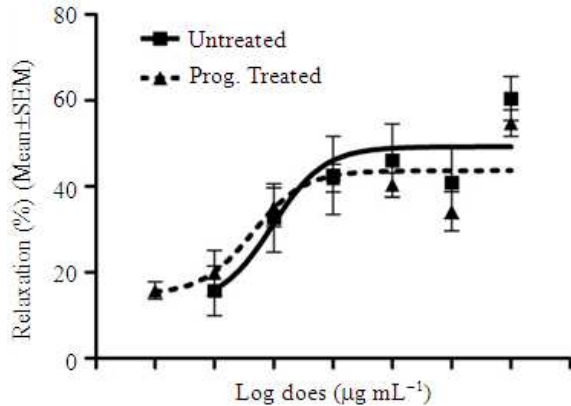


Fig. 4: Dose-response curves of the most active fraction AH2-11, untreated (continuous-lines) and progesterone-treated (dotted-line) on isolated late-pregnant rat uterus (Day22) in vitro against KCl-induced control contractions. Values presented are means of 6-8 observations, while vertical bars denote Standard Errors of the Mean (SEM)

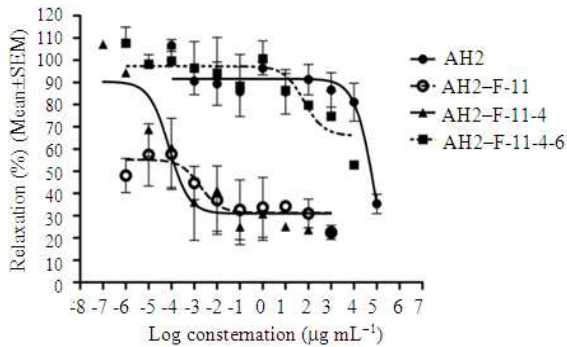


Fig. 5: The displacement curves of AH2 and its most active fractions on β -adrenergic receptors using Dihydroalprenolol [3H] DHA as a radioligand and isotopes on rat brain membrane preparation. Values presented are means of 6-8 observations, while vertical bars denote Standard Errors of the Mean (SEM)

Propranolol effect on the relaxant activity of the most active fraction on rat uteri: Propranolol in concentration of 10^{-6} M had no significant effect on neither the curve nor its parameters of the most active fraction AH2-11 on non-pregnant rat uterus (Fig. 3A and Table 3).

On late-pregnant rat uterus (Fig. 3B and Table 3), propranolol in concentration of 10^{-6} M exerts significant effect on the E_{max} % of the curve ($p = 0.0049$) but with insignificant ($p=0.184$) effect on the EC_{50} of the most active fraction AH2-11 under investigation.

Effect of AH2-11 on late-pregnant progesterone-treated rat uterus results: The progesterone treatment for late-pregnant rat uterus did not alter significantly neither the E_{max} % nor the EC_{50} of the curve of the most active fraction AH2-11 (Fig. 4).

Radio-ligand binding assay: The affinity of the Methanolic-extract and its most active sub fractions from different fractionation steps for β -adrenergic receptors were tested on rat brain membrane preparation, using Dihydroalprenolol [3 H] DHA (2 nM) as an isotopes radioligand. All of the ligands displace the radioligand from the target receptor.

The AH2 showed the displacement of the isotopes only in very high concentration ($10^4 \mu\text{g mL}^{-1}$) with unestimated K_i , while its other sub fractions showed better displacement affinities and the K_i -values of AH2-11-4 and AH2-11-4-6 were $(1.2 \pm 1.0) \times 10^{-2}$, $(1.1 \pm 1.1) \times 10^{-3}$ and $3.8 \pm 3.8 \mu\text{g mL}^{-1}$ respectively (Fig. 5).

DISCUSSION

Plants still a rich drug source (Wahab *et al.*, 2008), El-Hazha, is named locally in Sudan as “a plant of all disease”. Due to its extensive use traditionally this plant subjected to different studies in different directions, but the novelty of this study arises from many factors which can be summarized as; deep pharmacological study with detailed assay guided fractionation, while other studies either general or used only the crude extract without fractionation, It involve the use of a pharmacological methods like RLB-assay and It done on both non-pregnant and late-pregnant (D22) rat uterus.

Traditionally El-Hazha is used as an aqueous infusion. In our investigation we made a Methanolic-extract (AH2) from the plant, because the polarity of the two solvents are quite similar, the extract contains probably the same components. Previously, the aqueous extract of the plant was investigated by (Mohamed AH *et al.*, 1996) and gives potent contracting activity. The chloroformic fraction contains non-polar compounds and it was never used traditionally.

In general pilot screening, AH2 exerts relaxant effect in both uteri with insignificant difference in E_{max} %, but the EC_{50} was significantly differ, while the RLB assay revealed that the extract exerts binding affinity to β -ADR only in relatively very high concentration, these findings necessitates its fractionation to clarify this affinity.

Fractionation gives different sub-fractions with different efficacies on both isolated uteri, but we deal only with the most active ones. Fractions that produce relaxation $\leq 35\%$ on NP and $\leq 25\%$ on LP after first

fractionation and that produce relaxation activity $\leq 25\%$ on NP and 20% on LP after the 2nd fractionation process which calculated as $E_{\max} \pm \text{SEM}\%$ were mentioned and considered as active. Moreover, the fraction that showed best pharmacological activity was selected for the next steps.

The fractionation effect on the biological extract activity was achieved by relating AH2 to its most active fractions. In NP uterus, the extract activity was increased significantly ($p = 0.026$) by fractionation then markedly decreases, but still at a level (2X) greater.

In LP uterus the activity did not affected significantly by fractionation, but the original extract relaxant activity still exist. In spite that on this stage of pregnancy the sensitivity of the uterus to β -adrenergic activity was weaker than the non pregnant (Gaspar *et al.*, 2005).

These findings were supported with and confirmed by radio-ligand binding assay experiments results. In which at the beginning fractionation improved AH2 affinity to β -ADR, but after the 3rd fractionation process the affinity was deteriorated to a level similar to the AH2 before fractionation.

We continue the study using the most active sub fraction AH2-11, because we thought that this relaxant effect may be attributed to synergistic effects of the extract compounds that can be explain by the huge number of compounds that appears in the TLC plate (not shown here).

The selected Fraction AH2-11 as most active one when used to perform further experiments to verify the role of β -adrenergic receptor in mediating the above mentioned relaxant activity using propranolol as a blocker, both rat uteri revealed that; in NP, propranolol has no effect on the fraction's relaxant activity which may be taken as an evidence of a role of other mechanism(s) involves rather than β -ADR such as direct muscle effect (Ali *et al.*, 1992) or Ca^{+} -channel blocking activity. But in the LP data the β -ADR was clearly identified by the propranolol antagonistic effect on the fraction activity in which its dose -response curve was significantly ($p = 0.0049$) shifted to the right.

Further conformity test for the β -ADR role in this relaxation was done by pre-treatment of pregnant rats by progesterone because (Gálik *et al.*, 2008) reported that, Progesterone pre-treatment increases the expression of the β_2 -ADR during pregnancy and alters the effects of β_2 -ADR agonists on the pregnant myometrium. In addition, gestagen-induction increases in the myometrial β_2 -ADR density and the amount of activated G proteins coupled to β -ADRs.

Although (Gaspar *et al.*, 2005) found on the late stage of pregnancy the sensitivity of the uterus to β -

adrenergic activity was weaker than the non-pregnant, unfortunately the progesterone treatment did not potentiate the β -receptors sensitivity to this fraction, these findings can be explained by that, the β -ADR only partially participated in this relaxation besides other possible mechanism(s) or may be hide by the presence of other compounds in this semi-purified fraction.

TLC chromatogram showed the complexity of the active fractions. Even the sub-sub-sub fraction contains large number of structurally related compounds exists in a very small amount, thus their isolation is a very difficult process. These compounds may contribute the various traditional uses and the local name (plant of all diseases) of the plant. Finally, the separation may affect markedly its biological activity due to the well-known plant synergism phenomenon.

CONCLUSION

Finally we can concluded that, by these findings and demonstration we confirm and proof its mentioned traditional use, even it seems contradictory from the first point of view, The fractionation significantly affect the activity of its Methanolic-extract.

There is a partial role for β -ADR on mediating this relaxation activity or may be its complete, but inhibited by the existence of other contracting substances that needs further separation and isolation.

The suggested purification may lead to a discovery of a new novel natural therapeutic agent(s) useful to aid solving two major medical problems pre term labour and asthma.

REFERENCES

- Al-Burtamani, S.K., M.O. Fatope, R.G. Marwah, A.K. Onifade and S.H. Al-Saidi, 2005. Chemical composition, antibacterial and antifungal activities of the essential oil of *Haplophyllum tuberculatum* from Oman. *J. Ethnopharmacol.*, 96: 107-112. DOI: 10.1016/J.JEP.2004.08.039
- Ali, B.H., A.K. Bashir and R.A. Rasheed, 2001. effect of the traditional medicinal plants *rhazya stricta*, *balanitis aegyptiaca* and *haplophyllum tuberculatum* on paracetamol-induced hepatotoxicity in mice. *Phytother Res.*, 15: 598-603. <http://www.ncbi.nlm.nih.gov/pubmed/11746841>
- Ali, M.B., A.H.M., A.K. Bashir and A.M. Salih, 1992. Pharmacologica Investigation of *Haplophyllum tuberculatum*. *Pharmaceutica Biol.*, 30: 39-45. DOI: 10.3109/13880209209054628
- Boulus, L., 1983. *Medicinal Plants of North Africa*. Reference Publications Inc., Michigan, pp: 155-158.

- Clouse, A.K., E. Riedel, J.P. Hieble and T.D. Westfall, 2007. The effects and selectivity of beta-adrenoceptor agonists in rat myometrium and urinary bladder. *Eur. J. Pharmacol.*, 573: 184-189. <http://www.ncbi.nlm.nih.gov/pubmed/17632099>
- Gálik, M., R. Gáspár, P.Z.K.S. and G. Falkay, 2008. Gestagen treatment enhances the tocolytic effect of salmeterol in hormone-induced preterm labor in the rat *in vivo*. *Am. J. Obstet. Gynecol.*, 198: 319. <http://www.ncbi.nlm.nih.gov/pubmed/18313455>
- Ganguly, M., M. Kr Borthakur, N. Devi and R. Mahanta, 2007. Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino mice. *J. Ethnopharmacol.* 111: 688-691. DOI: 10.1016/J.JEP.2007.01.023
- Gaspar, R., 2005. Pregnancy-induced decrease in the relaxant effect of terbutaline in the late-pregnant rat myometrium: role of G-protein activation and progesterone. *Reproduction*, 130: 113-122. <http://www.ncbi.nlm.nih.gov/pubmed/15985637>
- Giles, W. and A. Bisits, 2007. Preterm labour. The present and future of tocolysis. *Best Pract. Res. Clin. Obstet. Gynaecol*, 21: 857-868. <http://www.ncbi.nlm.nih.gov/pubmed/17459777>
- Hannah, M.E., 2000. Search for best tocolytic for preterm labour. *Lancet*, 356: 699-700. DOI: 10.1016/S0140-6736(00)02626-X
- Khalid, S.A. and P.G. Waterman, 1981. Alkaloid, Lignan and Flavonoid Constituents of *Haplophyllum tuberculatum* from Sudan. *Planta Med.*, 43: 148-152. <http://www.ncbi.nlm.nih.gov/pubmed/17402027>
- Koucky, M., A. Germanova, Z. Hajek, A. Parizek and M. Kalousova, 2009. News in pathophysiology and management of preterm labour. *Ceska Gynekol*, 74: 54-63. <http://www.ncbi.nlm.nih.gov/pubmed/19408855>
- Mohamed AH, A.M., A.K. Bashir and A.M. Salih, 1996. Influence of *Haplophyllum tuberculatum* on the cardiovascular system. *Int. J. Pharmac.*, 34: 213-217. <http://informahealthcare.com/doi/abs/10.1076/phbi.34.3.213.13208>
- Mohammed, A., M.M.E.D. Al-Yahya, A. Ibrahim, Al-Meshal and S. Mansour, *et al.*, 1991. (+)-Dihydroperfamine: An Alkaloid from *Haplophyllum tuberculatum*. *Int. J. Pharmac.*, 29: 268-272. DOI: 10.3109/13880209109082895
- Monga, M. and R.K. Creasy, 1995. Pharmacologic management of preterm labor. *Semin Perinatol*, 19: 84-96.
- Mossa, J.S., M.A. Al-Yahya and I.A. Al-Meshal, 1987. *Medical Plants of Saudi Arabia*. pp: 1. <http://digital.library.ksu.edu.sa/ebook598.html>
- Owolab, O.J., Z.A. Nworgu, A. Falodun, B.A. Ayinde and C.N. Nwako, 2009. Evaluation of tocolytic activity of ethanol extract of the stem bark of *Ficus capensis* Thunb. (Moraceae). *Acta. Pol. Pharm.*, 66: 293-296. <http://www.ncbi.nlm.nih.gov/pubmed/19645329>
- Varamini, P.M.D., A. Mohagheghzadeh, M. Soltani and A. Ghaderi, 1992. Cytotoxic Evaluation of Four *Haplophyllum* Species with Various Tumor Cell Lines. *Pharmaceutical Biol.*, 45: 299-302. http://life-sciences.net/stories/206305/Cytotoxic_evaluation_of_four_Haplophyllum_species_with_various_tumor_cell_lines.html
- Pryde, P.G., R.E. Besinger, J.G. Gianopoulos and R. Mittendorf, 2001. Adverse and beneficial effects of tocolytic therapy. *Semin. Perinatol*, 25: 316-340. [http://www.seminperinat.com/article/S0146-0005\(01\)80037-9/abstract](http://www.seminperinat.com/article/S0146-0005(01)80037-9/abstract)
- Shih, H.C., C.S. Hsuand and L.L. Yang, 2009. *In vitro* study of the tocolytic effect of oroxylin A from *Scutellaria baicalensis* root. *J. Biomed. Sci.*, 16: 27. <http://www.ncbi.nlm.nih.gov/pubmed/19272127>
- Wahab, S.I.A., A.W.H. Mohamed, O.Y. Mohamed, M.M.E. Taha, A.B. Abdul and A.S. Al-Zubairi, 2008. Serotonergic properties of the roots of *clerodendron capitatum*. *Am. J. Biochem. Biotechnol.*, 4: 425-430. ISSN: 1553-3468.
- Tara, P.N. and S. Thornton, 2004. Current medical therapy in the prevention and treatment of preterm labour. *Semin. Fetal Neonatal Med.*, 9: 481-489. <http://www.ncbi.nlm.nih.gov/pubmed/15691786>
- Thaina, P., 2009. Uterine relaxant effects of *Curcuma aeruginosa* Roxb. rhizome extracts. *J. Ethnopharmacol.*, 121: 433-443. <http://www.ncbi.nlm.nih.gov/pubmed/19026735>
- Tsatsaris, V., D. Cabrol and B. Carbonne, 2004. Pharmacokinetics of tocolytic agents. *Clin. Pharmacokinet*, 43: 833-844. <http://www.ingentaconnect.com/content/adis/cpk/2004/00000043/00000013/art00001>
- Vermillion, S.T. and C.N. Landen, 2001. Prostaglandin inhibitors as tocolytic agents. *Semin. Perinatol*, 25: 256-262. <http://www.ncbi.nlm.nih.gov/pubmed/11561913>
- Zupko, I., A. Marki, R. Gaspar and G. Falkay, 1998. Correlation between alpha1/beta-adrenoceptor ratio and spontaneous uterine motor activity in the post-partum rat. *Mol. Hum. Reprod.*, 4: 921-4. DOI: 10.1093/molehr/4.9.921