Original Research Article

Effects of hydroethanolic extract of *Adenia lobata* (Jacq.) Engl. *(*Passifloraceae) on nociception and subsequent anxiety-like behavior: both anti-inflammatory and antioxidant approaches

ABSTRACT

Aims: *Adenia lobata* (Jacq.) Engl. (Passifloraceae) is widely used in Ivorian traditional pharmacopeia to heal various chronic diseases, relieve headache and pain of gingiva inflammation, and facilitate labor. Here, we investigated the effects of hydroethanolic extract *Adenia lobata* (HEAL) on nociceptive pain and subsequent anxiety-like behavior.

Materials and Methods: We used several experimental pain tests as the writhing, formalin and hot plate to evaluate both antinociceptive and anti-inflammatory actions of the extract. Anxiety related to nociception was tested with open field and elevated plus maze tests. Then, mice were sacrificed for assessing some oxidative stress markers.

Results: The extract of 30 mg/kg, p.o reduced in the similar manner as reference peripheral drug salcylicacetic acid (ASA, 200 mg/kg, i.p) the number of writhings induced by acid acetic. In both neurogenic and inflammatory phases of formalin test, the extract demonstrated an effective antinociceptive activity than ASA, but comparable to central analgesic tramadol (50 mg/kg, i.p). However, *Adenia lobata* reduced lesser thermal-induced pain than tramadol in hot plate test, but significantly compared to ASA. Furthermore, HEAL altered anxiety-like behavior in each case of the pain condition studied. Also, the extract showed the highest antioxidant activity by reduction oxide nitric (NO) and malondialdehyde (MDA), and increase non protein thiol (NP-SH) levels.

Conclusion: In conclusion, HEAL possesses antinociceptive and anti-inflammatory actions on peripheral and central mechanisms of pain. The phytochemicals components of the extract as Alkaloids and flavonoids suggest to interact with the opioid system and combat the oxidative stress, respectively. Our findings provide scientific basis for the use of *Adenia lobata* in traditional medicine against pain and related diseases.

Keywords: Adenia lobata, antinociceptive activity, anti-inflammation, antioxidant, anxiety-like behavior, Mice

1. INTRODUCTION

Pain acts as an alarm system allowing the organism to face to external or internal aggressive agents. While the nociceptive pain results from activation of nociceptive fiber nerves by chemical or thermal stimuli, the neuropathic pain is originating from lesions of either peripheral or central nervous system. Moreover, nociceptive pain is often considered as an acute inflammatory response leading to a protection of damaged tissues, a removal of the harmful stimuli and a

survival role for surrounding cells. By contrast, neuropathic pain is chronic with no beneficial biological effects, but described as allodynia, hyperalgesia, spontaneous mainly occurred in diabetic patients [1], and associated with anxiety [2], depression [3], or psychological and social factors [4].

It would seem that oxidative stress plays critical role in several mechanisms involved in nociception induction and central nervous system sensitization [5]. Indeed, a recent report suggested that oxidative stress contributes to the increase of expression of nociception-related inflammation mediators [6]. However, the interaction between pain and oxidative stress mechanisms remains controversial and not straightforward [7]. Otherwise, the analgesic drugs commonly found on the market present some negative actions including inefficiency and intolerable side-effects [8]. Thus, it is worthwhile to further promote alternative approaches of drugs-based on natural products with multiple pharmacological actions to relieve ailments.

Adenia lobata Jacq. (Passifloraceae) is a medicinal plant which is naturally growing in west African countries. In Ivory coast, as supported by an ethnopharmacological survey, it is used as traditional remedy against jaundice, malaria fever, infantile asthma [9]. Importantly, its extract is used to treat rheumatic pain, abdominal pain, headaches, and as an anti-cancer in some eastern communities of Nigeria, and also wellknown for antioxidant activity [10]. Those beneficial health effects of *Adenia lobata* extract suggest some analgesic properties. The present work aims to evaluate antinociceptive activity of HEAL in order to justify its ethnopharmacological use conditions. The nociceptive pain linked to inflammation process will be tested using various experimental models of pain followed by an assessment of abilities of *Adenia lobata* extract to alleviate anxiety-like behavior and oxidative stress-related to pain.

2. MATERIAL AND METHODS

2.1 Animals and acclimatation

Albinos males mice 3-month-old of age and weighing 30-32g were used in this study. They were divided into different group of 5 to 6 animals each and kept for acclimatation under normal condition of air temperature at 22 ± 2ºC, a relative good humidity at 60%, and on 12-h light /12-h dark cycle. They had access to tap water and food *ad libitum*. All experimental steps were carried out according to NIH guide for the care and use of laboratory animals, in order to minimize the number of animals used and their suffering.

2.2 Plant material and extraction method

Adenia lobata was harvested in the botanical garden of National Floristic Center (CNF). After an identification and authentication by a specialist of botany from CNF, the liana part was washed and dried at 55ºC in the stove for two weeks. The dried plant was crushed to obtain some powder (200g) which was soaked in 2 litres of 70% ethanol. The filtrate was concentrated and evaporated using a rotary evaporator at 40º C. The dry extract was stored at fridge + 4ºC.
Previous testing helped us to choose the effective dose of extract by oral route at 30 mg/kg.

2.3 Experimental design

The antinociceptive tests were used to assess the effects of plant extract in three nociception experiments.

2.3.1 Acetic aicd-induced writhing in mice as peripheral nociception

The writhing is nociceptive behavior of abdominal musculature contraction together with stretching of hindlimbs induced by intraperitoneal injection (i.p.) of acetic acid 0.6% (0.1 ml/10g, i.p.). The method used here is suited to that of Farouk et *al* [11]. Animals were grouped as the following: Group 1 as negative control mice (NaCl 0.9%, 0.1 ml/10g, i.p.), Group 2 was pre-treated with the dose of 30 mg/kg of HEAL (p.o.), and the analgesic effects was compared to the Group 3 as positive control of mice pretreated with standard drug acetylsalicylic acid (ASA, 200 mg/kg, p.o). Thirty minutes after the treatment, the nociception was induced with acid acetic 0.6% followed by 5 min of observation. The number of writhing and stretching movements was counted over a period of 5 min for 30 min. The percentage of nociception inhibition was calculated according the following: % inhibition = (1- number of writhing in treated / number of writhing in control) x 100.

2.3.2 Formalin test to assess both peripheral and central nociception

It consists to induce the pain by injecting subcutaneously 20µL of formalin 20% into the posterior right paw of the mice. The procedure of formalin test used is similar to that performed by Farouk et *al* [11]. The time spent for licking or biting the paw was considered as an indication of nociceptive behavior. We identified two responses consecutive to formalin injection. The initial response to pain was found during the first 5 min after formalin injection (neurogenic pain) and the second nociceptive behavior is peaked after 15-30 min (inflammatory pain). The mice were organized into 4 study groups: Group 1 as negative control mice (NaCl 0.9%, 0.1 ml / 10g, i.p.), Group 2 of mice were pre-treated with 30 mg/kg of HEAL, Group 3 of mice were pre-treated with ASA as positive control of peripheral antinociceptive drug (200 mg/kg, p.o), and Group 4 of mice were administrated before with the tramadol as positive control of central antinociceptive drug (50mg/kg, i.p.). All the treatments were performed 30 min prior the formalin injection. Then, after 5 min-habituation session, the nociceptive responses were recorded for 30 min.

2.3.3 Hot plate test

The effect of *Adenia lobata* extract on pain-induced by thermal factor was assessed with hot plate test. The test consists to leave the mice into a cylindric glass (20 diameters) on a hot plate maintained at 55 ± 0.2ºC [11]. Each mice was placed on heated surface and leave to observe the latency before licking (sucking) the paws or jumping on the wall of the cylindric glass, as an exhibition of thermal nociception response. To avoid any damage of the paw a cut-off time for latency response was taken as 20 s before their withdrawal. This experiment included 3 groups: Group 1 pre-treated with *Adenia lobata* crude extract (30 mg/Kg p.o.), Group 2 was pre-treated with ASA as positive control of peripheral antinociception molecule (200 mg/Kg, p.o), Group 3 was pre-treated with tramadol as positive control of central antinociception molecule (50mg/kg, i.p.). All the treatments were given 30 min before placing mice on the hot plate.

2.4 Testing of post-nociception anxiety behavior level

After antinociceptive tests, the mice were submitted to behavioral tests Open-field and Elevated Plus Maze (EPM) in order to assess the anxiety behavior-related to nociception.

2.4.1 Open-field test

The open-field test is dedicated to evaluate rodent's model of anxiety [12]. The open field apparatus is square arena (40-cm height, 100-cm width and 100-cm length) with the floor covered by a white consistent plastic. The floor was divided into 25 squares (5 rows of 5). The apparatus was illuminated under approximately 9lx. Animal was placed in the center and allowed 5 min to travel freely through arena. The central area of the open-field offers anxiogenic condition than to the peripheral. A video tracking recorded the spontaneous activity of the animal. The time spent in central square was taking into account. The apparatus was cleaned with 10% of ethanol solution in order to rule out the clues of previous animals.

2.4.2 Elevated Plus Maze (EPM)

The EPM is a behavioral test widely used to study animal's model of anxiety induced by an anxiogenic substances [13]
The apparatus consisted to four arms arranged in a form of cross. Two open arms (25-cm height cm x 10 cm width) opposed to two enclosed arms (40-cm walls), with an intersection as common central platform (5x5 cm). The EPM was raised to 50 cm above the floor and lighted with a halogen lamp of 9lx. The test began by placing animal onto the central square facing an open arm and allowed 5 min to explore freely. The time spent inside open arms was computed to evaluate anxiety related behavior. The apparatus was cleaned with ethanol 10% between test.

2.5 Oxidative stress markers assay

After behavioral testing, mice were anesthetized with choral (300 mg/kg) and killed by decapitation. Brain was removed and homogenized in ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 30 min at 4°C to obtain a supernatant that was used.

2.5.1 Analysis of Non-protein Thiols (NP-SHs) level

NP-SH level was assessed according to the method described by Ellman et *al* [14]. The supernatant was treated with 10% of trichloroacetic acid to precipitate proteins, and the sample was centrifuged at 2.000 rpm for 10 min. To the supernatant, was added 1 M of potassium buffer (pH 7.4) and 1mM of Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid). The NP-SHs levels were determined at 412 nm and expressed in nmol/g of tissue.

2.5.2 Nitrite oxide (NO) content

The quantification of NO content was determined by nitrite level in sample using the modified Griess method [15]. Briefly, 100 µl of supernatant was mixed with 50µl of Griess reagent [(1% sulphanylamide (A), and 0.1% N-1-naphtylethylenediamine dihydrochloride (B) in 2.5% orthophosphoric acid)], and the mixture was incubated at 37°C for 10 min in the dark. The reaction was performed in two steps. The first one consisted in a dinitrogenation reaction between the nitrite and Griess reagent A leading to a Diazonium salt by-product. The second step is the formation of stable chromophoric Azo product resulting by coupling between Griess reagent B and the Diazonium salt. The Azo product strongly absorbs at 543 nm at ELISA reader. The NO concentration was expressed in nmol/g of tissue.

2.5.3 Determination of Lipid peroxidation level

The assay Malondialdehyde (MDA) level, an important index of lipid peroxidation, was described in the method of Satoh et *al* [16]. Briefly, 500 µl of supernatant was mixed with 1.5 ml of trichloroacetic acid (10%), vortexed and incubated at room temperature for 10 min. Then it was added to the mixture 1,5 ml of thiobarbituric acid (0.67%), and heated in boiling bath water for 15 min. After a cooling, 1.5 ml of n-butanol was mixed to the solution and strictly vortexed. The sample was centrifuged at 800 rpm for 5 min, and the supernatant was collected. The absorbance was determined spectrophotometrically at 532 nm. The results were expressed as MDA level in nmol/g of tissue.

2.6 Data analysis

GraphPad Prism 6.0 version was used to record data and their analysis. The experimental results data were expressed as means ± S.E.M (Standard Error of Mean). Statistical analysis was done one-way analyses of variance followed by *Post hoc* Tukey test for multiple comparison. $P < .05$ considered as statistically significant.

3. RESULTS

3.1 Antinociceptive activity

3.1.1 Effects of HEAL on acetic acid-induced writhing in mice

After the injection of acetic acid to the control mice, the number of writhing recorded over the 30 min of observation was 34.33 ± 2.01. However, HEAL prevented acetic acid-induced nociception behavior through a significant reduction of number of writhing (25.67 ± 1.28; $P < .01$) as well as the ASA (21.83 ± 1.83; $P < .001$). The pre-treatment with plant extract and ASA caused an inhibition rate of nociception at 24.66% and 67.95%, respectively (Tab.1).

Table 1: Effects of HEAL pretreatment on acid acetic-induced writhing behavior

Group /dose	Number of writhings	inhibition (%)
Control	34.33 ± 2.01	
HEAL 30 mg/kg p.o.	25.67 ± 1.28**	25.22
ASA 200 mg/kg p.o	21.83 ± 1.83***	36.41

Data are represented as Mean ± SEM. One way ANOVA/ *Post hoc* Tukey. ** $P < .01$, *** $P < .001$ (vs control)

3.1.2 Effects of HEAL on formalin-induced nociception in mice

The antinociceptive action of HEAL on formalin-induced pain is consigned in Tab.2. In the control mice, formalin caused significant nociception reflected by the increased number of hind paws licking either 2.5 ± 0.342 or 8.66 ± 0.42 during the neurogenic and inflammatory phases of pain, respectively. However, the pre-treatment with the crude extract of *Adenia lobata* relieved the nociception; for the neurogenic phase (0.5 ± 0.211, $P < .001$ vs control) and for the inflammatory phase (1.167 ± 0.167, $P < .001$ vs control). Whereas the antinociception action of *Adenia lobata* was comparable to the standard drug tramadol in both neurogenic and inflammation phase, their action was prominent relative to the standard drug ASA during the inflammatory phase ($P < .001$).

Table 2: **Effects of HEAL pretreatment on formalin-induced hind paw licking**

Group /dose	Number of licking			
	Neurogenic pain (0-5 min)	Inhibition (%)	Inflammatory pain (15-30 min)	Inhibition (%)
Control	2.5 ± 0.342		8.66 ± 0.42	
HEAL 30 mg/kg, p.o.	0.5 ± 0.211***	80	1.167 ± 0.167***/+++	86.52
ASA 200 mg/kg, p.o	1.33 ± 0.211*	46.8	4.33 ± 0.558***	50
Tramadol 50mg/kg i.p.	0.16 ± 0.105***/+	93.6	0.33 ± 0.211***/+++	96.18

Data are represented as Mean ± SEM. One way ANOVA/ *Post hoc* Tukey test. * $P < .05$, ** $P < 0.01$, *** $P < .001$ (vs control); + $P < .05$, +++ $P < .001$ (vs ASA)

3.1.3 Effects of HEAL on heat-induced nociception in mice

The mice pre-treated with HEAL spent more time before reacting to thermal nociception by licking hindpaw when compared to the group of reference drug ASA ($P < .05$). By contrast, no difference was observed regarding the latency to Jump ($P > .05$). The mice pre-treated with the tramadol exhibited no reaction time to the heat-induced nociception (Tab.3.)

Table 3: Effects of HEAL pretreatment on heat-induced nociception

Group /dose	Thermal nociception reaction	
	Hindpaw Lick latency (sec)	Jump latency (sec)
HEAL 30 mg/kg p.o.	14.75 ± 1.23 $^{***/+}$	4.25 ± 0.97 ***
ASA 200 mg/kg, p.o	12.5 ± 1.12 ***	4.00 ± 0.53 ***
Tramadol 50mg/kg i.p.	more than 20	13.25 ± 1.26

Data are represented as Mean ± SEM. One way ANOVA/ *Post hoc* Tukey test. *** P < 0.001 (vs Tramadol); ^{+}P < .05 (vs ASA)

3.2 Post-nociception anxiety-like behavior

3.2.1 Anxiety level consecutive to acetic acid-induced writhing behavior in mice

In the open-field test (Fig. 1A), the group of mice pre-treated with HEAL spent highly significant time in central anxiogenic area of open-field (141.7 ± 18.45 sec, *P* < .001), relative to the control Mice (2.66 ± 0.21 sec) and reference drug ASA (31.23 ± 1.28 sec).

Regarding the EPM (Fig.1B), HEAL demonstrated considerably anxiogenic effect at the post writhing nociception period through the percentage of time spent into open arm (24.17 ± 5.84 %), in comparison to the ASA-treated mice (14.67 ± 1.52%; *P* < .01) or the control Mice (4.66 ± 0.95%; *P* < .001). However, the pre-treatment with reference drug ASA reduced anxiety level compared to control (*P* < .01).

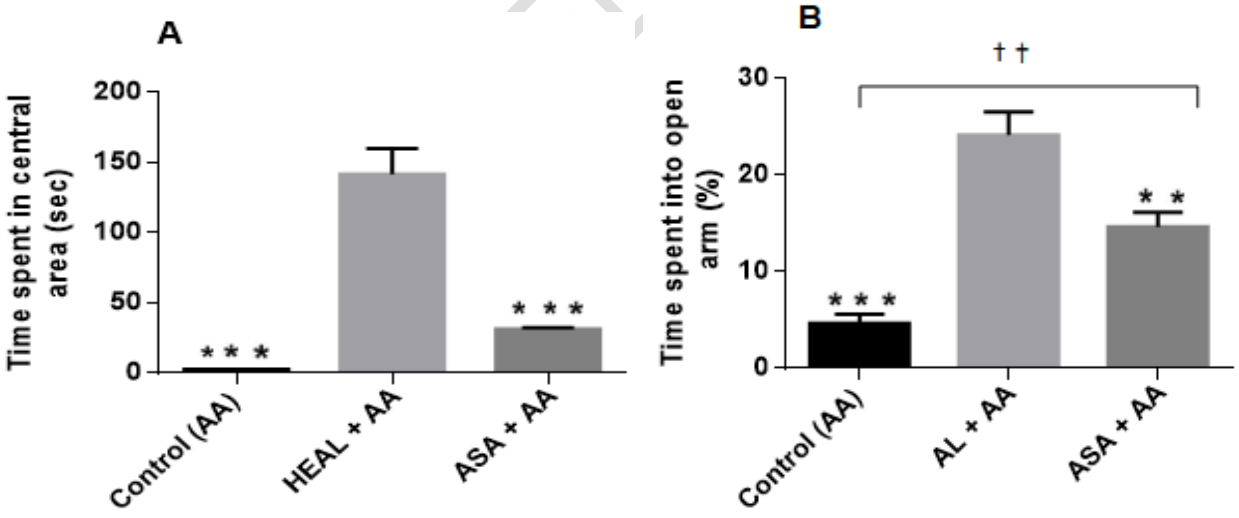

Fig.1. Effects of HEAL on anxiety level consecutive to acetic acid (AA)-induced writhing behavior in mice.

A, time spent in central area in open-field; **B**, time spent in open arms of EPM. Data are represented as Mean ± SEM. One way ANOVA / *Post hoc* Tukey test. $^{**}P$ < .01, $^{***}P$ < .001 (vs AL + AA), $^{++}$ *P* < .01 (Control vs ASA + AA)

3.2.2 Anxiety level consecutive to formalin-induced nociception in mice

In the open-field test (Fig. 2A), the pre-treatment of mice with reference drug tramadol prevent significantly anxiety related to formalin-induced nociception through the time spent in central area (205 ± 26,68 sec), incomparably to control (5.50 ± 0.56 sec; *P* < .001), reference drug ASA (32.50 ± 2.90 sec; *P* < .001), and the HEAL (61 ± 3.61 sec; *P* < .001). However, HEAL altered more significantly anxiety level in mice (*P* < .05 vs Control or ASA).

In the EPM (Fig. 2B), the beneficial effect of pre-treatment on formalin-induced post nociception anxiety was remarkably with HEAL (18.67 ± 1.22%) and tramadol (22.17 ± 1.7%) compared to the control (1.33 ± 0. 21; $P < .001$).

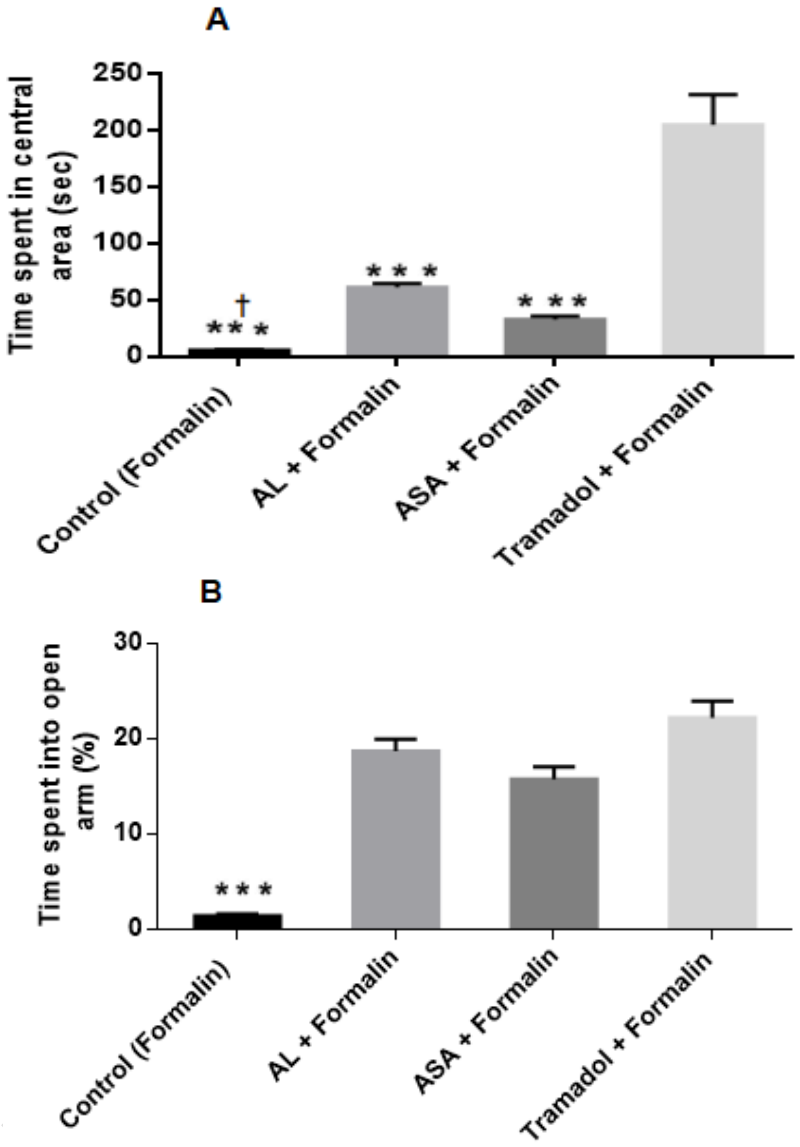

Fig.2. Effects of HEAL on Anxiety level consecutive to formalin-induced nociception in mice.

A, time spent in central area in open-field; **B**, time spent in open arms of EPM. Data are represented as Mean ± SEM. One way ANOVA / *Post hoc* Tukey test. $^{***}P < .001$ (vs Tramadol; Control vs experimental groups); $^{+}P < .05$ (vs AL + Formol)

3.2.3 Anxiety level consecutive to heat-induced nociception

In the open-field test (Fig. 3A), the mice pre-treated with HEAL did not exhibit anxiety after pain induced with thermal stimulus. They spent more time into anxiogenic area of open-field (126 ± 13. 92 sec) when compared to the mice pre-treated with tramadol (82 ± 4.26 sec, $P < .01$) or with ASA (62.88 ± 3.97 sec; $P < .001$).
In the EPM (Fig. 3B), the anxiogenic effect was confirmed HEAL in mice pre-treated through a reduced time spent into open arm (35.13 ± 3.36 %), compared to the tramadol (22.75 ± 2.59 %; $P < .01$) or ASA (22.50 ± 1.89 %; $P < .01$).

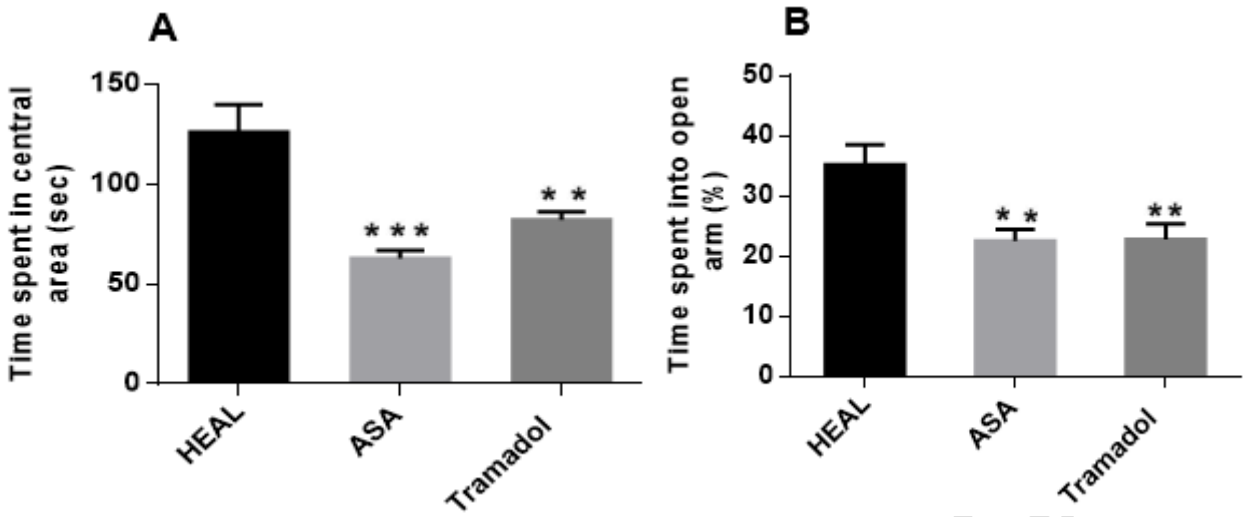

Fig. 3. Effects of HEAL on Anxiety level consecutive to heat-induced nociception

A, time spent in central area in open-field; **B**, time spent in open arms of EPM. Data are represented as Mean ± SEM. One way ANOVA / *Post hoc* Tukey test. $^{**}P < 0.01$, $^{***}P < 0.001$ (vs *Adenia lobata* extract)

3.3 Oxidative markers assay

The results of oxidative stress markers assayed are reported in the tab.1.

3.3.1 NP-SH level in Mice brain

In the case of AA-induced nociception, HEAL pre-treatment helped to generate higher level of the natural antioxidant NP-SH ($P < .001$ vs Control) than the reference drugs ASA ($P < .01$ vs control).
In the case of formalin-induced nociception, no significant difference was observed between the experimental groups.
Regarding the heat-induced nociception case, the level of NP-SH was significantly increased with both pre-treatments HEAL ($P < .05$ vs ASA) and reference drug tramadol ($P < .001$ vs ASA).

3.3.2 NO level in Mice brain

In the case of AA-induced nociception, the pre-treatment with *Adenia lobata* mitigated significantly free radical NO level in the brain ($P < .001$ vs control) comparatively to the reference drug ASA ($P < .01$ vs control).
In the case of formalin-induced nociception, only pre-treatment with HEAL showed a marked reduction of NO level ($P < .001$ vs other groups).
In the case of heat-induced nociception, again only the HEAL pre-treatment decreased significantly the NO production ($P < .001$ vs other groups).

3.3.3 MDA level in Mice brain

In the case of AA-induced nociception, HEAL prevented the lipid peroxidation in the brain of mice. The levels of MDA were at similar values as the reference drug ASA ($P < .001$ vs control).
In the case of formalin-induced nociception, the pre-treatment with HEAL demonstrated no significant effect on MDA level.
In the case of heat-induced nociception, both pre-treatments with HEAL and reference drug tramadol caused significant reduction of MDA level in the brain ($P < .001$ vs ASA).

Table 4: Effects of *Adenia lobata* extract pre-treatment on oxidative stress markers level

	Groups	Oxidative stress markers		
		NP-SH (nmol/g of tissue)	NO (nmol/g of tissue)	MDA (nmol/g of tissue)
AA Nociception	*Control (AA)*	657.34 ± 56.8	223.01 ± 21.52	25.12 ± 3.40
	HEAL *30 mg/kg, p.o.*	823.03 ± 45.7**	103.12 ± 15.13***	11.12 ± 2.01***
	ASA 200 mg/kg, p.o	795.54 ± 55.64*	175.25 ± 32$^{**/\ ++}$	10.32 ± 3.23***
Formalin Nociception	*Control*	798.3 ± 66.9	229.6 ± 8.16	12.29 ± 0.63
	HEAL *30 mg/kg, p.o*	808.8 ± 53.35	131±14.41***	13.06 ± 1.00
	ASA 200 mg/kg, p.o	816 ± 53.23	178.6 ± 6.21	12.98 ± 0.87
	Tramadol 50 mg/kg, i.p	810.6 ± 67.03	176.4 ± 7.41	6.61 ± 0.57***
Thermal Nociception	HEAL *30 mg/kg, p.o*	742.3 ± 24.85*	105.3 ± 6.50***	18.44 ± 1.45***
	ASA 200 mg/kg, p.o	699.4 ± 66.97	199.4 ± 16.80	35.87 ± 2.59
	Tramadol 50 mg/kg, i.p	885.9 ± 21.95***	201.8±11.53	23.43 ± 0.75***

Data are represented as Mean ± SEM. One way ANOVA/ *Post hoc* Tukey test. * $P < .05$, ** $P < .01$, *** $P < 0.001$ (vs control or ASA); $^{++}P < .01$ (vs control)

4. Discussion

The outcomes of the present study showed significant analgesic properties of crude extract of *Adenia lobata* using different experimental models of pain condition. We also highlighted that the HEAL exerted an anti-inflammatory effect and alleviating anxiety-like behavior related to pain. We investigated the both peripheral and central analgesic action by using three models of pain testing including AA-induced writhings, the formalin test and hot plate test.

The AA-induced writhings is specific to evaluate peripheral pain. In fact, AA-induced abdominal pain response is associated with an acute inflammation of the peritoneum. In our results, the HEAL (30 mg/kg) inhibited significantly writhing responses similarly to the reference drug ASA, suggesting that HEAL possesses some anti-inflammatory and antinociceptive activities as the reference drug ASA [17]. The mechanism action of ASA consists to irreversible inhibition of prostaglandin biosynthesis in peritoneal fluids via inactivation of both cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2). The peripheral mechanisms of pain which exacerbating nociceptive neurons are mediated concomitantly with various endogenous mediators as bradykinin, histamine, serotonin and substance P and prostaglandin. A previous research reported that the treatment with the medicinal plant *Kaempferia galanga* Linn (50 mg/kg) inhibited significantly AA-induced writhings comparable to ASA [18]. Those authors explained the anti-writhing responses of its bioactive compound (ethyl cinnamate) by a tonic inhibition of abdominal smooth muscles rather than its

any intrinsic analgesic activity. According to an ethnopharmacological survey, the liana of *Adenia lobata* is an useful medicinal plants to facilitate labor [19]. *Adenia lobata* has so an opposite effect relative to *Kaempferia galanga* due to its ability to stimulate muscular contraction. However, another previous study mentioned that hydroethanolic extract of *Solenostemon monostachyus* prevented AA-induced writhings when compared to the reference drug ASA [20]. Interestingly, the phytochemical screening of this specie revealed similar constituents to ours such as alkaloids, steroidal glycosides, saponins, flavonoids, tannins [10,21]. We suggest that flavonoid from *Adenia lobata* behaves as anti-inflammatory compound when it inhibits COX [22] and alkaloid as antinociceptive compound via the inhibition of opioid receptors activation [11]. Additional anti-inflammatory mechanism of *Adenia lobata* suggests to be a modulatory action of nuclear factor Kappa B cell (NFkB) which down-regulate inducible nitric oxide synthetase (i-NOS), as elucidated with ASA [23]. Our results demonstrated also an antioxidant action of *Adenia lobata* extract through a significant reduction of the level of NO and lipid peroxidation marker MDA as the reference drug ASA, and incomparably to that of the control mice. However, the specificity of writhing test to evaluate peripheral antinociceptive drug remains controversial [24]. Therefore, we continue our anti-nociceptive drug testing with formalin test, since it evaluates apart both peripheral and central pain.

The formalin test involves the assessment of two distinct phases namely the neurogenic phase due to a direct activation of peripheral nociceptive neurons by formalin, while the inflammatory phase is due to a sensitization of spinal cord neurons by endogenous opioid and inflammatory mediators [16]. The HEAL inhibited significantly both phases neurogenic and inflammatory responses of formalin-induced nociception. The anti-nociceptive action of the HEAL against the neurogenic pain was significantly higher than the reference drug ASA, but acted almost at the similar manner against inflammatory pain as the central acting analgesic drug tramadol. From the formalin test outcomes, we can conclude HEAL presents both peripheral and central analgesic properties, since it acts effectively than ASA on peripheral pain trough the inhibition of inflammatory phase of formalin-induced hind paw licking, and imitates the action of tramadol. From pharmacological view, tramadol plays antinociceptive role by activating the μ opioid M 1 receptors and enhancing GABAergic and glycinergic transmission at substantia gelatinosa site of spinal horn [23], and inhibiting the neural uptake of noradrenaline and serotonin 5-HT [26]. Moreover, it has been reported that central analgesic drugs such as opioid inhibit both phases central and peripheral pain equally [27]. Taking together, we suggest that the antinociceptive effect of HEAL is effective at the central and peripheral level, it also reduces delayed nociception of formalin test through anti-inflammatory action. Farouk et *al* [11] attributed the central and peripheral antinociceptive action to alkaloid extract mediated by opioid receptors.

We tested the antinociceptive activity of HEAL against pain related supra-spinal response with hot plate test. This model is selective to evaluate ability of central acting analgesic drugs. The HEAL reduced significantly the reaction time to thermal stimulus when compared to ASA, but less much comparatively to the tramadol. The effectiveness of central antinociceptive action of HEAL could be significantly confirmed dependently to dose level chosen. In fact, previous researches indicated central analgesic drugs from alkaloid-rich extract acted efficiently at least 200 mg/kg p.o using rodent's model of hot plate test [16;18].

Importantly, the pre-treatment with HEAL reduced significantly post-nociception anxiety-like behavior. The anxiolytic effect of HEAL suggest to be possibly due to the interaction with serotoninergic transmission. It has been reported that some types of alkaloids have various spectrum pharmacological action including interaction with 5-HT and benzodiazepine or glycine receptors [28-30]. Otherwise, the higher antioxidant activity of ethyl acetate extract fraction of *Adenia lobata* has been tested as a therapeutic approach for treatment of cancer [10].

5. Conclusion

In conclusion, our findings revealed that *Adenia lobata* extract treatment exhibits peripheral and central antinociceptive activity. Its action implies also anti-inflammatory pain. Interestingly, in all cases of the nociception testing, we found significant effects of HEAL on oxidative stress markers. However, additional investigations are needed to elucidate further pharmacological actions of HEAL-induced nociception pain inhibition such as those of opioid mechanisms.

CONSENT

Not applicable

ETHICAL APPROVAL

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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