Ph.D. Thesis Summary

University of Szeged - Faculty of Pharmacy

Department of Pharmacodynamics and Biopharmacy

Pharmacological investigations of natural β_2-adrenoceptors agonists on rat uterus *in vitro* and *in silico* studies

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1. INTRODUCTION

Premature labour as medical challenge and tocolysis as medical solution

labour may begin prematurely before the 37th week of pregnancy because uterine contractions cause the cervix to open earlier than normal. The baby is born premature and can be at risk for health problems. The specific causes of premature labour are not known and still medical challenge due to its incidence has not decreased recent several decades and no effective primary means of its prevention. Different risk factors were reported for premature labour such as self ability, Medicals and Life style.

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. Tocolytics are sometimes used to decrease the frequency of contractions, and may make women feel better. The main drugs used as tocolytics are indomethacin and other prostaglandins inhibitors, calcium channel blockers such as nifedipine, β- adrenergic agonists and oxytocin receptors antagonist, while the medical prevention consists of antibiotic or progesterone administration.

In clinical practice many drugs were used to inhibit the preterm labour incidence such as, magnesium sulfate. Whilst corticosteroids are given 24 hours before birth to help accelerate the baby's lung and brain maturity.

Numerous pharmacological agents have been utilized experimentally to inhibit preterm labour, but none has proven to be ideal. β_2-ARs agonists provide the best combination of safety and efficacy, also a combination of nifedipine and β_2-adrenoceptors agonists should be considered for the treatment or prevention of preterm birth.

A few β_2-ARs agonists were from natural origin. This information encourages us to attempt to isolate and test the pharmacological features of the isolated compound on β_2-adrenergic system.

El-hazha

During search for new β_2-adrenoceptors agonists from natural origin, a Sudanese herb El-hazha (*H. tuberculatum*) (Rutaceae); was selected as a target source due to its extensive traditional uses in this area.

Its essential oils were investigated for antimicrobial activity by Alburtamani *et al*., its cardiovascular effect were studied by Mohamed e*t al.*, its hepatoprotective activity was investigated by Ali *et al.* When its cytotoxic activity was checked against 11 tumor cell lines, strong cytotoxic activity was observed.

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 labour inhibition and asthma control).

On the other hands, many compounds were isolated from this plant, including alkaloids. Among these compounds, 6-methoxykaempferol-3-*O*-glucoside (6-MKG) and haplopine-3,3'dimethylallylether (HAP) were isolated and indentified early. But their pharmacological profile remains undetermined or controversial. In this study we attempt to proof and determine the pharmacological profile of both 6-MKG and HAP based on their uterus-relaxing activity using β_2-ARs as a main target.

β_2-Adrenoceptors

β_2-ARs are belong to the G protein-coupled receptors (GPCRs) which are the largest family of cell-surface receptors involved in signal transduction and are encoded by the largest gene family in most animal genomes. β_2-ARs represent one of the most important drug discovery targets. Among the GPCRs, β_2-ARs serve as the targets of 50% of drugs in the market.

The active binding site of β_2-ARs (finger print for optimum activity) was determined from the crystal structure of the β_2-AR–carazolol complex structure, whilst the ECL2 was found to be critical to ligand-binding kinetics due to its conformational flexibility.

β_2-ARs acts through a 2^{nd} messenger cAMP. GPCRs are in a conformational equilibrium between inactive and activating states [42] and binding of and activating ligand enables the receptor to catalyze the exchange of GTP for GDP in a heterotrimeric G protein.

β_2-ARs are an important pharmaceutical target for pulmonary and cardiovascular diseases. They are also useful in the treatment of nervous system injury and premature labour. Major pharmaceutical industries are investing enormous amounts of time and money

to achieve this object. This study is a bird's eye view on the some aspects β_2-adrenoceptors in drug discovery.

Molecular homology modelling and molecular docking

In drug discovery different techniques were used, the common direct one is once the target was identified, drug search starts in which a mixture of experimental and computer-based methods were involved.

Recently, *in silico* modeling of receptor-ligand interactions has become a common reference to biological studies carried out in the computer, joining the traditional terms *in vivo* and *in vitro* to describe the location of experimental studies, aimed to saving time and money in order to speed-up the research signals.

Nowadays, *in silico* homology modeling was used extensively to prepare the drug targets . The crystal structure of β_2- ARs was used in this study, as a template for homology modeling process. After building the target, molecular docking of ligands were performed to determine the interaction between the ligand and receptor molecule, because docking technique was a good predictor of molecule pharmacologic chaperoning capability.

Hetenyi *et al.* reported the use of *in silico* data, using the same useful equation (Eq. I) for the estimation equilibrium binding affinity (BA) of drug candidate calculation as the experimental free energy of binding (ΔG_E) from the experimental data to calculate the various Ligand efficiency (LE) values on the basis of a set of biologically relevant structural and thermodynamic experimental data.

2. AIMS

General aim:

- To find a new potential therapeutic natural tocolytic agent(s) or substantial lead substance from plant origin to aid in solving the medical challenge premature labour.

Specific aims:

- To investigate the relaxing activity of El-hazha herb regarding to its traditional uses on non-pregnant and late-pregnant rat uteri *in vitro*.
- Attempt to isolate pure active compound(s) based on this relaxing activity by biological-guided fractionation and by using β_2-adrenoceptors as a main target.

- To determine the pharmacological profiles of isolated compounds using *in vitro* and *in silico* techniques.

3. MATERIALS AND METHODS

Plant material

The aerial parts of *H. tuberculatum* (El-haza) were collected in the North part of Sudan and identified by (MAPRI), Khartoum, Sudan. A voucher specimen (No. M23/08) has been deposited at the Herbarium of MAPRI.

Animals

Sprague-Dawley rats (Charles-River Laboratories, Hungary) were used. Mature female (180-200 g) and male (240-260 g) rats were mated and vaginal smears were tested under a microscope, if positive, the female rats were separated and were regarded as first day-pregnant animals.

Softwares

The main softwares that used during our study were: AutoDock4 with AutoDockTools 1.5 were obtained freely via their official internet site. Molecular Operating Environment (MOE) was purchased from Chemical Computing Group Inc., Canada. The scientific graphing, curve fitting and statistics package, Prism 4.0 was purchased from GraphPad software Inc., USA.

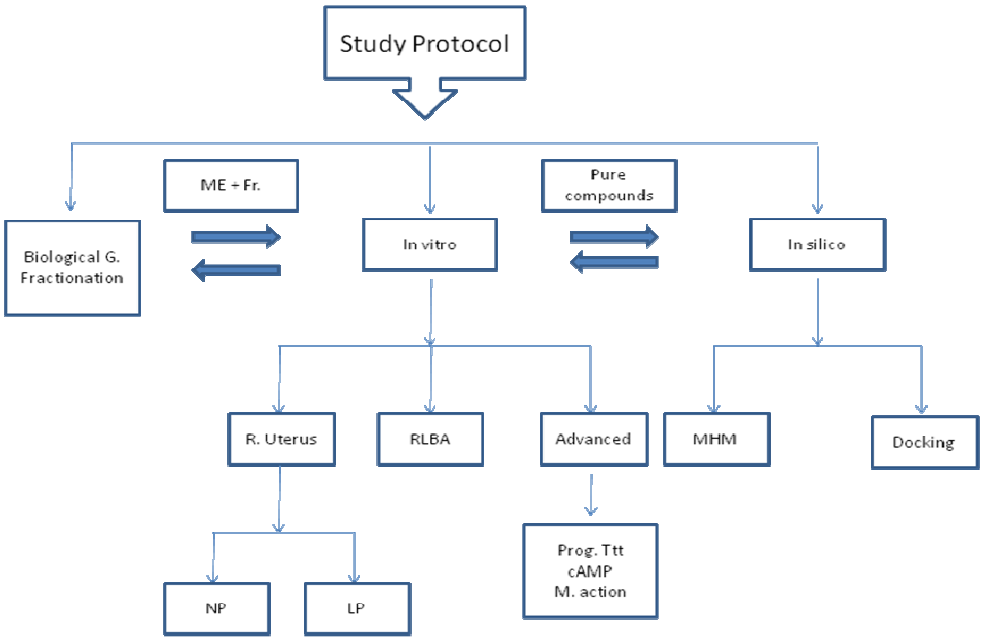
Study protocol

Different methods were used to achieve our aims, listed and diagrammed below:

1- biological guided fractionation for the ME depending on the uterus relaxing activity.

2- In vitro studies
- isolated rat uterus contractility
- cAMP measurement
- radioligand binding assay

3- In silico studies
-Ligand prepartion
-Building the receptor model
- Docking simulations and calculations



Extraction, Fractionation, Isolation and identification of the active compounds

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). At the end tow active compounds were isolated by biologically guided fractionation and their structures were determined by NMR spectral data analysis and pharmacologically tested.

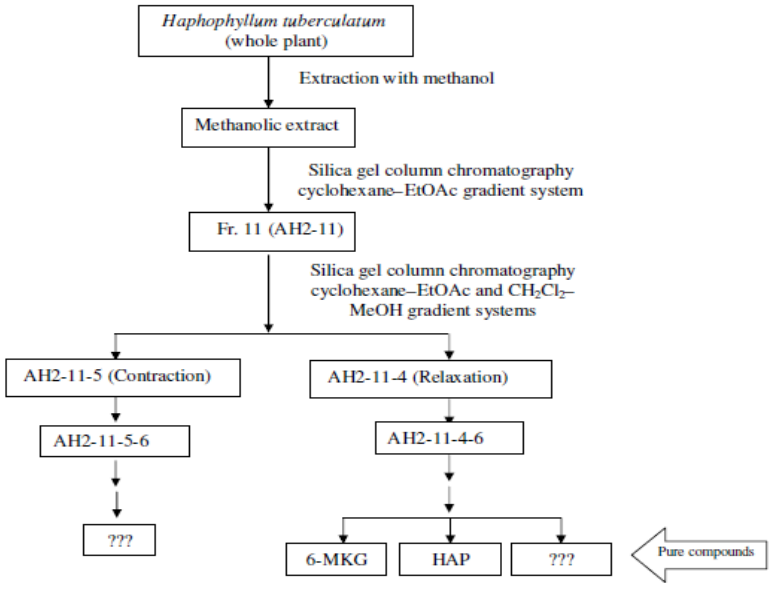


Fig. 1. Diagram illustrated the main steps of Bioactivity-guided fractionation of the methanolic-extract of El-hazha emphasized on the most active fractions resulted and used throughout the study. 6-MKG: 6-Methoxykaempferol-3-*O*-glucoside, HAP: haplopine-3,3'-dimethylallylether.

4. RESULTS

Isolated compounds

The two isolated active compounds from the most active fraction were:

1- 6-methoxykaempferol-3-O-glycoside (6-MKG), with M. wt of 478.40 g/mol and $C_{22}H_{22}O_{12}$ chemical formula.

2- Haplopine-3,3'-dimethulallylether (HAP), with M. wt of 313.13 g/mol and $C_{18}H_{19}O_4$ chemical formula.

In vitro results

Contractility studies on non-pregnant and late-pregnant rat uteri

Methanolic extract reasonable relaxant effect in a dose dependent manner for both (NP) and (LP). This effect was changed reasonably after several fractionations processes (Fig. 2). Also the two isolated compounds 6-MKG and HAP alone or in combination appears similar relaxant activity in both uteri, with 50% and 80% of the E_{max} of terbutaline, whilst the EC_{50} was lower than that of terbutaline. Fig. 3

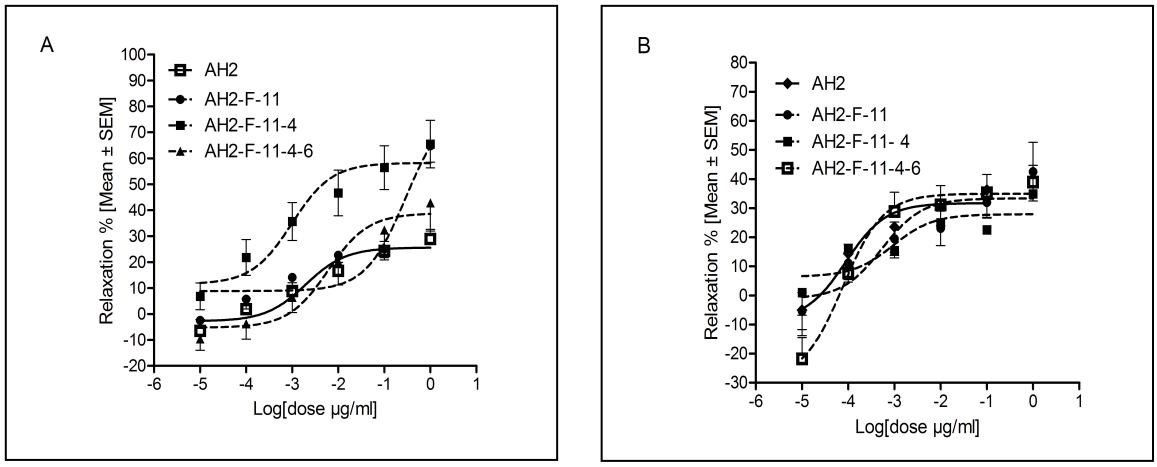


Fig. 2: Showed the (AH2) and its best fractions relaxant effects on (**A**) non-pregnant (NP) and (B) late-pregnant (LP) isolated rat uterus *in vitro*.

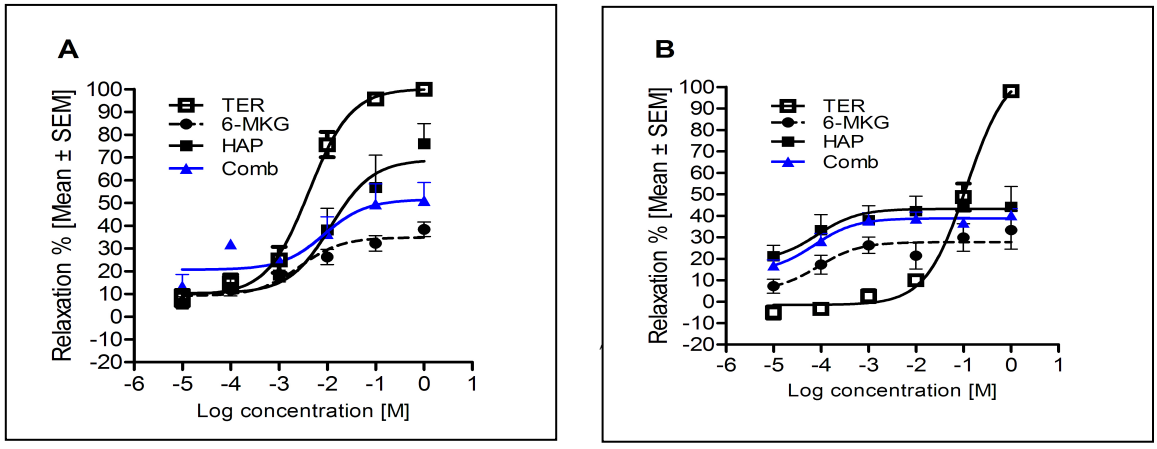


Fig. 3: Comparison of the relaxant effect of 6-MKG, HAP and their combination to the standard terbutaline on (**A**) non-pregnant (NP) and (**B**) late-pregnant (LP) isolated rat uterus *in vitro*.

Our study found this relaxant effect was mediated by β-ARs. The non-selective β-ARs blocker propranolol blocks competitively the effect of the most active extract fraction at preliminary steps of our study, while progesterone pre treatment of the (LP) rat uterus did not alter significantly the effect of this fraction.

The β_2-ARs antagonist ICI118,551 competitively antagonized the relaxing effect of both 6-MKG and HAP comparable to terbutaline (Table 1)

Table 1: Effect of ICI118,551 on the relaxing activities of terbutaline, 6-MKG, HAP and their combination on non-pregnant and late-pregnant rat uteri *in vitro*

Ligand	pA_2 [mean ± SEM], N=6-8		Emax [mean ± SEM]%, N=6-8			
			NP		LP	
	NP	LP	without ICI	with ICI	without ICI	with ICI
TER	**4.8 ± 0.3**	**2.9 ± 0.1**	**101.8 ± 0.5**	**74.6 ± 8.2**	**112.1 ± 1.7**	**25.2 ± 4.4**
6-KMG	4.5 ± 0.2	7.7 ± 0.4***	35.9 ± 2.3***	27.2 ± 3.9**	40.1 ± 1.3***	15.1 ± 3.6
HAP	4.7 ± 0.1	3.3 ± 0.02	80.8 ± 11.8*	16.5 ± 4.5**	51.8 ± 8.4***	35.2 ± 4.3
Combination	2.8 ± 0.6***	1.6 ±0.2***	52.1 ± 7.8***	40.9 ± 1.5*	39.0 ± 2.6***	33.4 ± 4.5

Terbutaline was used as positive control to compare and relate other to it throughout the table. TER: terbutaline, HAP: isolated compound; pA_2: negative logarithm of the antagonist concentration that reduces an agonist effect to its $E_{max}/2$; N: total number of observations; ICI: ICI18,551 *: p>0.05, **: p>0.01.

When the 6-MKG, HAP were added together, 6-MKG reduced significantly the activity of HAP in NP rat uterus Fig. 4A, whilst the addition of two compounds in the same time with terbutaline, both decreased the terbutaline effect in both uteri, Fig. 4B

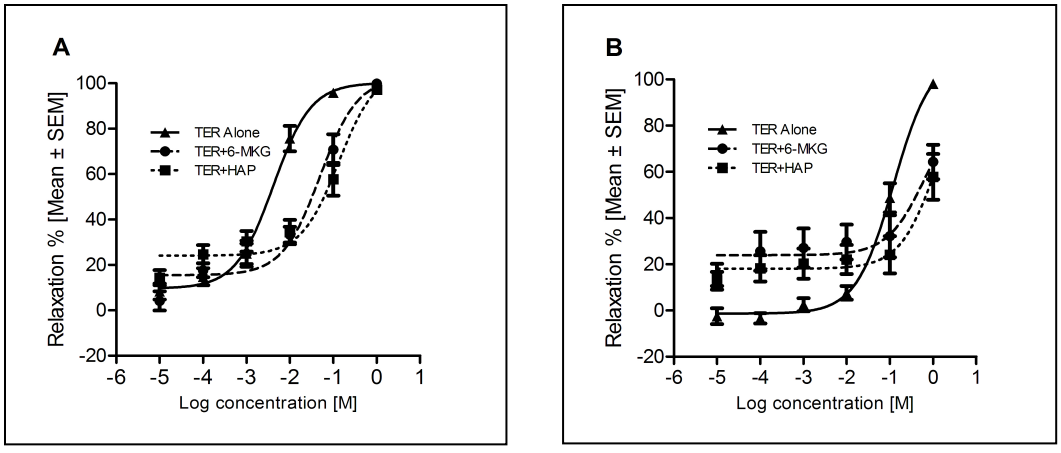


Fig. 4: Effect of 6-MKG, HAP on to the standard terbutaline relaxant effect on (**A**) non-pregnant (NP) and (**B**) late-pregnant (LP) isolated rat uterus *in vitro*.

Effect of 6-MKG on cAMP level

6-MKG induced the level for uterine cAMP on late-pregnant rat uterus, Fig. 5

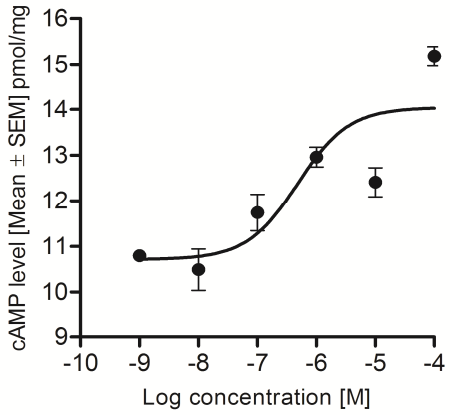


Fig. 5: Dose-response curves of the effect of 6-MKG on the cAMP induction level of the late-pregnant rat uterus in vitro. Values are means of 6-8 observations; vertical bars denote standard errors of the mean (S.E.M.).

Radioligand binding assay

The affinity of the methanolic-extract (AH2) and its most active sub fractions, The AH2 displace the radiligand only in very high concentration (10^4 µg/ml), whilst its other sub fractions showed better displacement affinities (Fig. 6A). The affinity of the terbutaline, ICI 118,551 and 6-MKG for β_2-ARs were tested on LP rat uterine membrane preparation, All ligands displace the radioligand from the target receptor, (Fig. 6B).

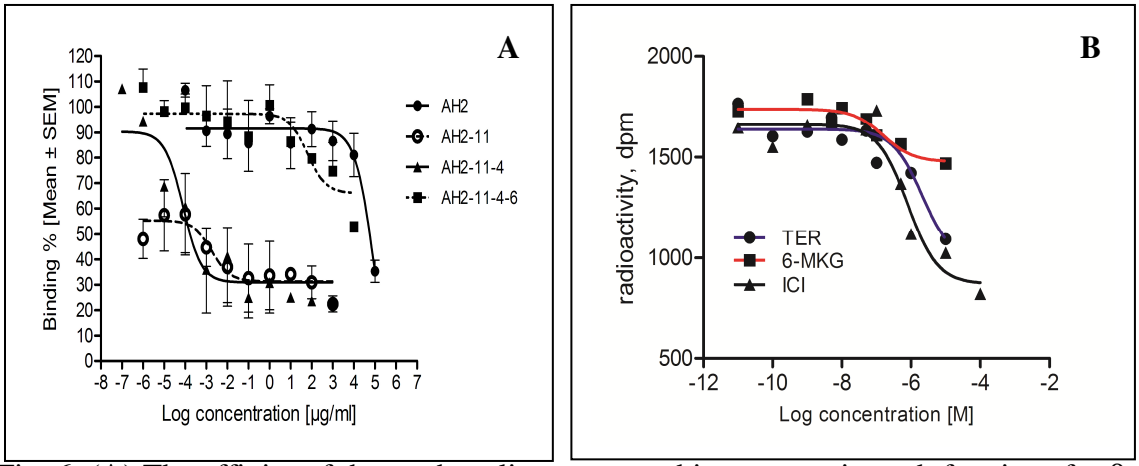


Fig. 6. (**A**) The affinity of the methanolic-extract and its most active sub fractions for β-ARs on rat brain membrane preparation, using Dihydroalprenolol [^{3}H] DHA (2 nM) as an isotopes radio ligand, (**B**) The affinity of the terbutaline, ICI 118,551 and 6-MKG for β_2-ARs on 22-day pregnant rat uterine membrane preparation, using [^{3}H]ICI 118,551 as radio ligand (2 nM).

***In silico* results**

Homology modeling

The Customized rat homology modeling for the β_2-AR that modeled from the template of the modified human β_2-AR, resulted in 91.5 % homology identity.

The applied restraints kept the orientation of the Ser^{203} and Ser^{204} side chains toward the active site. The model was validated by docking of noradrenaline that detected all of the important interactions (Asp^{113}, Phe^{193}, Ser^{203}, Ser^{204}, Asn^{312}) therefore this receptor model was suitable for molecular docking calculations.

Molecular Docking

The results of docking studies of the six standard ligands and our compounds 6-MKG and HAP summarized in the Table 2.

Table 2. Interaction points, estimated free energy of binding (ΔG_{bind}) and calculated K_d of docked ligands for the rat β_2-adrenoceptor. All of the mentioned interactions were H-bonds, except for that of Phe^{193}, which was a benzyl-benzyl interaction. ICI: ICI 118,551; PROP: propranolol; TER: terbutaline; ISO: isoproterenol; nADR: noradrenaline; ADR: adrenaline.

Interactions		Tested Ligands		Standard ligands					
Residue	Type	6-MKG	HAP	ICI	PROP	TER	ISO	nADR	ADR
Asp^{113}	acidic	+		+	+	+	+	+	+
Thr110	polar		++						
Thr^{118}	polar					+			
Cys^{191}	polar	+							
Phe^{193}	polar			+	+	+	+	+	+
Ser^{203}	polar						+	+	+
Ser^{204}	polar						+	+	+
Asn^{293}	polar						+	+	+
Asn^{312}	polar	+		+	+	+	+	+	+
Tyr^{316}	polar	+							
His93	basic		+						
$-\Delta G_{bind}$, kcal/mol		-11.53±0.06	-8.12 ± 0.12	-9.10±0.05	-10.30±0.07	-8.18±0.05	-8.45±0.04	-5.66±0.12	-6.65±0.02
calculated K_d, nM		3.55±4.5	1.12±0.5 μM	214.28±57.2	28.28±4.4	1.01±0.3 μM	641.71±75.6	19.7±3.5 μM	13.38±2.5 μM

Comparative *in vitro* - *in silico* ligand efficiency estimation results

Our isolated compounds 6-KMG and HAP exhibited ΔG/NHA value deeper than -0.24 kcal/mol, molecular weight lower than 500 g/mol and with the number of heavy atoms of 34 and 23 respectively.

Ligand efficiency was calculated for 6-KMG, HAP and terbutaline for both *in vitro* and *in silico* data regarding the number of heavy atoms Table 4, the value was similar to the exact E_{max} value that calculated by Prism software from the *in vitro* results as shown in Fig. 3.

Table 4. comparison of calculated Ligand efficiency (LE) value based on number of heavy atoms *in vitro* and *in silico* for terbutaline and isolated compound HAP, *In vitro* E_{max} value.

compound	NHA	*In vitro* (NP) N=6-8		*In silico*		E_{max}±SEM
		EC_{50}	LE[a]	ΔG, kcal/mol	LE[b]	*In vitro*
TER	**16**	**5.94 ± 3.2 E-07**	**-5.31E+02**	**-8.18 ± 0.05**	**-0.511**	**101.8 ± 0.5**
6-MKG	34	6.11± 2.4 E-07	-2.41E+02	-11.53 ± 0.06	-0.339	35.93 ± 2.9
HAP	23	6.34 ± 3.9 E-07	-3.68E+02	-8.12 ± 0.12	-0.353	80.8 ± 11.8
Ratio of 6 -MKG/TER			**45.39%**		**66.34%**	**35.29%**
Ratio of HAP/TER			**69.30%**		**69.08%**	**79.37%**

TER: terbutaline, HAP: isolated compound; NP: non-pregnant rat uterus; NHA: Number of heavy atoms; MW: Molecular weight; ΔG_{bind}: estimated free energy of binding; E_{max}: the maximal relaxing effect of TER or HAP against KCl-induced contraction, EC_{50}: the concentration of the TER or HAP producing 50% of their maximal relaxing effect against (KCl-induced contraction) in the system; S.E.M.: standard errors of the mean; N: total number of observations.

[a] $LE=-RT\ln(IC_{50})/NHA$, [b] $LE=\Delta G/NHA$.

5. DISCUSSION

Finding therapeutics to act on potential drug targets is a challenging and often very expensive process, huge work and efforts were done on developing and producing biotechnology drugs from protein origin, these drugs have many disadvantages, which redirected the researchers back to the nature looking for small molecules from medicinal herbs, because medicinal plants are a rich drug source.

Premature labour still a health challenge world-wide, thus huge efforts were done and required to found a solution for it, this work was an attempt to find a natural solution.

However, the efficacy and safety of tocolytics are not adequate, new agents are therefore required including substances from natural sources.

In general pilot screening, AH2 exerts relaxant effect in both NP and LP uteri, whilst the radioligand binding assay revealed that the extract exerts binding affinity to β-AR only in relatively very high concentration, these findings necessitates its fractionation to clarify this affinity.

Fractionation affect significantly the biological extract activity, These findings were supported with radioligand binding assay experiments results.

The most active fraction (AH2-11) was used to perform experiments to verify the role of β-adrenergic receptor in mediating this relaxant activity, in the LP the β-AR role was clearly identified by the significant propranolol antagonistic effect on the fraction activity.

Progesterone treatment did not potentiate the β- ARs sensitivity to this fraction, although progesterone pre-treatment increases the expression of the β_2-ARs during pregnancy and alters the effects of β_2-AR agonists on the pregnant myometrium, these findings can be explained by, the β-AR was only partially participated in this relaxation.

β-AR activity of the semi-purified fraction was not strong, even after the isolation of its two main compounds which were found to be a partial agonist after comprehensive further investigations.

Pharmacodynamics of the isolated compounds

Both 6-MKG and HAP evidently have agonistic features, because they produced approximately 50% and 80% of the maximum activity of terbutaline on the isolated rat uteri, with a higher binding affinity for the β_2-ARs in both *in vitro* radioligand for 6-MKG and *in silico* docking experiments.

On the other hands, 6-MKG exhibited a lower EC_{50} on LP rat uterus than that of terbutaline (half), but bound to the β_2-ARs with higher affinity and lower efficacy, and was therefore a weak agonist. Whilst in NP rat uterus, the E_{max} of 6-MKG was 25% of that for terbutaline, while EC_{50} was higher for 6-MKG than for LP, where it was 1000x that for terbutaline.

ICI 118,551 in the LP uteri completely blocked the effect of 6-MKG unlike terbutaline (partially), suggesting that 6-MKG is only a weak agonist.

The β_2-ARs are GPCR that acts through cAMP, 6-MKG enhanced the induction level of cAMP significantly in a dose-dependent manner in the LP uteri, and thus 6-MKG is an agonist, because Klukovits *et al.* revealed that terbutaline as agonist induced a cAMP level in similar mannor. Besides, the radioligand binding assay revealed that 6-MKG has better affinity than terbutaline and ICI118,551 for the β_2-ARs.

HAP exerts uterus relaxant activity less than that of terbutaline, which was in line with *in silico* results. HAP possibly has β_2-AR agonistic activity showing similar binding affinity than that of terbutaline. This hypothesis has been supported by the action of β_2-ARs antagonist ICI118,551 which blocked the relaxant effect of both HAP and terbutaline with same pA_2 value. However, the E_{max} of HAP was more depressed by the antagonist, which suggests that HAP has a lower efficacy feature compared to terbutaline.

The comparison of the isolated compound 6-MKG and HAP to the original herb methanolic extract revealed that 6-MKG and HAP should play a major role in the relaxant effect of the extract. However, we can only compare the E_{max} values, because 6-MKG and HAP were used in molar concentration, while the methanolic extract was used in µg/ml, so the EC_{50} values cannot be compared to each other.

β_2-ARs has only one binding site, so addition of two agonist at the same time compete with each other and interferes with others leading to synergistic additive or partial agonistic pharmacological effects. To check this our isolated compounds were added alone or in combination to the standard terbutaline, where they decreased significantly the terbutaline relaxing effect on rat uteri, thus serves as partial agonists.

On other hands 6-KMG significantly decreased the maximal effect of HAP from 80% to 60 % and thus considered as partial agonist. Besides, the ICI118,551 competitively blocked the combination effect in both uteri.

In silico studies

Homology modeling was required for rat receptor from human one, because our study was carried in *in vitro* rat uterus as main target and to compare the results logically. The receptor structure obtained by homology modelling with its RMSD values and the

interaction points (Asp^{113}, Phe^{193}, Ser^{203}, Ser^{204}, Asn^{312}) with noradrenalin proved that it is a good starting point for molecular docking calculations.

In case of the reference molecules we have identified 3 common interaction points (Asp^{113}, Phe^{193} and Asn^{312}), and other two residues (Ser^{203} and Ser^{204}) represented by ligands containing the catechol ring (adrenaline, noradrenaline, isoprenaline). These findings confirm the efficiency of our homology model.

The 6-MKG binds to the rat β_2-ARs with low ΔG_{bind} and K_d values and has different interaction points than that of terbutaline, adrenaline, noradrenaline and ICI 118,551. The position of glycopyranoside ring is stabilized by Asn^{312} (electrostatic interaction), Cys^{191} (H-bond) and the Tyr^{316} (two strong H-bonds), while the Asp^{113} forms an H-bond with the 5-hydroxy part of the flavone ring. Therefore the benzopyrane ring of 6-MKG anchors far from the Ser^{203} (3.4 – 5.2 Å), Ser^{204} (3.8 – 4.7 Å) and Ser^{207} (4.6 – 6.0 Å) and it was not able to showed these typical catechol interactions.

In silico findings revealed that HAP resembles the standard terbutaline in ΔG_{bind} and K_i, and differ from it on the orientation within the active pocket and the replacement of the acidic interaction at Asp^{113} by the basic interaction at His^{93}.This slight difference leads to observe slight activity difference consequently, because activity similarly feature is matter of binding energy and affinity constant (K_d) property.

Although partial-agonist is a compound that can activate receptors but is unable to elicit the maximal response of the receptor system, both 6-KMG and HAP were partial β_2-ARs agonists, because partial agonists may have 50% response doses lower or higher than full agonists. Information regarding the binding sites of partial agonists is still not sufficient to explain why they cannot fully activate the receptor.

Partial agonists were of therapeutic essentials, nicotine receptor partial-agonists may help people to stop smoking such as cytisine, a drug widely used in central and eastern Europe for smoking cessation, while partial agonists of dopamine receptors was used in schizophrenia and psychosis. Lastly the β-adrenergic partial agonist alifedrine implicated in the management of cardiac performance with acute ischemic left ventricular failure.

Comparative *in silico* - *in vitro* functional studies

Ligand efficiency (LE) was a useful metric for measuring the impact on activity of the addition of more molecular bulk where molecules that achieve a given potency with fewer heavy atoms are by definition more efficient. It allows the combination of pharmacodynamic (IC_{50}, ΔG_{bind}) and pharmacokinetic (MW, NHA, NoC, etc) properties into unique measures Bemebenek *et al.* To Identifying a drug candidate the ΔG/NHA value must be deeper than -0.24 kcal/mol, the molecular weight lower than 500 g/mol and the number of heavy atoms between 20 and 70. In our case, both isolated compounds 6-KMG and HAP satisfies this feature.

Although in this study we only emphasizing on the pharmacodynamic property of the isolated compound so only three parameters (MW, ΔG_{bind} and NHA) were used while other factors related to pharmacokinetics features was out of our aims.

For *in silico* LE calculation the experimental free energy of binding (ΔG_{bind})was used, whilst for *in vitro,* inhibitor concentration at 50 % inhibition (IC_{50}) was used as BA pharmacodynamics representing parameter.

The correlation of our calculated LE and the E_{max} revealed that there was a direct strong relation between the efficiency and *in vitro* efficacy, because we got similar value in both cases when comparing our compounds 6-KMG and HAP to the standard terbutaline.

6. SUMMARY & CONCLUSION

Our work can be summarized in a brief informative way in which, El-hazha as a herb was used many years in Sudan to treat different aliments, by these findings its ethno-pharmacology in the field of gynaecology has been proofed to some extent and two new natural tocolytic agents were isolated from it in order to aid in solving the problem of premature labour.

The traditional use for El-hazha as relaxing agent may be due to the presence of active compounds that act on β_2-Adrenergic receptor.

Our results revealed that the fractionation affect significantly the relaxant activity of the plant methanolic extract and there were strong evidences that showed the presence of a partial role for β-AR 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 purification leads to a discovery of a new novel natural therapeutic agent(s), considered as a useful tocolytic.

The isolated compound, 6-MKG exerts weak β_2-adrenoceptor agonistic activity, whilst HAP exhibited strong activity. 6-MKG and HAP serve as a starting point in future drug development aimed at the production of a new safe, effective and bio-accessible therapeutic agent, and can be considered as natural compounds of potential significance for the treatment and prevention of premature labour.

Both 6-KMG and HAP were categorized as partial β_2-adrenoceptors agonist due to they cannot fully activate the receptor, but still of therapeutic essentials that implicated in the management of several medicinal disorder such as premature labour, bronchial asthma and even cardiac performance with acute ischemic left ventricular failure.

Future work is recommended to investigate the pharmacokinetics and toxicological features for the isolated compounds in order to be available for evidence-based clinical therapy.

Finally we can concluded that, plants still a rich source for drugs, and a recent trend in medicine to return to the nature in treating disease and to discover new therapeutic agents.

We investigated the plant methanolic extract and found it showed significant relaxant activity in which β_2-adrenergic receptors participate substantially in mediating it. These findings stimulate the isolation and the pharmacological characterization of its active compounds.

Confirmation and scientifically proof of its mentioned traditional use, even it seems contradictory from the first point of view.

The fractionations significantly affect the activity of its methanolic-extract, existence of partial role for β-AR on mediating the plant relaxant activity

Both isolated compounds, 6-KMG and HAP were β_2-AR agonists with potential therapeutic value in preventing premature labour.

6-KMG and HAP can be categorized as partial β_2-ARs agonists.

Both pure compounds can serve as a starting point in future drug development aimed at the production of a new safe, effective and bio-accessible therapeutic agent.

Future work was recommended to investigate the pharmacokinetics and toxicological features for the isolated compounds in order to be available for evidence-based clinical therapy.

Publications list

Publications related to the PhD thesis

1. **Aimun Abdelgaffar Elhassan Ahmed**, Robert Gaspar, Arpad Marki, Andrea Vasas, Mahmoud Mudawi Eltahir Mudawi, Judit Hohmann and George Falkay.
   Uterus-Relaxing Study of a Sudanese Herb (El-Hazha).
   American J. of Biochemistry and Biotechnology 6 (3): (2010) 231-238, …….. **IF: 1.493**
2. **Aimun AE. Ahmed**, Arpad Marki, Robert Gaspar, Andrea Vasas, Mahmoud M.E. Mudawi, Balázs Jójárt, Judit Verli, Judit Hohmann, and George Falkay.
   β_2-Adrenergic activity of 6-methoxykaempferol-3-O-glucoside on rat uterus: *in vitro* and *in silico* studies.
   European Journal of Pharmacology 667 (2011) 348–354……..... **IF: 2.737**
3. **Aimun AE. Ahmed**, Arpad Marki, Robert Gaspar, Andrea Vasas, Mahmoud M.E. Mudawi, Balázs Jójárt, Renáta Minorics, Judit Hohmann, and George Falkay.
   In vitro and *in silico* pharmacological investigations of a natural alkaloid.
   Medicinal Chemistry Research, DOI:10.1007/s00044-011-9946-0,……..... **IF: 1.058**

Abstract and presentation related to the thesis

In vitro and *in silico* pharmacological investigations of natural β_2-Adrenoceptors agonists. The 3rd PharmSciFair Conference, June 13-17, 2011, Prague, Czech Republic.

BIOGRAPHICAL SKETCH

Aimun AE. Ahmed was born on Dec. 22, 1975 in Shandi, Sudan. In July 2001, he obtained his B.Pharm degree in Pharmacy, Khartoum University. In July 2006, he obtained his M.Pharm degree from Faculty of Pharmacy University of Khartoum at the department of Pharmacology. He joined Department of Pharmacodynamics and Biopharmacy at faculty of Pharmacy Szeged University as a graduate student in Sept. 2008. He will defend his Ph.D. thesis in Pharmacology in February 2012.