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Effects of decoction products of *Lavandula angustifolia*, *Laurus nobilis,* and *Artemisia herba-alba* on depression and anxiety behaviors in Wistar rats

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Abstract Medicinal and aromatic plants have very substantial emotional effects on rats, which is part of the current study. Decoction products of three Moroccan plants (*Lavandula angustifolia L., Laurus nobilis L., and Artemisia herba-alba*) were used to be tested on Wistar rats in the laboratory. The goal was to check if they had an anti-depressant and/or anti-anxiety action on the animals' tests. Wistar Rats were born and bred in the pet store of the Faculty of Science, Kenitra. The anti-depressant and anti-anxiety effects were assessed according to three animal models: Open Field, Elevated Plus-maze for anxiety, and the Forced Swimming animal model for depression. The results showed that drinking water containing plant extracts has anti-depressant and anti-anxiety effects. Rats have overcome depression by reducing downtime during forced swimming. Concerning anti-anxiety, the open-field test showed an increase in the rat's locomotor activity, which indicates unhesitating exploratory behavior in the form of confident movement through the number of visits to the central part and the total distance crossed. The anxiety reduction was also corroborated by the elevated Plus-maze test, where the rats displayed no aversion to open spaces on account of the prolonged time they spent in the open arms and platform of the raised cross maze. In light of the findings from the experiments conducted in the study, the decoction products of the three plants appear to be viable candidates for the treatment of depression and anxiety.

Keywords: anxiety, depression, forced swimming, Moroccan plant extracts, open field, raised cross maze

1. Introduction

Anxiety is an adaptive response or sensation to imminent dangers of undetermined origin, which can normally induce stress; indeed, it can negatively affect daily activity. Therefore, anxiety disorders are linked to high emotional distress and are characterized by an intense, irrational fear strongly associated with adult panic disorder (Alkozei et al 2014). Recently, the use of natural herbal "green substances" as a remedy in the form of dietary supplements has increased to treat mild to moderate anxiety disorders (Saeed et al 2007). Motor tension, sweating, intense heart, dizziness, fear, and concentration difficulty could be common symptoms (Brown et al 2001; Fricchione et al 2004). In Morocco, especially in rural areas, plants like *Laurus nobilis, Lavandula angustifolia,* and *Artemisia herba-alba* are very popular for various problems, including their use as a tonic nerve (Hmamouchi 1997). So, it seems relevant to study the effects of these plants on anxiety. The Open field and elevated plus-maze tests - to assess locomotor activity and anxious behavior -are the best experimental methods to measure anxiety (Herskin et al 2002; Mansouri et al 2014; Caravan et al 2016).

Depression could be characterized by psychosomatic, neuroendocrine, or somatic symptoms. A few models are generally used to assess the antidepressant effects of different agents. Among others, the Forced Swimming test is a technique commonly used to suggest a depressive state in animals, as some aspects of human depression correspond to the behavioral immobility of rats during swimming (Porsolt et al 1977; Porsolt et al 1987; Wilner 1984; Petit-Demouliere et al 2005; Carbajal et al 2009; Bourin et al 2007). First, *Laurus nobilis L*. spontaneously grows in the North of Morocco. It is appreciated for its capacity to preserve olives and flavor meat or fish meals. Moroccan tagine is an example (Bellakhdar 1997). Second, *Lavandula angustifolia* L. is used by rural people to fight against nuisances caused by insects in hot weather, flavor drinking water, and produce detergents. (Bellakhdar 1997; Jan volák and jiří soldata 1997). Third, *Artemisia herba-alba* is used against dehydration and increases digestion. It is tonic, depurative, and antidiabetic. It acts against all diseases of the cold season, vertigo, and leishmaniasis (Bellakhdar 1997; Mohamed et al 2002).

Besides the properties above, we will, through the present study, assess the emotional and behavioral effects of the three plants described above on wistar rats.

2. Materials and Methods

2.1. Plant material

In July, leaves of individual herbs of *L. nobilis* were collected from a forest site known as MoulayABdessalam (Tetouan, the North of Morocco). At the same time, the flowers of *L. angustifolia* were collected from a forest site near a lagoon commonly known in Morocco as Dayat Awa. In August (towards the period of grain maturation), the flowers of *A. herba-alba* were collected in Gulmima, Errachidia (North-East Morocco). The parts of the plants were carefully separated from the rest of the plants after having collected them. They were cleaned and left to dry for 7 to12 days.

After drying, 10g and 20g of specimens were put successively in 1000ml of distilled water in a flask to heat up to a boil (100 °C) for 40 to 45minutes. After adapting the water refrigerant on the balloon, we allowed the Decoctant. The solution obtained after filtration is stored in a refrigerator at 5 °C.

2.2. Breeding

Wistar rats were born and bred at the pet store of the Department of biology, Faculty of Sciences-Kenitra, IbnTofail University – Morocco. The rats, aged between 12 and 18 months, were divided into seven homogeneous groups and placed in a collective standard cage (5rats/cage) at 23 °Cand a 12/12 hour-light-dark cycle. Six groups are given food and a bottle containing mixed water of the decoctant to be tested according to the concentration (i.e., 10g/l or 20g/l for each plant); in addition, the seventh group, the control group, which drinks tap water with the same food as the other groups. All groups were administered orally daily for 45 days; during this period, the rats were weighed every five days, and the bottles were checked daily.

During the breeding period, the rats did not undergo any physiological anomalies. The body weight monitoring of different groups was expressed in grams.

2.3. Behavior tests

2.3.1. Open field test (OF)

The OF test targets emotional reactions that exploit locomotor activity as an index of emotional responsiveness (Hall 1934; Hall 1938). The arena used is an open rectangular box(1 m^2×40 cm) with white background and black lines and is subdivided into 25 equal tiles, 16 peripheral and nine central tiles. Indeed, the rat would place itself in the center of the device by holding its tail (Albrechet-Souza 2005; Karl et al 2003). After 10 min (the duration of the manipulation), the rat was returned to their home cage, and the arena was cleaned with 70 % ethanol solution. The behavior will be evaluated according to the measurement of the following parameters: the time spent in the central area (TSC), the number of returns to the center (NRC), and the number of total tiles (TT).

2.3.2. Elevated Plus-Maze (EPM) Test

The EPM is one of the rodents most commonly used behavioral tests as a device to measure anxiety in rats (Catherine and Guy 2001; Thiebot 1989). It consists of two open arms (50 cm × 10 cm) and two closed arms (50 cm × 10 cm × 40 cm), which extend from a common central platform 10cm^2 (Pellow et al 1985). It was elevated to a height of 50cm above floor level. The EPM test is carried out for 10mins (Gonzalez and File 1997) after the rat's location at the central area facing one of the open arms. We measured the behavioral parameters, reflecting the anxiety of the animals: the number of entries in the open arms (EOA), the number of entries in the closed arms (ECA), the total number of entries in all arms (EAA), the time spent in the open arms (TSOA), and the time spent on the central platform (TSCP).

2.3.3. Forced swimming test (FS)

The FS test, or Porsolt test (Porsolt 1987; David et al 2007), predicts the effectiveness of antidepressant treatment (David et al 2007). It consists of a Plexiglas cylinder 50 cm in height and 30 cm in diameter, filled with water at 40cm height to ensure that the rat will not be able to escape by clinging to the edges of the device, nor will it use its lower limbs to escape to the surface. This forces the rat back to swim, knowing that the aquarium water is at a temperature between 10 and 15 °C (Borsini 1988).

The procedure involves placing rats individually in a transparent cylindrical water-filled tank for 5 minutes (Porsolt et al 1978). The rat cannot get out. It starts swimming vigorously and tries to pass over the edge, then it gives up and stops in more or less lengthy periods during which only the movements allow it to hold its head out of the water. The time of immobility is the parameter that will enable us to measure the antidepressant effects of our extracts on rats. A camera recorded all these tests.

2.4. Data analysis

Data entry and analysis were performed using Superior Performance Statistical Software (SPSS) version 20. The taken values are collected in Table 1 and the graphic in Figure 1. Data are presented as a mean ± SEM with a 95 % confidence interval. ANOVA followed by post hoc is performed for comparisons of values with control: values of non-significant (NS; $P > 0.05$); significant difference (*$P < 0.05$); very significant difference (**$P < 0.01$); and highly significant difference (***$P < 0.001$).

3. Results

The results analysis and monitoring of the weight evolution showed - after having compared all groups of the treated rats (*L. nobilis* 10g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. herba-alba* 10g/l, and *A. heba-alba* 20g/l)to the control group according to this parameter - that the growth of all rats remains normal.

Table 1 The weight (g) evolution of the different groups of rats during the breeding period.

The rats groups	Days									
	0	5	10	15	20	25	30	35	40	45
control	145.08 ± 2.73	163.82 ± 3.62	185.74 ± 1.90	195.30 ± 206	204.68 ± 2.71	213.52 ± 2.63	222.20 ± 3.08	230.50 ± 2.66	235.78 ± 2.13	242.88 ± 1.88
L. nobilis 10g/l	97.88 ± 2.78	107.44 ± 2.53	118.82 ± 2.73	131.90 ± 2.87	144.32 ± 2.74	158.14 ± 2.34	164.88 ± 1.80	172.68 ± 1.62	182.5 ± 2.06	195.48 ± 1.46
L. nobilis 20g/l	97.52 ± 1.95	110.62 ± 3.08	128.40 ± 1.44	138.80 ± 2.75	150.42 ± 2,50	167.06 ± 3.72	179.24 ± 3.51	191.36 ± 3.20	205.18 ± 2.73	215.88 ± 2.63
L. angustifolia 10g/l	93.4 ± 3.80	105.86 ± 8.84	122.72 ± 6.31	134.38 ± 5.50	146.58 ± 6.72	153.04 ± 6.71	158.92 ± 6.96	168.72 ± 6.97	178.9 ± 7.50	191.50 ± 7.00
L. angustifolia 20g/l	98.7 ± 3.32	111.44 ± 3.80	128.42 ± 2.65	141.22 ± 2.59	154.88 ± 3.74	166.18 ± 4.86	175.32 ± 3.10	185.64 ± 4.70	191.64 ± 4.74	202.32 ± 3.42
A. herba-alba 10g/l	151.48 ± 3.67	163.14 ± 5.82	175.68 ± 6.38	183.96 ± 6.40	192.46 ± 6.88	200.22 ± 7.03	209.44 ± 7.85	217.50 ± 7.64	229.32 ± 6.81	235.68 ± 7.95
A. herba-alba 20g/l	159.36 ± 3.20	176.18 ± 4.51	191.58 ± 5.34	208.14 ± 4.35	221.28 ± 4.53	234.36 ± 4.45	248.44 ± 5.95	261.56 ± 6.32	275.24 ± 6.26	289.54 ± 4.21

Similarly, the conditions of breeding and treatment do not adversely affect the biological quality of these animals. In other words, the conditions of the breeding and the treatment technique would have more effects on the future results through the behavioral tests (Selye 1936).

Table 2 shows the effect of *L. angustifolia, L. nobilis,* and *A. heba-alba* on the behavior of Wistar rats in OF. There was a variation in the results compared to animals in the control group according to the nature of the plants as well as the concentration of the extract.

Figure 2 represents the rats' results obtained during the behavior OF test. Indeed, Figure 2a shows that none of these decoctants tested have any effect on the parameter TSC of the OF test, but Figure 2b shows that the difference is significant for decoctant *L. nobilis* 10g/l, *A. heba-alba* 10g/l, and *L. angustifolia* 10g/l; very significant for the decoctant *L. angustifolia* 20g/l and *A. heba-alba* 20g/l, and highly significant for the decoctant *L. nobilis* 20g/l. Figure 2c, which presents the parameter TT of the OF after the chronic treatments by the different substances, shows a very significant difference for the decoctant *L. angustifolia* 20g/l and a highly significant difference for the decoctant *L. nobilis* 10g/l and *L. angustifolia* 10g/l.

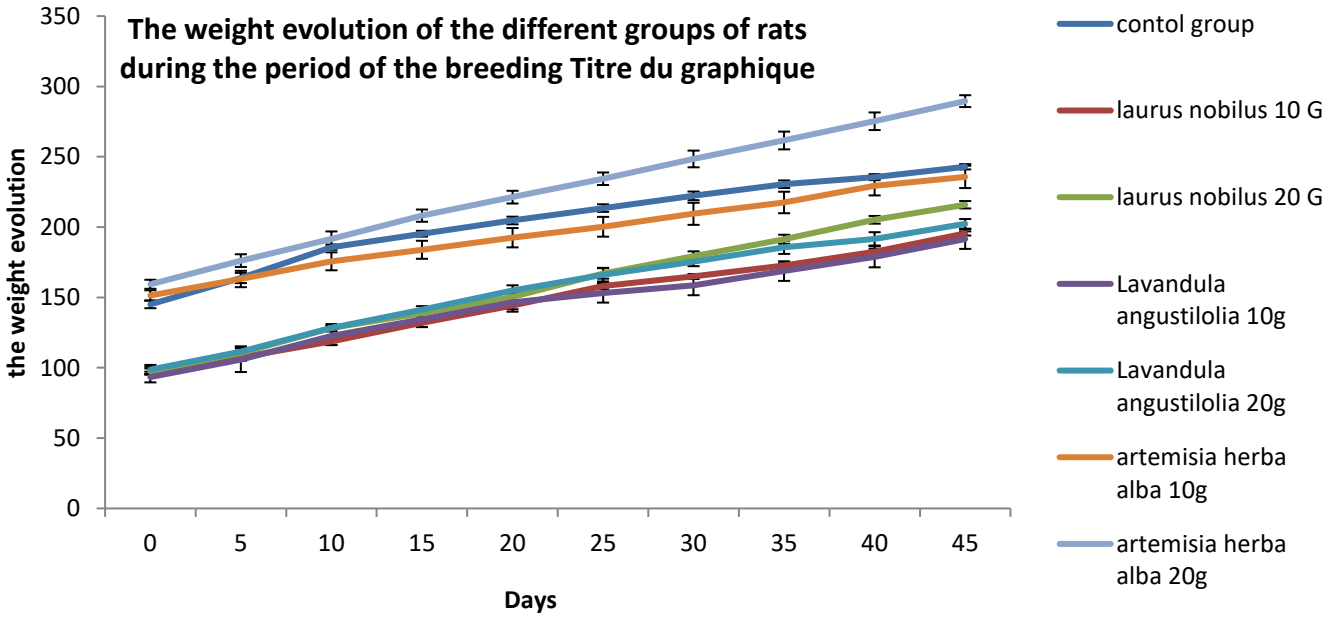

Figure 1 Rat weight evolution in control and treated rats.

Table 3 shows the effect of *L. angustifolia*, *L. nobilis,* and *A. herba-alba* on the behavior of Wistar rats in EPM. There was a variation in the results - compared to animals in the control group according to the nature of the plants - and the concentration of the extract.

Figure 3 represents the results obtained during the test of the behavior of Wistar rats in the EPM. The treatment of the results obtained by the parameter EOA of the test of the EPM (Figure 3a) shows that there are highly significant differences for the decoctant of the *A. heba-alba* 10g/L and

20g/l, as well as a very significant difference for the decoctant of *L. angustifolia* 10 g/l and 20g/l.

Table 2 Effect of chronic treatments by different substances; *L. nobilis* 10 g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. heba-alba* 10g/l, and *A.heba-alba* 20g/l, on the various open field parameters studied.

Parameters	Groups	Mean ± S.E.M
TSC	control	37.8± 14.68
	L.nobilis 10g/l	67.2± 15.76
	L.nobilis 20g/l	45.8± 16.51
	L.angustifolia 10g/l	58.6± 4.13
	L.angustifolia 20g/l	45.2± 6.66
	A.heba-alba 10g/l	69.4± 14.47
	A.heba-alba 20g/l	77.4± 16.47
NRC	control	11.2 ± 1.47
	L.nobilis 10g/l	22.2 ± 3.9
	L.nobilis 20g/l	23.6 ± 1.6
	L.angustifolia 10g/l	20 ± 1.82
	L.angustifolia 20g/l	31 ± 4.65
	A.heba-alba 10g/l	24 ± 5.10
	A.heba-alba 20g/l	31.2± 5.44
TT	control	92.8 ± 2.46
	L.nobilis 10g/l	117.8 ± 1.99
	L.nobilis 20g/l	114.4 ± 11.5
	L.angustifolia 10g/l	134.4 ± 6.7
	L.angustifolia 20g/l	143.6± 11.06
	A.heba-alba 10g/l	119.2 ± 12.18
	A.heba-alba 20g/l	106.4 ± 13.37

After that, the results of the follow-up of the parameter ECA of the EPM test (Figure 3b) of different groups and their comparison to the results obtained from the control group show a significant difference for The *L. nobilis* 10g/l decoctant, *L. nobilis* 20g/l, *L. angustifolia* 20g/l, and *A. heba-alba* 20g/l; and a very significant difference in *A. heba-alba* 10g/l.

On the other hand, the follow-up results EAA (Figure 3c) show no significant difference in the results obtained between the different groups of the decoctants tested and the control group.

Indeed, the comparison of the results obtained from the different groups with those of the control group of the parameter TSOA (Figure 3d) of the EPM test shows that there is a significant difference between the decoctant *L. nobilis* 10g/l and *A. heba-alba* 10g/l, and a very significant difference of *L. angustifolia* 10g/l decoctant and *L. angustifolia* 20g/l, and also a highly significant difference of the *A. heba-alba* 20g/L.

The variation of the parameter TSCP (Figure 3e) of the EPM test after the chronic treatments by the different substances in different groups shows a significant difference in decoctant *L. nobilis* 10g/l, *L. angustifolia* 10g/l, *A. heba-alba* 10g/l, and *A. heba-alba* 20g/l.

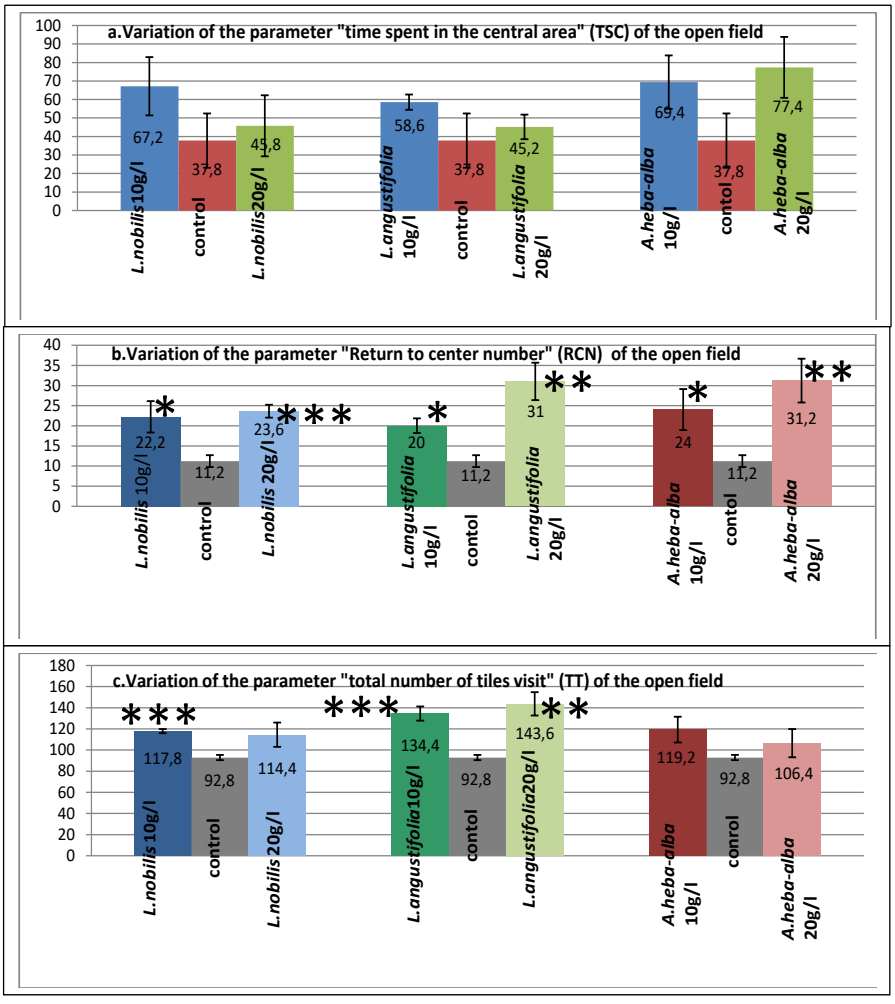

Figure 2 Variation of the parameter "time spent in the central area" (TSC), "Return to the center number" (RCN), and "total number of tiles visit" (TT) of the open field after the chronic treatments by the various substances, *L. nobilis* 10g/L, *L. nobilis* 20g/L, *L. angustifolia* 10g/l, *L. angustifolia 20g/l, A. heba-alba* 10g/l and *A. heba-alba* 20g/l in different groups. The data are represented on Mean ± SEM. Non-significant difference (NS; $P > 0.05$); Significant difference (*$P < 0.05$); Very significant difference (**$P < 0.01$); Highly significant difference (***$P < 0.001$).

Table 4 summarizes the variation in the immobility time, and Figure 4 represents the graphic of the obtained results. The comparison of the results shows a highly significant difference between decoctant *L. nobilis* 20g/l, *L. angustifolia* 10g/l, and *L. angustifolia* 20g/L.

4. Discussion

The present study provides evidence of the beneficial effects of *L. nobilis*, *L. augustifolia*, and *A. herba-alba* against anxiety and depression in rats, as they reduced symptoms associated with these disorders. These plants were tested for their effects on spontaneous motor activity, anxiety, and depression, by administering individual oral decoctant of Trojan plants at two doses, 10g/l and 20g/l, for 45 days. These plants induce neither toxic effect no significant influence on body weight evolution.

Indeed, the animals during rearing do not suffer from any factor that may trigger depression, anxiety, or other equivalent disturbance. In other words, the emotional and environmental effects during rearing are almost negligible or absent and do not negatively influence the behavior of the rats; that is, they are subjected to normal situations daily. Besides, rearing conserves conditions to ensure animal wellbeing (Hacène et al 2010; Elizalde et al 2008). This is what arouses our curiosity to study the effects of these decoctants on the behavior of rats through the OF, EPM, and FS tests.

Table 3 Effect of chronic treatments with different substances (*L. nobilis* 10 g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. heba-alba* 10g/l, and *A. heba-alba* 20g/l) on the different parameters of Elevated cross labyrinth test studied.

Parameters	*Groups*	Mean ± S.E.M
EOA	control	5.8 ± 0.86
	L. nobilis 10g/l	8.2 ± 1.47
	L. nobilis 20g/l	7 ± 1
	L. angustifolia 10g/l	9.6 ± 0.51
	L. angustifolia 20g/l	10 ± 0.84
	A. heba-alba 10g/l	8.6 ± 0.51
	A. heba-alba 20g/l	8 ± 0.32
ECA	control	10 ±1.31
	L. nobilis 10g/l	6 ±0.45
	L. nobilis 20g/l	6 ± 0.32
	L. angustifolia 10g/l	7.2 ±0.86
	L. angustifolia 20g/l	6.6 ±0.68
	A. heba-alba 10g/l	4.6 ± 0.25
	A. heba-alba 20g/l	6 ± 0.32
EAA	control	15.8 ± 1.32
	L. nobilis 10g/l	14.2 ±1.83
	L. nobilis 20g/l	13 ± 1.14
	L. angustifolia 10g/l	16.8 ±1.24
	L. angustifolia 20g/l	16.6 ±1.44
	A. heba-alba 10g/l	13.2 ±0.38
	A. heba-alba 20g/l	14 ±0.55
TSOA (seconds)	control	74.8 ± 9.55
	L. nobilis 10g/l	178.4 ±25.18
	L. nobilis 20g/l	101 ± 23.06
	L. angustifolia 10g/l	163 ± 14.35
	L. angustifolia 20g/l	141.6 ± 8.18
	A. heba-alba 10g/l	147.4 ± 16,63
	A. heba-alba 20g/l	141 ± 5.9
TSCP (seconds)	control	22.2 ± 5.53
	L. nobilis 10g/l	42 ±3.12
	L. nobilis 20g/l	30 ± 3.65
	L. angustifolia 10g/l	39.8 ± 2.34
	L. angustifolia 20g/l	27.6 ± 1.72
	A. heba-alba 10g/l	41.8.± 1.99
	A. heba-alba 20g/l	45 ± 4.56

In this work, we will evaluate the effects of these decoctant plants on the rats' anxiety through the OF test and EPM test, that is, their behavior in the face of anxiety-inducing situations. In addition, we will also evaluate the effects of the same herbs on depression, but this time through the FS test. As a reminder, the tests to be used in this work are based on the innate and spontaneous reaction of the rat when faced with an anxiety-inducing or depressant situation.

4.1. Open field test

In OF test, all rats in the substance groups to be tested and those in the control group spend almost equal time in the central area (TSC)of the OF. On the other hand, the number of returns to the center (NRC) of all the groups of rats with substances to be tested is better than the control group of rats. Then, the number of total tiles visited (TT) in the rats of the groups with substances to be tested was greater than in the control group of rats. This shows an increase in locomotor activity in these groups of rats. What matters is the crossed distance.

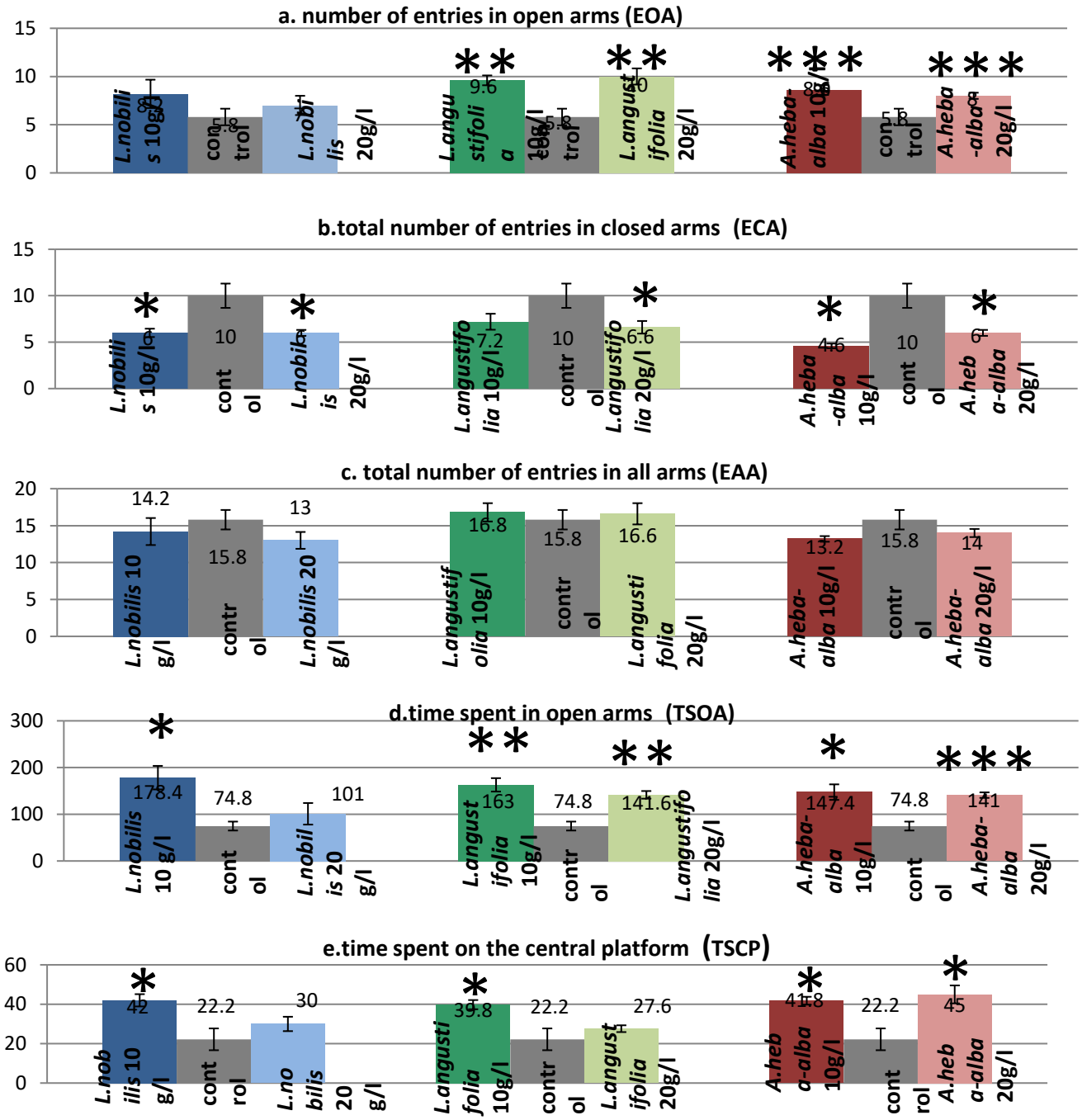

Figure 3 Variation of the parameter "number of entries in the open arms (EOA)", "number of entries in the closed arms (ECA)", "total number of entries in all arms (EAA)", "the time spent in the open arms (TSOA) and "and the time spent on the central platform (TSCP)" of the Elevated cross labyrinth test after the chronic treatments by the different substances, *L. nobilis* 10g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. heba-alba* 10g/l *and A. heba-alba* 20g/l in different groups. The data are represented on Mean ± SEM. Non-significant difference (NS; $P > 0.05$); Significant difference (*$P < 0.05$); Very significant difference (**$P < 0.01$); Highly significant difference (***$P < 0.001$).

Indeed, the statistical analysis shows no significant difference in the "TSC" parameter of the device Figure 2a. ($P > 0.05$). After that, these results indicate a decrease in the motivation to explore a new environment due to an increase in the anxiety level of the rats (Kevin et al 2004).

However, statistical analysis showed that the parameter "(NRC)" of the substances groups of rats tested with *L. nobilis* 10g/l, *L. angustifolia* 10g/l, and *A. herba-alba* 10g/l have a significant difference (*$P < 0.05$) compared to that obtained in the group of control rats as shown in the figure 2b. We observe in the same figure that the groups of rats with substances tested, namely *L. angustifolia* 20g/l and *A. herba-alba* 20g/l, have a very significant difference (**$P < 0.01$) compared to the group of control rats. Finally, we record a highly significant difference (***$P < 0.001$) in the rats

with *L. nobilis* 20g/l substance test compared to the control group.

After that, the statistical analysis of the parameter "TT" shows: first, a very significant difference in the group of rats with *L. angustifolia* 20g/l substance test compared to the control group of rats (**$P < 0.01$) and, second, a highly significant difference (***$P < 0.001$) in the substance test groups of rats such as *L. nobilis 10g/l* and *L. angustifolia 10g/l* compared to the control group. On the other hand, we do not note any significant difference in the rest of the groups compared to the control group. This parameter indicates that the tested substances increase the crossed distance. This suggests that our extracts have a positive effect on increasing locomotor activity in rats and decreasing anxious behavior (Czech Donald 2002; Kennett et al 1987).

Table 4 Effect of chronic treatments with different substances (*L. nobilis* 10g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. heba-alba* 10g/l, and *A. heba-alba* 20g/l) on the different parameters of forced swimming studied.

Parameter	Groups	Mean ± S.E.M
The time of immobility (seconds)	control	43.87 ±1.63
	L. nobilis 10g/l	38.89 ± 7.42
	L. nobilis 20g/l	114.6 ± 9.77
	L. angustifolia 10g/l	24.31± 2.68
	L. angustifolia 20g/l	16.49 ±0.73
	A. heba-alba 10g/l	43.99 ± 3.21
	A. heba-alba 20g/l	44.64 ± 3.81

4.2. Elevated Plus-Maze test

The EPM Rats, afraid of heights, naturally take refuge in closed arms, offering them more security due to the walls. The more anxious the rat is, it will hesitate before exploring with an open arm; the less it ventures, it will rely on an aversive exploration/avoidance conflict. (Larrère 2007; Katz et al 1987; Rodgers and Dalvi 1997; Van Gaalen and Stickler 2000).

This test is based on the rats' natural aversion to new, open, lighted, and high spaces. Generally, the rat is afraid of empty and high spaces. The rat exploring open arms will be a testament to less anxious behavior. On the other hand, the more the animal is localized or going into closed arms, its behavior is designated as anxious; which means that the avoidance of "open arms" with a clear preference for closed arms is a behavior manifested in anxious rats (Brummelte et al 2012; Dawson et al 1995). Its behavior is suppressed in the substance groups: *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. herba-alba* 10g/l, and *A. herba-alba* 20g/l. And this shows that these groups have better locomotor activity than the control group, as shown in Figure 3-document (a). The same figure shows that entry into open arms has a highly significant difference (***$P < 0.001$) in substance-tested groups: *A. herba-alba* 10g/l, *A. herba-alba* 20g/l; then, we notice a very significant difference (**$P < 0.01$) in substance tested groups: *L. angustifolia* 10g/l and *L. angustifolia* 20g/l all compared to the control group.

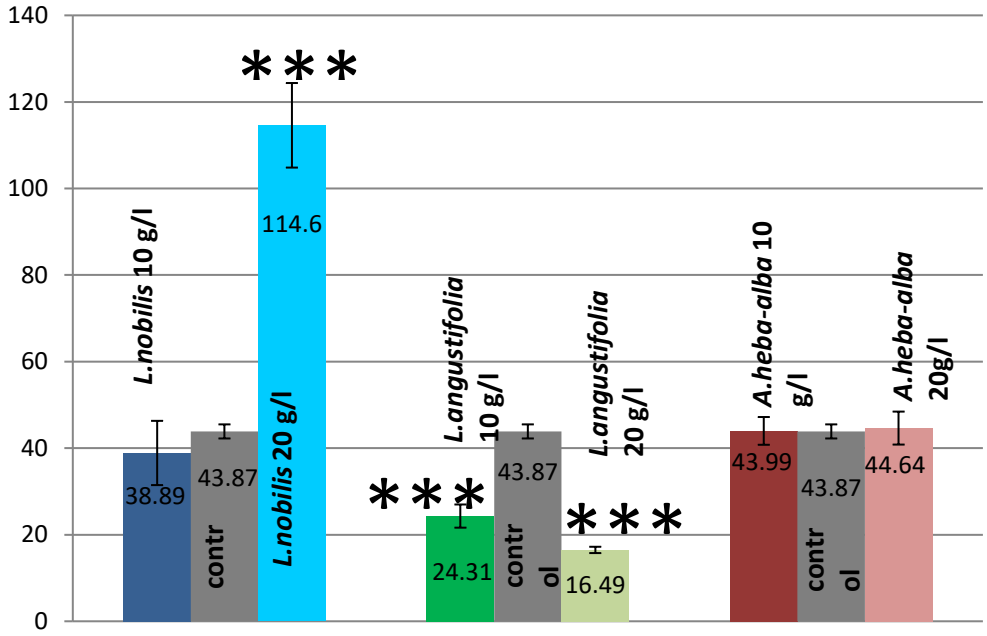

Figure 4 Variation of the parameter "The time of immobility", in the test of swimming forced after the chronic treatments by the different substances, *L. nobilis* 10g/l, *L. nobilis* 20g/l, *L. angustifolia* 10g/l, *L. angustifolia* 20g/l, *A. heba-alba* 10g/l and *A. heba-alba* 20g/l in different groups. The data are represented on Mean ± SEM. Non-significant difference (NS; $P > 0.05$); Significant difference (*$P < 0.05$); Very significant difference (**$P < 0.01$); Highly significant difference (***$P < 0.001$).

It could be suggested that the increase is likely to take place due to general stimulant activity; therefore, anti-anxiety behavior can be determined by measuring spontaneous motor activity; i.e., total entries into open arms (Mansouri et al 2014; Pellow et al 1986; Budzynska et al 2013).

The results displayed in Figure 3b show that the parameter "ECA" has a significant difference (*$P < 0.05$) in the substance tested groups: *L. nobilis* 10g/l, *L. nobilis* 20g/l, *L. angustifolia* 20g/l, and *A. herba-alba* 20g/l. Likewise, results reflect a very significant difference (**P <0.01) in substance tested groups: *A. herba-alba* 10g/l, all compared to the control group. These results confirm the obtained results at the parameter "between open arms" level.

In the same context, this test, for all the tested extracts, shows that the labyrinth parameter "EAA" of the labyrinth displays no observed difference in all groups compared to witnesses. This parameter was used as an index of locomotor activity, which suggests that it is not affected by the treatment of the different tested extracts. (Espejo et al 1997)

Rats usually spend more time in closed arms than on the central platform, while they spend little in open arms, indicating a penchant for relatively safe sections of the maze. Afterward, anxious rats avoid darkness and more easily stay in a dark compartment. They are readier to stay in protected aisles by walls than in non-protected aisles (Cruz et al 1994). This behavior is absent in the substance-tested groups*: L. nobilis* 10g/l and *A. herba-alba* 10g/l, whose difference is significant. Likewise, there is a very significant difference (**$P < 0.01$) in *L. angustifolia* 10g/l and *L. angustifolia* 20g/l groups. Then, we notice a highly significant difference (***$P < 0.001$) in the *A. herba-alba* 20g/l group, all compared to the control group, as shown in Figure 3d.

Ultimately, the statistical analysis of the results obtained in Figure 3e reveals a significant difference (*$P < 0.05$) of "TSCP" of the device in the substance tested groups, namely *L. nobilis* 10g/l, *L. angustifolia* 10g/l, *A. herba-alba* 10g/l, and *A. herba-alba* 20g/l all compared to the control group. These results confirm that our substances have an anti-anxiety effect and freedom from hesitation.

4.3. Forced swimming

Depression could be defined as a multifaceted condition of psychosomatic, neuroendocrine, and somatic symptoms that is difficult to reproduce in animals. Since some aspects of human depression correspond to the behavioral immobility of rats, we have opted for the FS test. This behavioral test was deployed for rats (rodents in general) to evaluate antidepressant drugs, the antidepressant efficacy of new compounds, and experimental manipulations aiming to make or prevent depression-like.

The index used is the rats' mobility. Generally, immobility is characterized by an almost zero speed of movement; a floating position considered in the absence of any movement except for the movement of the legs necessary to keep the head above water (Porsolt et al 1977; Porsolt et al 1987; Wilner 1984; Petit-Demouliere et al 2005; Carbajal et al 2009) without taking into account the active and voluntary behavior of wall climbing, the deflection in the caudal suspension, which gives way after a few minutes to periods of passive immobility.

The validity of this test stems from their sensitivity to antidepressants that are effective in the clinic, which significantly reduce the duration of passive behavior and promote behavior oriented towards flight (Taiwo et al 2012; Mora et al 2005).

The classical interpretation is that the animal loses all hope of escaping the situation, but other hypotheses are possible. According to one of them, immobility is neither a sign of despair nor a model of depression but a sign of motor inhibition and, therefore, a model of motor slowing down in depression conditions. On the other hand, the animal's behavior is in no way pathological; it is a passive adaptive response in a situation where active responses are ineffective.

The results in Figure 4 show an increase in the immobility of the rats treated with the decoctant of *L. nobilis* 20g/l (114.6±9.77) compared to the control group (43.87±1.63) and a reduction or decrease in the immobility time of the rats treated with the decoctants *L. angustifolia* 10g/l (24.31±2.68) and *L. angustifolia* 20g/l (16.49±0.73) compared to the control group (43.87±1.63). In contrast, we do not observe any significant difference ($P > 0.05$) in the rest of the treated groups. Statistical analysis has shown that the increase and decrease in immobility time were highly significant (***$P < 0.001$). These results indicate that *L. angustifolia* reduces the duration of immobility or has an antidepressant effect. Moreover, at the concentration level of 20g/l, *L. nobilis* increases the duration of immobility and consequently has a depressant effect.

5. Conclusions

To conclude, through a multidisciplinary approach and targeting alternatives to chemical uses for the treatment of depression and anxiety, trials on *L. nobilis*, *L. angustifolia,* and *A. herba-alba* provide converging evidence, demonstrating that all these three herbal remedies have an anxiolytic effect on Wistar rats. Subsequently, they show that *L. angustifolia* has an antidepressant effect; on the other hand, *L. nobilis,* at the concentration level of 20 g/l, has depressant effects.

This experimental design does not allow us to determine exactly the active ingredients to which pharmacological effects can be attributed. Still, it will enable us to experimentally reproduce a situation akin to including decoctants from three plants studied in the human diet, short of *L. nobilis,* which should be included in foods with a controllable dose.

Conflict of Interest

The authors declare no conflict of interest.

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References

Albrechet-Souza L, Oliveira AR, De Luca MCZ, Tomazini F, Santos NR, Brandao MLA (2005) Comparative study with two types of elevated plus-maze (transparent vs. opaque walls) on the anxiolytic effects of midazolam,

onetrial tolerance and fear-induced analgesia. Prog Neuropsychopharmacol Biol Psychiatr 29:571–9.

Alkozei A, Cooper PJ, Creswell C (2014) Emotional reasoning and anxiety sensitivity: associations with social anxiety disorder in childhood. J Affect Disord 152–154:219–228.

Bellakhdar J (1997) La pharmacopée marocaine traditionnelle, dixième édition.

Bourin M, Benoit P, Brid ND, Martine H (2007) Animal models of anxiety in mice; Fundamental & Clinical Pharmacology 21:567–574.

Borsini F, Meli A (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl.) 94:147–160.

Brown TA, O'Leary TA, Barlow DH (2001) Generalized anxiety disorder. In: Barlow DH (ed) Clinical handbook of psychological disorders: A step-by-step treatment manual. 3rd edn. Guiford, New York, pp. 154–208.

Brummelte S, Lieblich SE, Galea LAM (2012) Gestational and postpartum corticosterone exposure to the dam affects behavioral and endocrine outcome of the offspring in a sexually-dimorphic manner. Neuropharmacology 62:406-418.

Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Woziniak K, Michalak A, Musik I, Biala G (2013) Effects of imperatorin on nicotine induced anxiety and memory related responses and oxidative stress in mice Physiol Behav 122:46–55.

Caravan I, Sevastre Berghian A, Moldovan R, Decea N, Orasan R, Filip GA (2016) Modulatory effects of caffeine on oxidative stress and anxiety-like behavior in ovariectomized rats. Can. J. Physiol. Pharmacol 94:1–12.

Carbajal D, Ravelo Y,Molina V, Mas R (2009) Arruzazabala ML D-004 a lipid extract from royal palm fruit, exhibits antidepressant effects in the forced swim test and the tail suspension test in mice; Pharmacol Biochem Behav 92:465–468.

Catherine B, Guy G (2001) Measuring normal and pathological anxiety-like behaviour in mice: a review. Behavioural Brain Research 125:141–149.

Czech Donald A (2002) A simple integrated circuit device for measuring distances traveled and determining speed in open-field environments. Pharmacol Biochem Behav 72:73–75.

Cruz AP, Frei F, Graeff FG (1994) Ethopharmacological analysis of rat behavior on the elevated plusmaze. Pharmacol Biochem Behav 49:171–176.

David DJP, Klemenhagen KC, Holick KA, Saxe MD, Craig DA, Swanson C (2007) Efficacy of the MCHR1 antagonist N-[3-(1-{[4-(3,4-difluorophenoxy)phenyl]methyl}(4-piperidyl))-4-methylphenyl]-2-methylpropanamide (SNAP 94847) in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J Pharmacol Exp Ther 19:237-48.

Dawson GR, Rupniak NM, Iversen SD, Curnow R, Stanhope KJ (1995) Lack of effect of CCKB receptor antagonists in ethological and conditioned animal screens for anxiolytic drugs. Psychopharmacology (Berl) 121:109–117.

Elizalde N, Gil-Bea FJ, Raminez MJ, Aisa B, Lasheras B, Del Rio J (2008) long-lasting behavioral effect of antidepressant treatment. psychopharmacology (Berl) 199:1-14.

Espejo EF (1997) Structure of the mouse behaviour on the elevated plus-maze test of anxiety. Behavioural Brain Research 86:105-112.

Fricchione G (2004) Generalized anxiety disorder. N Engl J Med 351:675–682.

Hacène F, Réda D, Bachir A R, Nabila F, Abdelkrim T, Abdel MB (2010) Le kètoconazole antagonise les effets immuno-gonadotropes au test de la nage force´e chez le rat mâle Wistar. Revue canadienne de physiologie et pharmacologie 88:733-744.

Hall CS (1934) Emotional behaviour in the Rat. I. Defecation and urination as mesures of individual differences in emotionality. J.Comp. Psychol 385-403.

Hall CS (1938) The inheritance of emotionality. Sigma Xi Quarterly 26:17-27.

Herskin MS, Jensen KH (2002) Effects of open field testing and associated handling v. handling alone on the adrenocortical reactivity of piglets around weaning. Anim Sci 74:485–491.

Hmamouchi M (1997) Plantes alimentaires, aromatiques, condimentaires, médicinales et toxiques au Maroc. In Heywood (V.H.) Skoula (M.) (Eds.) Identification of wild food and non-food plants of the Mediterranean region. Chania: CIHEAM-IAMC 89-108.

Jan volák et jiří soldata (1997) plantes médicinales;.dixième édition 319 pp.

Karl T, Pabsat R, Stephan VHS (2003) Behavioral phenotyping of mince in pharmacological and toxicological research. EXP. ToxicolPathol 55:69-83.

Katz RJ, Roth KA, Carroll BJ (1987) Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neuroscience and bio behavioral reviews 5:247-51.

Kennett GA, Dourish CT, Curzon G (1987) Antidepressant-like action of 5-HT1A agonists and conventional antidepressants in an animal model of depression. Eur J Pharmacol 134:265–274.

Kevin SL, Craig A, Dana C, Torpey C (2004) Impact of healthy aging on awareness and fear conditioning. Behavioral Neuroscience 118:905-915.

Larrère R (2007) Justifications éthiques des préoccupations concernant le bien-être animal. INRA Prod. Anim 20:11-17.

Luis EG, Sandra EF (1997) A Five Minute Experience in the Elevated Plus-Maze Alters the State of the Benzodiazepine Receptor in the Dorsal Raphe Nucleus, The Journal of Neuroscience 15:1505–1511.

Mansouri MT, Soltani M, Naghizadeh B, Farbood Y, Mashak A, Sarkaki A (2014) A possible mechanism for the anxiolytic-like effect of gallic acid in the rat elevated plus maze. Pharmacol Biochem Behav 117:40–46.

Mohamed B, Hassane M, Abdelkhaleq L, Abderrahim Z (2002) Ethnopharmacology Forum Medicinal plants used in the treatment of diabetes in Morocco; Int J Diabetes & Metabolism 10:33-50.

Mora S, Diaz-Veliz G, Lungenstrass H, Garcia-Gonzalez M, Coto-Morales T, Poletti C (2005) Central nervous system activity of the hydroalcoholic extract of Casimiroa edulis in rats and mice. J Ethnopharmacol 97:191–197.

Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149-167.

Pellow S, Sandra FE (1986) Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: Anovel test of anxiety in the rat. Pharmacol Biochem Behav 24:525–529.

Petit-Demouliere B, Chenu F, Bourin M (2005) Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology 177:245–255.

Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266: 730-732.

Porsolt RD, Anton G, Blavet N et Jalfre M (1978) Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol 47:379–381.

Porsolt RD, Anton G, Blavet N, Jalfre M (1987) Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 47:379–391.

Rodgers RJ, Dalvi A (1997) anxiety, defence and the elevated plus-maze. Neuroscience Biobehav 21:801-810.

Saeed SA, Bloch RM, Antonacci DJ (2007) Herbal and Dietary Supplements for Treatment of Anxiety Disorders. Am Fam Physician 76:549–556.

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138:32.

Taiwo AE, Leite FB, Lucena GM, Barros M, Silveira D, Silva MV (2012) Anxiolytic and antidepressant-like effects of Melissa officinalis (lemon balm) extract in rats: Influence of administration and gender. Indian J Pharmacol 44:189–192.

Thiebot MH (1989) Les modèles animaux de l'anxiété. Psychologie Médicale 21:97-108.

Van Gaalen MM, Stickler T (2000) Behavioural analysis of four mouse strains in an anxiety test battery. Bahav Brain Res 115:95-109.

Wilner P (1984) The validity of animal models of depression; Psychopharmacology 83:1–16.