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Original Article

Study of the Anti-Anxiety Properties of the Phaseollidin, An Isoflavonoid Isolated from Erythrina droogmansiana (Leguminosae)

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ABSTRACT

The anti-anxiety properties of the phaseollidin, isoflavonoid isolated from *Erythrina droogmansiana*, were studied in the white mice. Various experimental models (Elevated Plus-Maze, Open-Field, Hole-Board, Hyperthermia Induced Stress), were used to highlight these properties. The results showed that the phaseollidin involved a significant increase of the time spent in the open arms of the raised labyrinth in cross, with 97.8 ± 21.64 seconds for a dose of 50 mg/kg and 115.4 ± 34.03 seconds for the dose 25 mg/kg. These results thus testify to the fall of the aversion of the rodents for enlightened spaces. In the tests of the hole-board and open-field, used to evaluate the exploration and locomotion activities of mice, the results showed a significant increase of the locomotion (crossing) with 42.2 ± 10.30 for the dose 25 mg/kg and 60 ± 9.05 for the dose 100 mg/kg of the phaseollidin. The doses 25 mg/kg and 100 mg/kg of the phaseollidin also showed a significant increase of "rearing". These results show that the phaseollidin has anti-anxiety properties.

Keywords: phaseollidin, *Erythrina droogmansiana*, Anxiety.

INTRODUCTION

In Africa, phytotherapy plays a significant role in the treatment of diseases and the use of medicinal herbs by the world populations has greatly increased with time because of their proven effectiveness (Ngo Bum *and al*., 2009b; WHO, 2008). Numerous species of plant and molecules coming from plants, have already shown their healing power on many ailments. More and more plants are introduced in the treatment of psychiatric diseases like alternative or complementary drugs (Woode *and al*., 2010).

A good amount of these drugs of vegetable origin were tested on animal models, which made it possible to understand their biological activity. Concerning the therapeutic interests, the barks and the roots of *Erythrina* plants gender, play a part in the traditional pharmacopeia, where they are used in form of infusion, of paste or decoction for the treatment of various diseases such as rheumatism, cognition, fever, cough, tooth ache (Wandji, 1987). Abyssinone V-4'-methyl ether, a flavonoid isolated from *Erythrina droogmansiana*, showed its anti-inflammatory (Sokeng *et al*., 2013) and antioxidative (Yaya *and al*., 2014) properties. The phaseollidin secondary metabolite also isolated from *Erythrina droogmansiana*, is an isoflavonoid whose anti-anxiety properties are studied here. In parallel, the exploring and locomotor activities of the mice are also evaluated.

MATERIAL AND METHODS

Plant Material: The phaseollidin, secondary metabolite of *Erythrina droogmansiana* De Willd

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and T. Durand, were isolated at the University of Yaounde I starting from the barks from the trunk, the barks of the roots, wood from the roots and sheets collected in Nkomekoui in the center region of Cameroon in August 2010 and, preserved in the form of crystals at room temperature in a hermetically closed tube.

Extraction and isolation of the compound: The powders of the barks of the roots and the barks of the trunk separately underwent extractions by simple maceration at room temperature. The extraction for each powder was initially done with ethyl acetate during 72 hours three times. The extract obtained was concentrated dry under reduced pressure by means of a rotary evaporator. The dried powder residue was extracted with methanol during 72 hours three times and 225 grams of acetic extract was obtained from the barks of the roots, 185 grams of methanolic extract from the barks of the roots. Then 175 grams of this powder, were fixed on average grain sand and series 1 obtained was chromatographied on silica gel column (granulometry….) and eluted with hexane, the hexane / ethyl acetate and the ethyl methanol acetate mixtures. After evaporation, several fractions crystallized and then washed with hexane, the compound YG7 was obtained as a reddish amorphous (80mg).

Experimental Animals: The experimentation was carried out on white mice *Mus musculus* Swiss of the family of Muridae. The mice weighing between 24 to 26 ± 2 grs and approximately 2 months old were provided by the National Veterinary Laboratory (LANAVET) of Garoua. . Mice were taken along to the laboratory 72 hours before the experimentation for acclimatization.

Drugs and Chemicals: The chemical substances of reference used were Diazepam (Valium ®) 5 mg/mL in the form of bulbs and phenobarbital (Phenobarbitone ®) 200 mg/mL in the form of bulbs.

PHARMACOLOGICAL TESTS

Elevated plus-maze test (Rodgers and al, 1997): Anxiety in animals is evaluated by the elevated plus-maze test. It is baised on the innate aversion of the rodents for open and lit spaces, vacuum and innovations (Handley and al, 1984; pellow *and al*., 1985). The experiment was carried out on five groups of mouse comprising five mice each. The mice were led to the laboratory 48 hours before the test for acclimatization. The positive control group received diazepam at a dose of 3 mg/kg intra-peritoneally, while the negative control group received distilled water (p.o) and the three test groups received the different doses of phaseollidin, 25 mg/kg, 50 mg/kg and 100 mg/kg (p.o) respectively. One hour after administration, each mouse was placed in the central zone of the labyrinth, the head directed towards an open arm, and left to freely explore the labyrinth for 5 minutes. During the five minutes of observation, we noted the number of visits and time spent in each type of arm, the number of "rearing" (the mouse draws up itself in driving position, resting against its rear limbs and the forelimbs are placed on the edge of the device), the number of grooming (the mouse puts itself in ball and is cleaned), the number of head-dipping (the mouse leans and passes its head over the edge of an open arm of the labyrinth).

Hole-Board (Takeda *et al*., 1998): Five groups of five mice each were constituted. The positive control group received diazepam at the dose of 0.5 mg/kg intra-peritoneally and the negative control received distilled water (p.o), the remaining three group received three different doses of phaseollidin : 25 mg/kg, 50 mg/kg and 100 mg/kg respectively (p.o). After each treatment, the mice were put in their cages and an interval of approximately thirty minutes was respected between administrations to two consecutives groups. One hour after administration, each mouse was placed in the central zone, and was left freely explore the surface for 5 minutes. During the five minutes of observation, parameters such as the latency time for the appearance of the 1st head-dipping, the number of head-dippings (the mouse leans and passes its head by one of the holes of the device), the number of rearing, the number of crossings (the mouse crosses lines during the exploration of the surface) and the number of grooming (the mouse puts itself in ball and is cleaned) were noted.

Open-Field (Belzung, 1999): The rodents tend to avoid enlightened and open spaces. When they are placed in the luminous enclosure of the arena, the rats and the mice prefer to move at the level of the periphery against the ramparts of the device. The activity in the arena represents for this reason, a valid measure of the anxious behavior in the laboratory animals (Tronche, 2009). For the experimentation, twenty-five mice were distributed in five homogeneous groups. Two controls groups in which the positive control group received diazepam at the dose of 0.3 mg/kg intra-peritoneally and the negative control group received distilled water (p.o). The three remaining groups received three doses of phaseollidin (25, 50 and 100 mg/kg, p.o) respectively. After each treatment, the mice were kept in their cages and an interval of thirty minutes was respected between the administrations in two consecutive groups. One

hour after administration, each mouse was placed in the central zone of the arena, and was let to explore it during 5 minutes (Royce, 1977). During the observation, parameters such as the time spent in the center, the number of rearing (the mouse draws up itself in driving position, resting against its rear limbs and the forelimbs are posed on the edge of the device), the number of crossing (the mouse crosses lines during the exploration of surface) and the number of grooming.

Stress Induced hyperthermia test (Borsini *and al*., 1989): The stress induced hyperthermia test is a simple and fast measurement of anxiety. For the realization of this test, fifty mice were distributed homogeneously in five groups. Two controls groups in which positive control group received Phenobarbital at the dose of 20 mg/kg intra-peritoneally and the negative control group received distilled water by cramming. The remaining three test groups received three different doses of phaseollidin 25, 50 and 100 mg/kg; p.o. After each treatment, the mice were kept in their cages and interval of thirty minutes was respected between the administrations in two consecutive groups. One hour after administration, they were withdrawn, one after the other at a rate of one mouse per minute. The rectal temperature was taken by introduction of a probe (2 mms diameter and 2 cm length) into the rectum of the mouse. Before each temperature recording, the probe was maintained in NaCl 9 ‰. The hyperthermia induced by stress (HIS) was given by making the difference between the rectal temperature of the three last mice and those of the three first of each group.

Statistical analysis: Calculation of the percentage time and number of entries on the open arms with 95% confidence limits and comparisons of the results were performed using computerized linear regression analysis, using GraphPad Prism (version 4.00, GraphPad Soft- ware Inc., San Diego, CA, USA). The statistical analysis of data was performed by one-way analysis of variance (ANOVA) followed by dunnet's multiple comparison test. In all cases differences were considered significant if $p < 0.05$.

RESULTS

Structure elucidation of phaseollidin: The compound responds positively to the test of phenolic and cyanhydrinic compounds, that which is characteristic of flavonoids. After rigourous analysis of the different spectral data, the formular $C_{20}H_{20}O_4$ with 11 insaturations was attributed to this compound.

The presence of aliphatic signals on its RMN^{1}H spectrum has 3,45 (1H, dd, *J* = 9,6 ; 10,7 Hz); 3,43 (1H, ddd, *J* = 4,5 ; 6,5 ; 10,7 Hz); 4,17 (1H, dd, *J* = 4,5 ; 9,6 Hz) and 5,38 (1H, d ; *J* = 6,5 Hz), which we can deduce that this compound is a pterocarpan. The appearance at 6.49 ppm (1H,dd, J= 8.3Hz) which couples in COSY through an AB system with on aromatic proton at 7.30 ppm of the cycle A of pterocarpans. and this cycle is monosubstituted. The appearance of signals at 1.72 and 1.63 ppm, 3.23 and 5.33 ppm indicating the presence of a unique isoprenic unit.

Its RMN^{13}C spectrum reveals: Quatenary carbons: 159.5; 156.7; 159.4; 112.3; 157.5; 112.9 and 132.8 which are amongst others are carbons carrying hydroxylgroups or linked to oxygen atoms, ethylinic carbons and carbons with aromatic rings. Tertiary carbons: 40.9; 78.9; 131.3; 110.1; 103.6; 118.7; 122.2; 107.9 and 123.4 ppm which correspond to methines and oxymethines of the cycle B of pterocarpans, to ethylinic methines of the isoprenyl group and to methines of the A and D aromatic rings. Secondary carbons: 23.2 and 67.2 ppm which correspond to methylenes of the isoprene group and to cycle B pterocarpans.
Primary carbons: 17.4 and 25.5 ppm corresponding to the methyl groups of the two isoprene groups.
The comparison of these data with those reported in the literature permits that we attribute the structure of YG7 to phaseollidin recently isolated from *Erythrina addisoniae* by Watjen and al., 2007.

Elevated Plus-Maze test
Effects of the phaseollidin on the percentage of the number of entries in the open arms. The doses 25, 50 and 100 mg/kg of the phaseollidin and diazepam, increased the percentage of number of entries in the open arms. The percentage of number of entries in the open arms increased from 19 ± 7, 87 for the negative control group to 50.3±4.35, 47.9±5.78 and 40.7±5.74% at the doses of 25; 50 and 100 mg/kg of phaseollidin, respectively. This value is 44.8±6.73 for the positive control (fig 1).

Effects of the phaseollidin on the percentage of the time spent in the open arms. The amounts 25 mg/kg and 50 mg/kg of the phaseollidin as well as Diazepam, increase the percentage of time spent in the open arms. The percentage of time spent in the open arms varies from 7.53±2.6 for negative control group to 38.5±5.07 and 32.6±3.23 % at the dose of 25 and 50 mg/kg of phaseollidin (fig 2). This value is of 32.7±8.41 % for diazepam (3 mg/kg).

Effects of phaseollidin on the percentage of the number of entries in the closed arms.

The dose of 25 and 50 mg/kg of phaseollidin and Diazepam (3 mg/kg), decrease the percentage of the number of entries in the closed arms of the labyrinth compared to negative control. The percentage of entrance in closed arms is 49.7±4.35, 52.1±5.78 and 55.2±6.73 % at the doses of 25, 50 mg/kg of phaseollidin and diazepam, respectively. This value is 81±7.87 for negative control.

Effects of phaseollidin on number an time in open and closed arms, Head-dipping, rearing, stretched attend posture (SAP) and grooming. The administration of extract results in the significant increase of the number of entries into open arms from 2.25 in the control group to 9.20 at the dose of 25 mg/ kg (table 1). Diazepam and phaseollidin (50 mg/kg) also induced significant reduction in the close arms entries from 8.2±1.3 in the negative control group to 5.2±1.5 and 5.0±1.58 % respectively. The number of rearing and stretched attend posture were reduced both by diazepam and the extract (table 1). The number of grooming was increased by the extract from 0.6 for the negative control to 1.6±0.8, 1.6±0.49 and 1.6±0.49 at the dose of 25, 50 and 100 mg/kg of phaseollidin solution (table 1).

Hole-Board test: The doses 25, 50 and 100 mg/kg of phaseollidin and Diazepam decreased the latency time of appearance of the 1st head-dipping. The latency time of appearance of the 1st head-dipping is 10.2 ± 2.28 sec, , 9.8 ± 2.28 sec and 8 ± 1.58 sec at the dose 25 mg/kg,50 mg/kg, and 100 mg/kg of phaseollidin, respectively and 20.6±5.13. This value is of 7.8 ± 1.64 for positive control.

The doses of 25 mg/kg and 100 mg/kg of phaseollidin increased the number of crossing compared to the negative control. The number of crossing varies from 18.2±2.77 for negative control group to 42.2 ±10.3 and 60±9.06 at the dose of 25 and 100 mg/kg of phaseollidin. The number of rearing was also increased in animal treated with phaseollidin and diazepam. This number went from 5.4±3.5 for the negative control group to 14.2±4.02, 6.8±1.33, 14.6±2.42 and 14.4±1.42 for the doses 25, 50, 100 mg/kg of phaseollidin and diazepam (0.5 mg/kg) (table 2).

Open-Field test: The doses of 25 mg/kg and 50 mg/kg of the phaseollidin, increased the number of crossings compared to the negative control. The number of crossing varied from 38.3±5.4 for negative control group to 62.2 ± 18.8 for the dose of 50 mg/kg and 71.2 ± 11.84 for the dose of 25 mg/kg of phaseollidin. The number of crossing was 55.2±10.2 for positive control group. The doses of 25 mg/kg of the phaseollidin, increased the time spent in the center compared to negative control. The time spent in the center is 6.4 ± 1.67 for the amount 25 mg/kg of the phaseollidin and 2.8±0.86 for the negative control group (table 3).

Hyperthermia Induced Stress: The doses 50 mg/kg and 100 mg/kg of phaseollidin decreased the hyperthermia induced by the stress. This value is of 0.63 for the dose of 50 mg/kg and of 0.06 for the dose of 100 mg/kg of the phaseollidin compared to the negative control group in which HIS is 1.23 (fig 5). The temperature is reduced in the group who received phenobarbital compared to the negative control group; 33:7±0:270 and 36.0±0.306 respectively (fig 6).

DISCUSSION

The elevated plus-maze test is one of the models of anxiety most used in the study of anxiety in animals (Pitchaiah *and al*., 2008). phaseollidin increases the number of entries and the percentage of time spent in the open arms and, decreases the number of entries in the closed arms. The increase in the number of entries and the percentage of time spent in the open arms and the reduction in the number of entries in the closed arms, indicate the increase in exploration and aversion of the animal drops for the open arms of the labyrinth. These results suggest the presence of anxiolytic properties (Pitchaiah *and al*., 2008).

The hole-board test is a simple method of measurement of the response of the animals ina new environment and, is generally used to understand the emotional anxiety and/or reactions to the stress of the animals (Nolan *and a*l, 1973; Takeda *and al*, 1998). The phaseollidin decreases the latency time of appearance of the 1st head-dippingand increases the number of crossing. This reduction in the latency time of appearance of the 1st head-dipping and the increase of crossings, shows an increase in the locomotory and exploratory activities in the animal. These results suggest that the phaseollidin would act on the exiting neuronal systems such as the glutaminergic system, by inhibiting them or would act by exciting the inhibiting neuronal ways via the γ-aminobutyric acid (GABA) (Moses *and al*., 2011). Knowing that the drugs which help to control the disorders of the anxiety modify the concentration of the principal chemical messengers such as gamma-aminobutyric acid or GABA which play a part in anxiety (Boulenger, 2007), the phaseollidin would thus have anxiolytic properties. The open-field test is today one of the most popular methods used to study the emotional and psychological variation in animals. Indeed, the anxious behavior in the open-field is reflected by two factors: the separation of the animal from its social group and agoraphobia

(Prut *and al*, 2003). The number of crossing carried out in the open one, would make it possible to measure the exploratory behavior and the level of anxiety in the animal (Brown *and al.,* 1999). The phaseollidin increases the number of crossing and the time spent in the center of the open-field. This increase of crossing and the time spent in the center of the open-field indicates the increase in exploration and the locomotion in animal. As the tranquillizers reduce the aversion of the rodents for enlightened spaces and increase the number of crossing and /or the time spent in the center of the open-field (Oliver *and al*., 2000), this result suggests the presence of the anti-anxiety properties. The hyperthermia induced stress test offers a better parametric analysis of the physiological reaction of the anxiety (Cryan *and al*., 2003). The rectal temperature of the rodents tends to increase in situation of stress (Cryan *and al*., 2003). The phaseollidin involves a fall of hyperthermia in the mice. This result suggests that phaseollidin would have antipyretic properties.

STATISTICAL ANALYSIS

For the statistical analysis of the effects of the phaseollidin, an analysis of variance (ANOVA) followed by the multiple comparison test of Dunnett (bilateral) was carried out to compare the values of the various parameters obtained in the mice of the various test batches and those of the negative control batch. From $p \leq 0.05$, the values were regarded as significant.

CONCLUSION

The study conducted on the phaseollidin, secondary metabolite isolated from *Erythrina droogmansiana*, made it possible to highlight its effects on the central nervous system of the white mice. This secondary metabolite have an effect in the anxious state on elevated plus maze test, increased locomotory and exploratory activities of the mice. These results revealed the anti-anxiety properties of phaseollidin.

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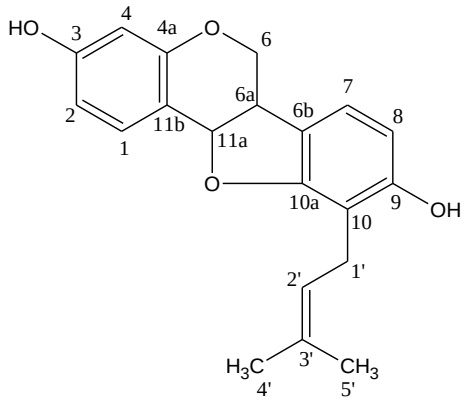
Table 1: ^{1}H, ^{13}C NMR and HMBC data of compound 1 in $CDCl_3$-MeOD

Carbon N°	^{13}C (δ in ppm)	^{1}H (δ in ppm)
1	131,3 (d)	7,30 (1H, d, J=8,3 Hz)
2	110,1 (d)	6,49 (1H, dd, J=8,3 ; 2,3)
3	159,5 (s)	
4	103,6 (d)	6,31 (1H, d, J=2,3Hz)
4a	156,7 (s)	
6	67,2 (t)	
6ax		3,45 (1H, dd, J=9,6 ; 10,7 Hz)
6eq		4,17 (1H, dd, J=4,5 ; 9,6 Hz)
6a	40,9 (d)	3,43 (1H, ddd, J=4,5 ; 6,5 ; 10,7 Hz)
6b	118,7 (d)	
7	122,2 (d)	6,86 (1H, d, J=8,4 Hz)
8	107,9 (d)	6,30 (1H, d ; J=8,4)
9	159,4 (s)	
10	112,3 (s)	
10a	157,5 (s)	
11a	78,9 (d)	5,38 (1H, d ; J=6,5)
11b	112,9 (s)	
1’	23,2 (t)	3,23 (2H, d ; J=7,1 Hz)
2’	123,4 (d)	5,33 (1H, t ; J=7,1 Hz)
3’	132,8 (s)	
4’	25,5 (q)	1,72 (3H, s)
5’	17,4 (q)	1,63 (3H, s)



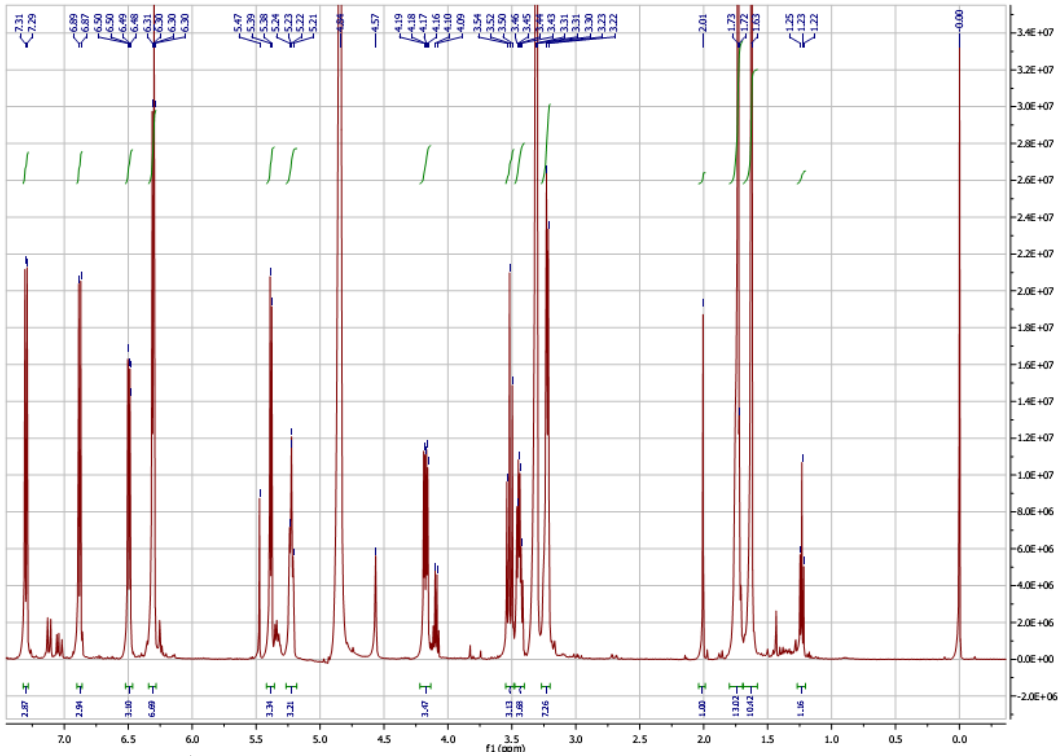


Figure 1 : RMN[1]H Spectrum (500 MHz, MeOD) de YG_7

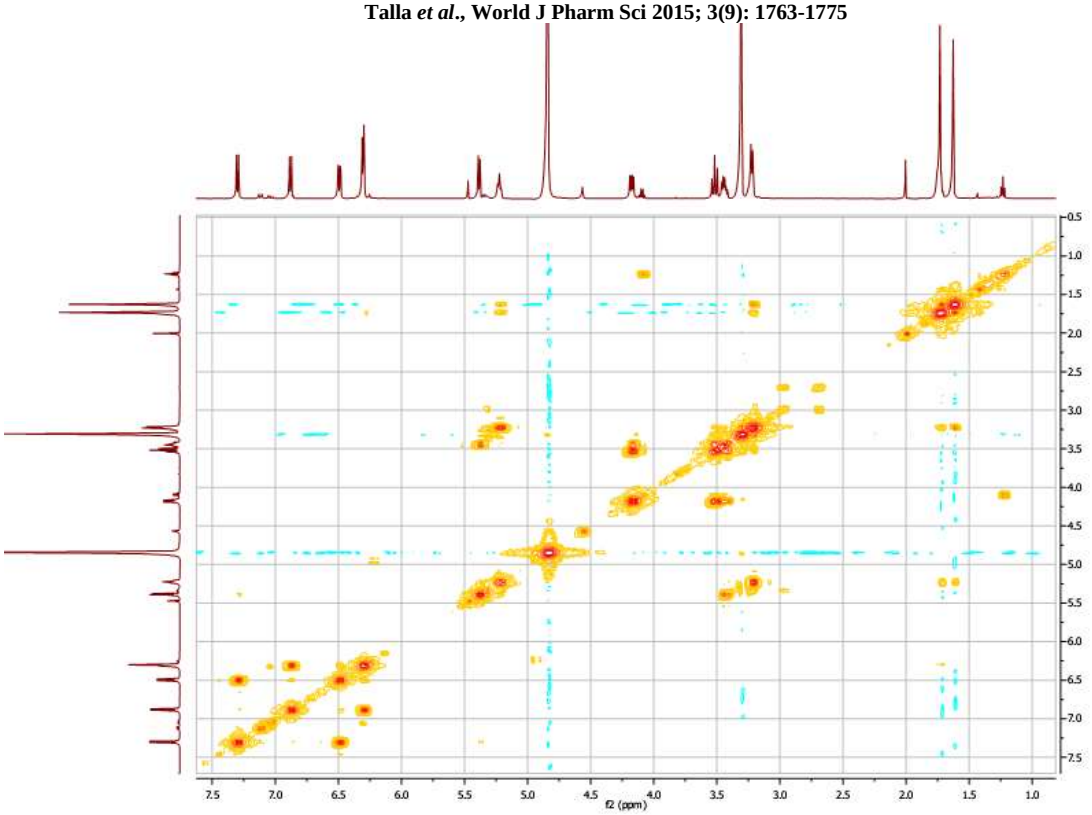


Figure 2 : COSY Spectrum (500 MHz, MeOD) de YG_7

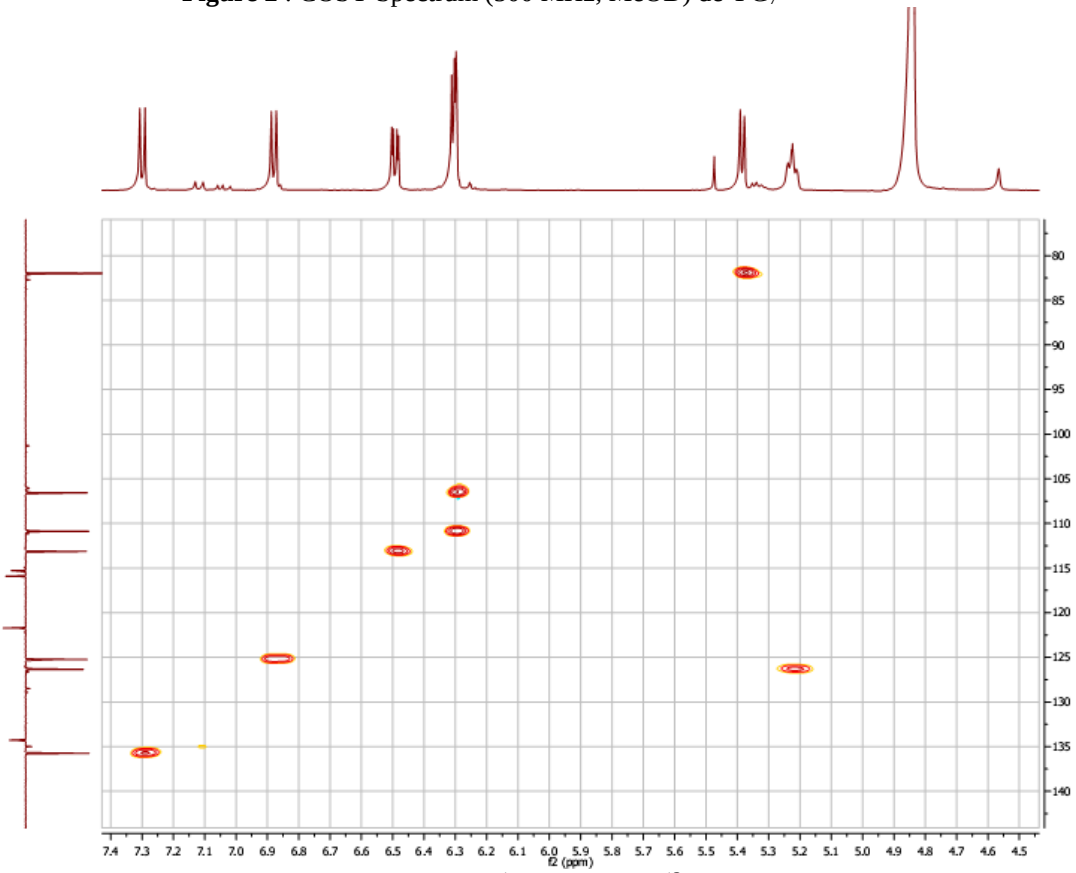


Figure 3 : HSQC Spectrum (^{1}H : 500 MHz, ^{13}C : 125 MHz, MeOD) de YG_7

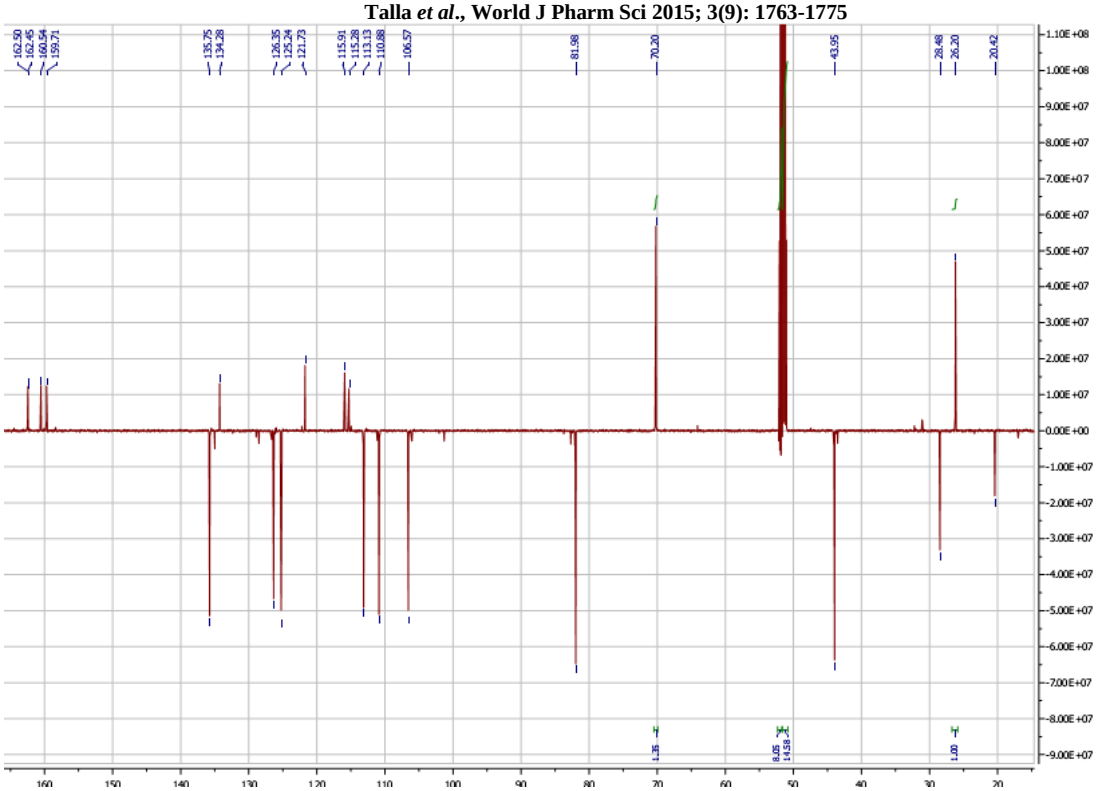


Figure 4 : RMN^{13}C Spectrum type DEPT (125 MHz, MeOD)

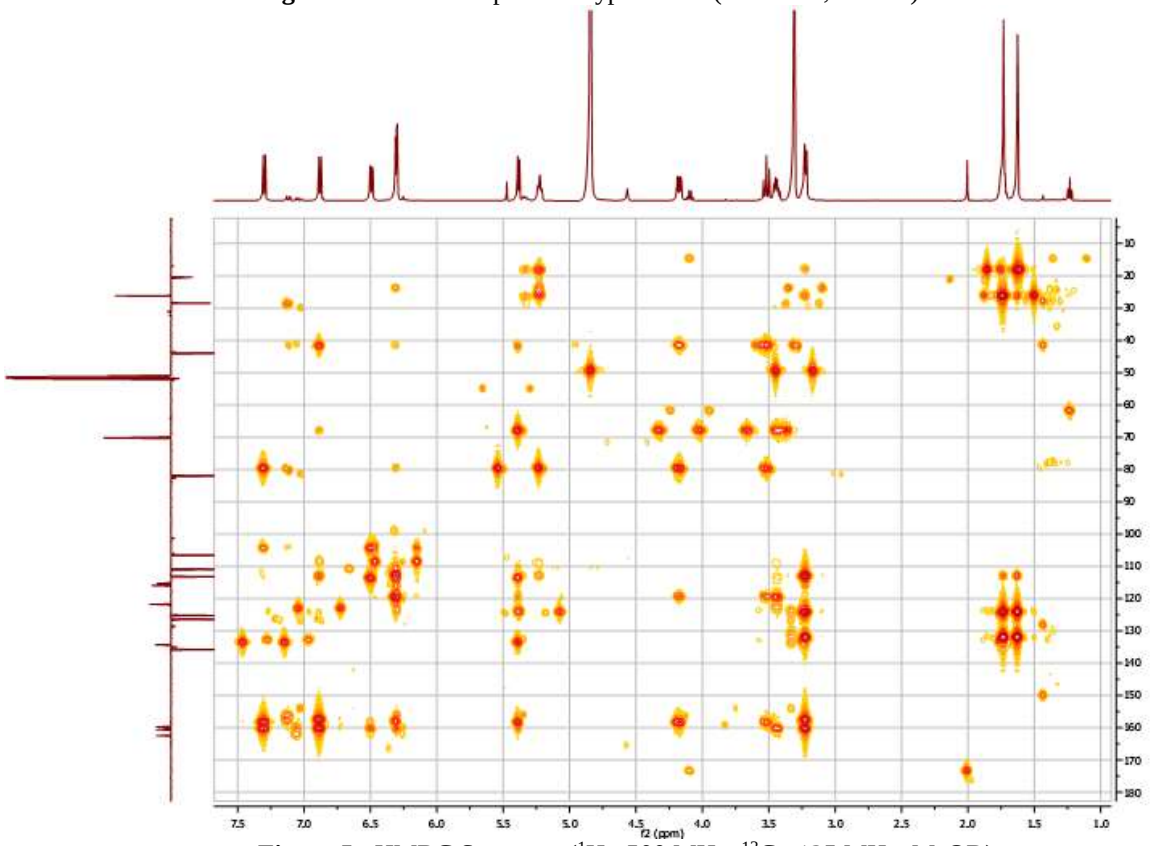


Figure 5 : HMBC Spectrum (^{1}H : 500 MHz, ^{13}C : 125 MHz, MeOD)

Table 2: The number of open arms entries, closes arms entries, rearing, head- dipping and grooming and SAP on EPM

(mg/kg)	Distilled water	Diazepam	25	50	100
Open arms entries	2.25± 2.39	5.20 ± 3.11	9.20 ±2.77**	5.20 ± 3.56	4.60± 1.97
Closes arms entries	8.20 ± 1.30	5.6 ± 1.52*	8.83 ± 1.3	5.00 ± 1.52**	6.40±1.41
Rearing	12.2 ± 2.59	2.0 ± 2.12***	12.8 ± 1.79	8.00 ± 1.41*	11.2 ± 2.28
Head- dipping	15.8 ± 5.45	12.8 ± 2.77	21 ± 2.4	17.4 ± 3.85	10.0 ±1.41*
Grooming	0.6 ± 0.49	2.00 ± 2.12***	1.6 ± 0.8*	1.6 ± 0.49*	1.6 ± 0.49*
SAP	20.6 ± 6.66	7.80 ± 1.92***	10.2 ± 2.28***	9.80 ± 2.28***	9.8 ± 1.58***

Data are mean, S.E.M., n= 5, *< 0.05, ** < 0.01, ***< 0.001, ANOVA following by Dunnett

Table 3: The number of crossing, rearing, grooming and center time on OF

	Crossing	Rearing	grooming	Time in center (sec)
Distilled water (10 mg/kg)	38.80 ±2.24	23.2±5.67	1.8±0.88	2.8 ± 0.85
Diazepam	52.80 ±10.04	11.2 ±2.77*	0.80±0.80***	1.8 ± 0.837
25 (mg/kg)	71.2 ± 11.8***	24.2± 6.76	1.40 ± 0.55	6.40 ± 1.67***
50 (mg/kg)	62.2± 18.8***	18.6 ± 8.66	2.60 ±1.14	2.80 ± 0.447
100 (mg/kg)	49.4 ± 5.73	15.8 ±16***	3.20 ± 1.30	1.66 ± 0.548

Data are mean, S.E.M., n= 6, *< 0.05, ** < 0.01, ***< 0.001, ANOVA following by Dunnett

Table 4: The number of crossing, rearing, head-dipping and first head-dippings(sec) on HB

	Crossing	Head-dipping	First head-dipping (sec)	rearing
Distilled water (10 mg/kg)	12.80 ±2.77	17.0±4.30	20.6 ± 5.13	5.4 ± 3.35
Diazepam	30.80 ±8.44	19.6 ± 2.70*	7.8 ± 1.64***	14.4 ± 2.42*
25 (mg/kg)	42.2 ± 10.3***	17.6± 2.70	10.2 ± 2.28***	14.2 ± 4.02***
50 (mg/kg)	18.2± 5.54	14.4 ± 3.91	9.8 ± 2.28***	6.0 ± 1.33
100 (mg/kg)	60 ± 9.06***	18.6 ± 2.97	8.0 ± 1.58***	14.6 ± 2.37***

Data are mean, S.E.M., n= 5, *< 0.05, ** < 0.01, ***< 0.001, ANOVA following by Dunnett

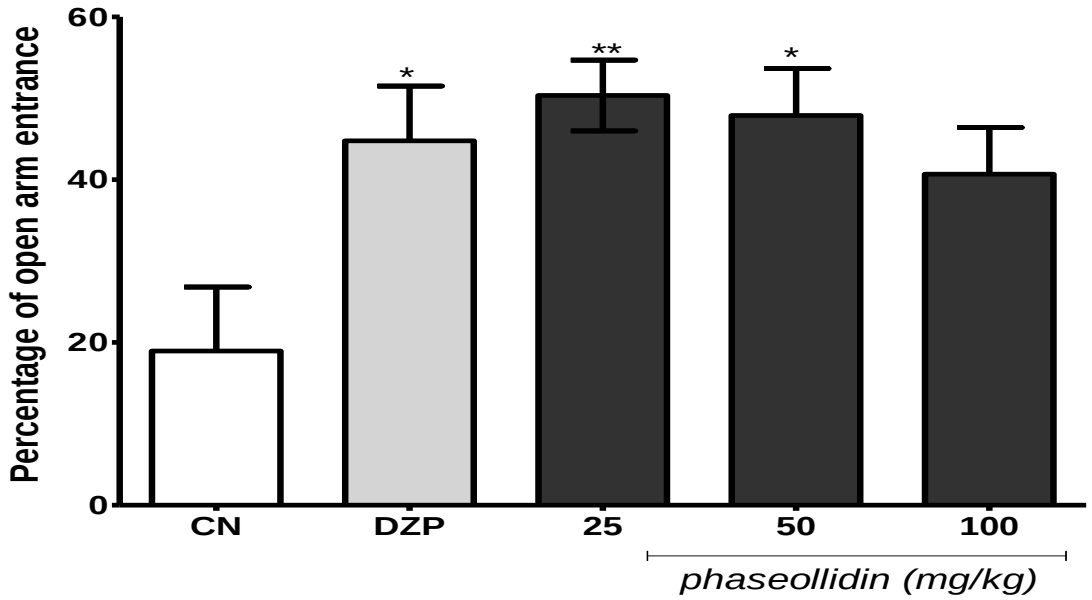


Figure 6: Effect of phaseollidin (mg/kg) on percentage of open arm entrance. The bar represents the percentage of open arms entries / total arms entries of session time. n= 5 per dose. *< 0.05, **< 0.01 and *** < 0.001, ANOVA followed by Dunnet. CON = distilled water. DZP = diazepam 3

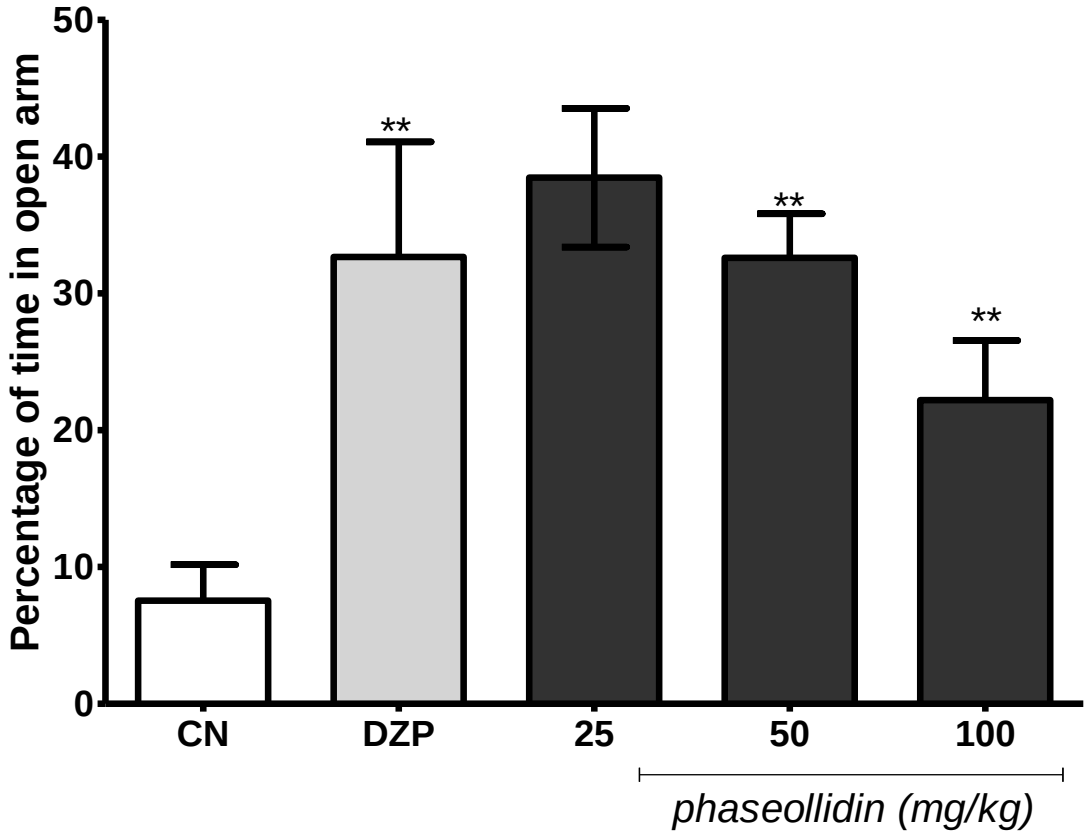


Figure 7: Effect of phaseollidin (mg/kg) on percentage of time in open arms.
The bars represent the percentage of time in open arms. n= 5 per dose. *< 0.05, **< 0.01 and *** < 0.001, ANOVA followed by Dunnet. CON = distilled water. DZP = diazepam 3

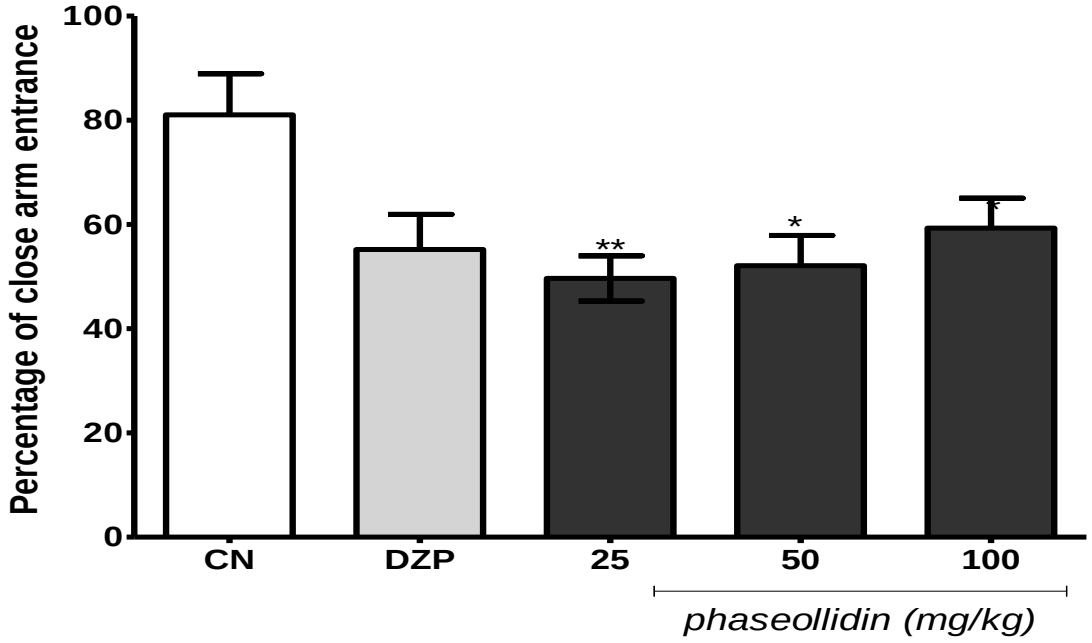


Figure 8: Effect of phaseollidin (mg/kg) on percentage of close arms entrance
The bars represent the percentage of close arms entries / total arms entries of session time. n= 5 per dose. *< 0.05, **< 0.01 and *** < 0.001, ANOVA followed by Dunnet. CON = distilled water. DZP = diazepam 3

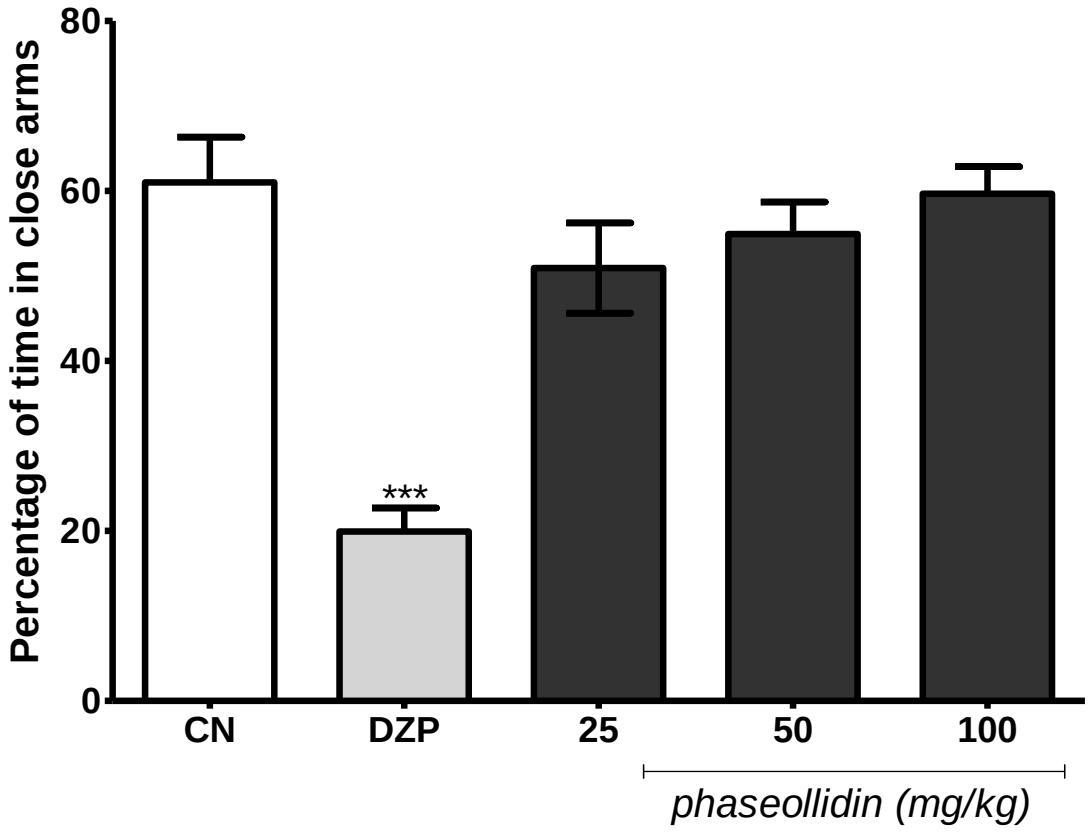


Figure 9: Effect of phaseollidin (mg/kg) on percentage of time in close arm
The bars represent the percentage of time in closes arms. n= 5 per dose. *< 0.05, **< 0.01 and *** < 0.001, ANOVA followed by Dunnet. CON = distilled water. DZP = diazepam 3

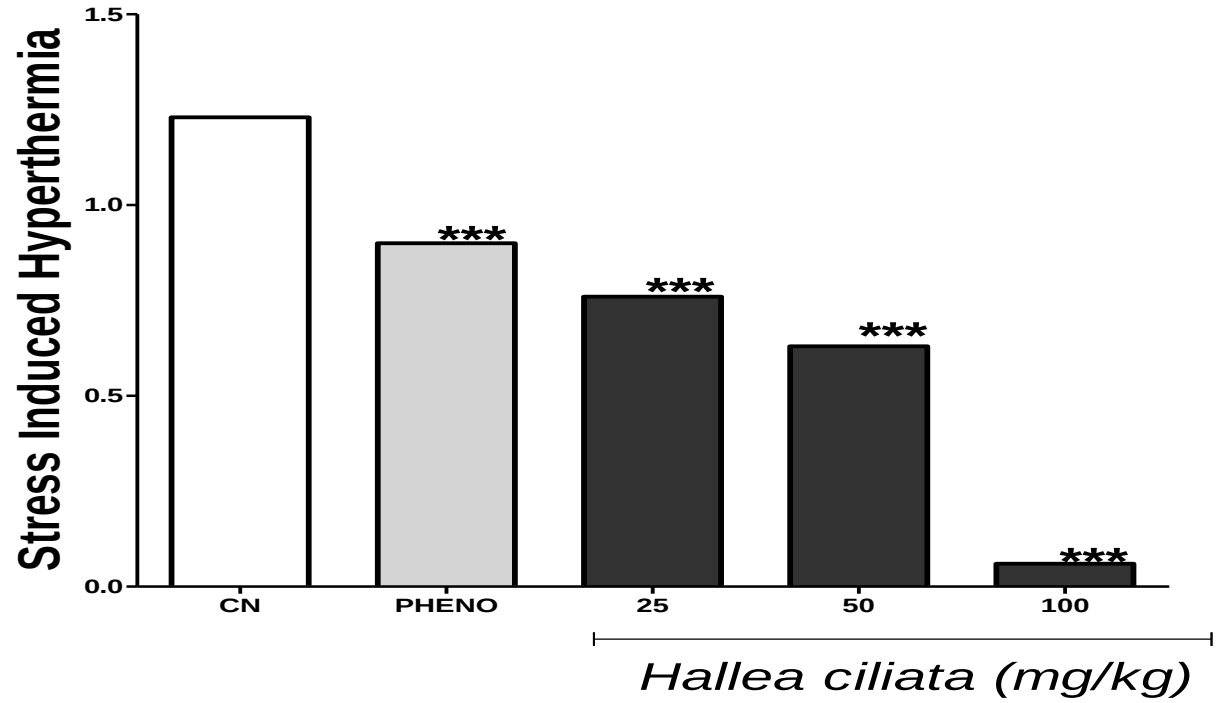


Figure10: Effects of phaseollidin on the stress induced hyperthermia. Each bar represents hyperthermia. n=6. *** p≤ 0,001 significant difference compared to PHENO group. CN: negative control made up by the mice having received distilled water, PHENO: positive control made up by the mice having received Phenobarbital (20 mg/kg).

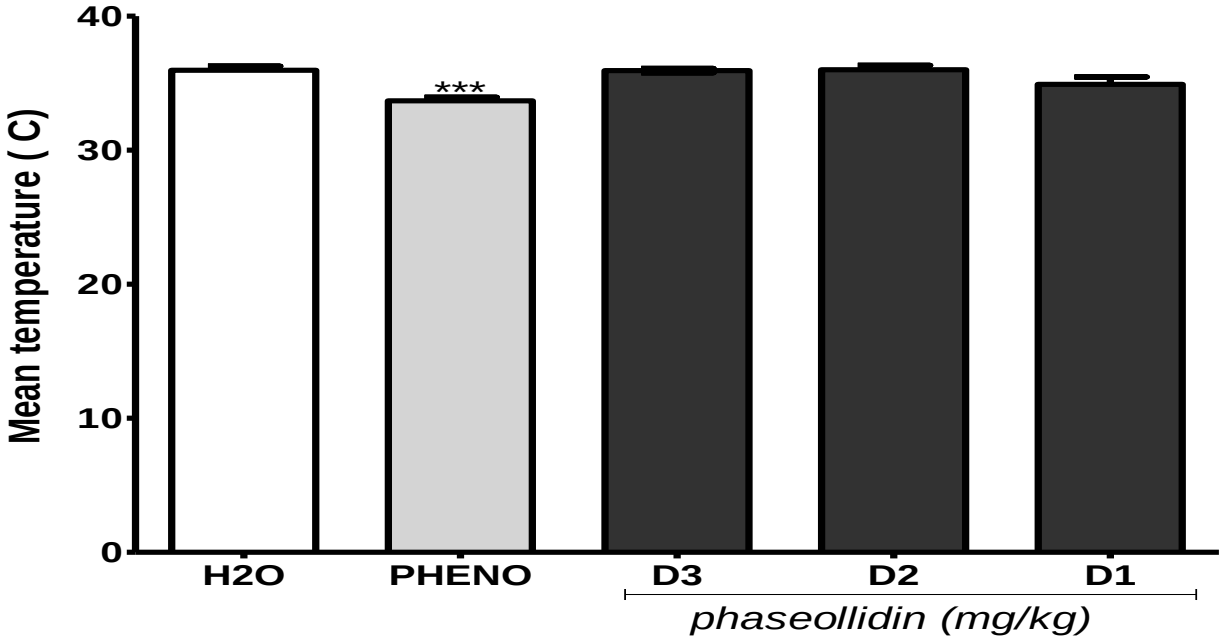


Figure 11: Effects of phaseollidin on mean temperature in HIS test. Each bar represents mean temperature ± ESM. n=10. *** p≤ 0,001 significant difference compare to CN group. CN: negative control made up by the mice having received distilled water, PHENO: positive control made up by the mice having received Phenobarbital (20 mg/kg).

REFERENCES

1. Belzung C. Measuring rodent exploratory behavior. In Crusio W. E., and Gerlai (eds), Handbook of Molecular-Genetic techniques. Brain and Behavioural Research 1999; 11: 738-49.
2. Borsini F and al. A model to measure anticipatory anxiety in mice. Psychopharmacology 1989*; 98:207*-11.
3. Boulenger J., De L'anxiété Normale Aux Troubles Anxieux, Programme 2e Cycle Psychiatrie; Faculté De Médecine Montpellier-Nîmes 2007 ; pp.15.
4. Brown R. and al. Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behavior Genetics 1999; 26:263-71.
5. Cryan F. and al. Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. European Journal of Neuroscience 2003; 17: 2409-17.
6. File E.and al. Raised corticostérone in the rat afterexposure to the elevated plus-maze. Psychopharmacology (Berl) 1994; 113(3-4):543-6.
7. Handley S.and Mithani S., Effects of alpha-adrenoceptor agonists and antagonists in a maze -exploration model of 'fear'-motivated behaviour. Naunyn Schmiedebergs Arch. Pharmacol 1984; 327(1):1-5.
8. Moses and al. Neuropharmacological Effects of Nigerian Honey in Mice. Afr J Tradit Complement Altern Med. 2011; 8(3): 230–49.
9. Ngo Bum and al. Anticonvulsivant, anxiolytic, and sedative properties of the roots of *Nauclea latifolia* Smith in mice. Epilepsy Behav 2009b; 15:434-40.
10. Nolan N. and Parks W., The effects of benzodiazepines on the behavior of mice on hole-board. Psychopharmacologia 1973 ; 29:277-86.
11. Oliver B.and al. 5-HT3 receptor antagonists and anxiety; a preclinical and clinical review. Eur Neuropsychopharmacol 2000; 10: 77-95.
12. World organization of Health., 2008. Strategy of the WHO on the traditional medecine; Geneva ,65p.
13. Pellow S. and al. Validation of open: closed arm entries in an elevate plus-maze as a mesure of anxiety in the rat. J Neurosci Methods 1985; 14(3):149-67.
14. Pitchaiah G. and al. Pharmacological Evaluation Of Alcoholic Extract Of Stem Bark Of *Erythrina Variegata* For Anxiolytic And Anticonvulsant Activity In Mice. Pharmacology Online 2008; 3: 934-47.
15. Prut L. and Belzung C., The open field as a paradigm to measure the effects of drugs on anxiety-like behaviours: a review. Euro J Pharmacol, 2003; 463:3-33.
16. Rodgers R. J. and Dalvi A., Anxiety Defence and the Elevated Plus Maze. Neuroscience and Biobehavioural Reviews 1997: 21 (6): 801-10.
17. Royce R., On the construct validity of open-field measures. Psychological Bulletin 1977; 84, 1098–106.
18. Sokeng D. and al. Anti-inflammatory effect of abyssinone V-4'-methyl ether on acute and chronic inflammation models. *Hygeia.J.D.Med*. 2013; 5 (1): 121-8.
19. Takeda M. and Matsumiya T., Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol. 1998; 350:21-29.
20. Tronche C., Effets d'un stress aigu sur le rappel mnésique : approches comportementale et endocrinienne chez la souris jeune et âgée. Thèse de doctorat de l'Université Bordeaux I; Ecole doctorale : Sciences de la vie et de la santé; Spécialité Neurosciences, 2009 ; pp.205.
21. Wandji J., 1987. Thèse de Doctorat de 3ème cycle, (1987), Université de Yaoundé, Cameroun.
22. Watjen, W. and al. Pterocarpans phaseollin and neorautenol isolated from *Erythrina addisoniae* induce apotopic cell death accompanied by inhibition of ERK phosphorylation. Toxicology 2007; 242: 71-9.
23. Woode E. and al. Anxiolytic and antidepressant effects of a leaf extract of *Palisota hirsute* K. schum. (Commelinaceae) in mice. Int. J. Pharmacol. 2010; 6: 1-17.
24. Yaya A. and al. Antioxidant activity of compounds isolated from the root woods of *Erythrina droogmansiana*. International Journal of Pharmaceutical Sciences and Drug Research 2014; 6 (2): 160-3.