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Corrigendum

CORRIGENDUM TO "TOXICITY, ANALGESIC AND ANTI-PYRETIC ACTIVITIES OF METHANOLIC EXTRACT FROM HYOSCYAMUS ALBUS' LEAVES ON ALBINOS RATS"

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ABSTRACT

Objective: The aim of this work is to evaluate *in-vivo* the analgesic and antipyretic activities of the methanol extract of *Hyoscyamusalbus* (HAMeOH) on Wistar rats.

Methods: An acute toxicity test effected according to OECD – 420. After asubacute toxicity was realisedby testingthe therapeutic doses during 28 days.The analgesic activity was effected by two methods: the acetic acid test and the formalin test. The antipyretic activity was effected by inducing fever by Brewer's yeast.

Results: In the acute toxicity, the administration the dose of2000 mg / kg body weight (b.w) of *H.albus* did not induce any death of rats and any signs of toxicity were observed that's for the (LD50) was considered greater than 2000 mg / kg b.w. In the subacute toxicity, the therapeutic doses chosen 100 and 200 mg / kg b. wdid not give any mortality for 28 days, also no significant difference (P>0.05)noted in the body weight of rats during subacute toxicity. The results of the analgesic activity show that HAMeOH (100 mg / kg and 200 mg / kgb.w) have a peripheral analgesic activity by decreasing the number of licks inducing by the acetic acid and a significant analgesic effect by reducing the licking time during the second phase of formalin test. In the antipyretic activity, HAMeOH decreased the body temperature that induced by Brewer's yeast infection in significant way and dose-dependent manner from the 3rd hour using paracetamol as reference.

Conclusion: Our results show that the methanol extract of *H.albus* have peripheralanalgesic effect, also antipyretic effect.

Keywords: *Hyoscyamusalbus*, Methanolic extract, Toxicity, Analgesic activity, Antipyretic activity, Formalin test.

INTRODUCTION

Pain sensation due to the internal or external nociceptive stimulus [1].Current treatment of inflammation or pain concentrate on narcotics such us opioidsor non- narcotics as silicates, but these last have adverse effects, the peopleused substances of natural origin [2]. *H.albus*is a plant which belongsfor Solanaceae family, it used in traditional medicine as a nervous sedative and para sympatholytic [3]. They could isolate some tropane alkaloids such as scopolamine, hyoscyamine, atropine and also with spectral technics they isolated 2,3 – dimethyl nonacosane [4]. The actual work is toevaluate analgesic and antipyretic activities of the methanol extract of *H.albus* (HAMeOH) as well as acute and subacute toxicity.

MATERIALS AND METHODS

Chemicals, Formadehyde CH_2O (EDEN LABO), Acetic acid puriss glacial (Sigma-Aldrich), Indomethacin and acetylsalicylic acid (Sigma-Aldrich), were used in the present study.

Plant Material

The leaves of this plant was collected from Bouzinacity, Batna, Algeria. It Was Identified by Dr. OUDJHIH, Laboratory of Botanic, Department of Agronomy, Batna algeria. Plant leaves were dried for 40 days at an ambient temperature under shade, after; the leaves were crushed to obtain a fine and homogeneous powder and conserved in dry place.

Extracts preparation

1 Kg of powdered leaves was extracted with petroleum ether three times 5 L for each time. Then, the marc was dried and extracted with chloroform three times 5 L for each time and with methanol three times 5 L for each time and the supernatants were filtered sequentially using cloth filter, cotton wool, and What man filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature(30C) using a vacuum rotary evaporator (BuchiRotavapor).

Phytochemical Screening

The phytochemical screening of HAMeOH was performed using standard method [5]. Phytochemical constituents such as phenolic compounds, terpenoids, saponins, alkaloids, steroids and tannins were qualitatively analysed.

Experimental animals

Wistar rats weighted (140-170g) provided by the Pasteur Institute – Algiers. These rats were allowed a favorable conditions before and during the experiment: Temperature (23 ± 2) ° C, relative humidity 50 -55 %, with 12 day / night cycle respectively. The food and water were given *ad libitum*.

The acute toxicity study

The acute toxicity of the methanol extract of *H.albus* was evaluated on female Wistar rats weighted (150-170) according to OECD guideline 420 (OECD, 2001) which limits the dose at 2000 mg / kg body weight [6].Female rats (140-160 g b. w) were divided in groups. Four groups of six rats received HAMeOH by gavage using the concentrations (500, 1000,1500 and 2000) respectively, with a volume of 10 ml / kg, the control group received the distilled water (10 ml / kg b.w). The observations were followed 30 min, 1, 2 and 4 hours each day for 14 days. The changement of the skin, morbidity, respiratory movements, the number of death of animals were recorded for each group. LD50 was calculated by the arithmic method of Karbar.

Subacute toxicity

Both doses of 100, 200 mg / kg were selected for subacute toxicity. Three groups were used each group contains six rats (140 –160g) (3 males + 3 females). Group I considered as negative control and received distilled water with a volume of 10 ml / kg b. w. Group II and III received HAMeOH 100 and 200 mg / kg b.w/ day respectively with the volume of (10 ml / kg b.w. in distilled water) every day for 28 days. The body weight and mortality were recorded during this

period and were considered as toxic manifestations. After 28 days, the animals werefastedall night and dissected after anesthesia with chloroform. Biochemical analyses were performed for the blood samples which were taken from ocular sinus. Theinternal organs as:liver, kidneys, lungs and heart were removed, weighted and analysedfor the lesions.Finally they conserved in formalin 10% for the histopathological study.

Assesment of Biochemical analyses

Blood is collected in heparin tubes and centrifuged at 3500 rpm for 5 min. After centrifugation, the biochemical analyses were effected of some parameters like: Aspartase aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, triglycerids, urea, glucose, creatinine, Biliribin (Bil).

Histopathologicalstudy

The histopathological study was done according to the method of Lamb [7]. Organs (liver and Kidney) were fixed in a formalin solution 10%, after these organs are embedded in the paraffin, the tissues were cutted with microtome 5 µm of thickness and are mounted on slides and stained with the hematoxylin-eosin.

Biological activities

Analgesic activity

a. Evaluation of peripheral analgesic activity

Method of acetic acid is used to evaluate the peripheral analgesic activity according to (Seighmund *et al*., 1957**)** [8] The acetic acid induced Stretches and licks after its injection. A solution of acetic acid 0.6% was injected intra peritoneally in the posterior paw of rat (1 ml / 100 b.w). Knowing that 30 min before the induction of the pain, Four lots, each one contains six rats(n=6), each lot received the following treatments: Group I and II received HAMeOH (100 and 200 mg / kg, b.w) respectively, Group III received product (acetylsalicylic acid) as reference and group IV received distilled water as negative control. After 5 min of the injection, the number of stretches was counted during 15 min. And Analgesic Activity was assessed using the relationship: Inhibition Percent = (1 - Ec / Et) × 100. which: Et= Number of stretches in the test group. Ec= Number of Stretches in the control group.

b. Formalin Test

This method used according to Hilaly*et al.* 2004 [9]., pain was induced by injecting subcutaneously 20 µl of formalin solution 5% in the posterior paw of the rat.

30min before the induction of pain, rats were given orally the following treatments: Group I and II were received HAMeOH (100 and 200 mg / kg b.w), Group III received standard reference indomethacin (10 mg / kg, b.w) and Group IV received distilled water and served as a negative control. These rats were individually placed in the cages and the time taken for licking was counted during the period 0 - 5min (first phase or neurogenic phase) and during the second phase (inflammatory phase) and inhibition percentage was calculated like the method of acetic acid.

Evaluation of the antipyretic activity

The antipyretic activity was evaluated by inducing pathogenic fever using Brewer's yeast [10]. Fever was induced by injecting subcutaneously the aqueous suspension of Brewer's yeast 20% (20ml/Kgb.w). Four groups were received orally the following treatments: control group I received distilled water, Group II and III were received HAMeOH with the two concentrations (100 and 200 mg / kg b.w) respectively, and Group IV was received paracetamol (150 mg / kgb.w)as a positive control, these treatments were administered 17 hours after the induction of fever. After; rectal temperature was taken 1,2,3,4 and 6 hours after administration of treatments..

Statistical Analysis

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

RESULTS

Screening phytochimique

The phytochemical screening of HAMeOH revelated the presence of alkaloids, terpenoids,saponins, condensed tannins and steroids, the phenolic compounds and also flavonoids were very answered in the extract (Table 1).

Table1: Phytochemical constituents of methanolic extract from *H.albus'*sleaves.

Sample	Phytochemicalconstituents	Result
HAMeOH	Alkaloid	+++
	Saponin	+++
	Flavonoid	++
	Tannins and polyphenolic compounds	++
	Terpenoids	+++
	Steroid	++

+++: Very positive reaction, ++: positive reaction, -: negative reaction

Test of Toxicité

The acute toxicity test showed that the LD50≥ 2000 mg / kg b.w.The concentrations (500, 1000.1500 and 2000) did not cause any death of animals within lots. Also, no signs of toxicity in animals after administration of these doses (Table 2). In the subacute toxicity test, the two selected therapeutic doses (100 and 200 mg / kg b.w) were not recorded any deaths during the treatment period of 28 days, either in the control or treatment groups. The animals did not show changes in general behavior and other physiological activities. The body and organ weight of the rats after 28 days are given in (Table3).

The results show that there is no significant difference (p> 0.05) in body weight and organ weights of treated groups compared to control group (Table3).

Table 2: LD50 determination by arithmetic method of Karbar

Groups	Number of rats	No. of animalsdead	Dose difference (a)	Meanmortality (b)	Probit (a×b)
Control group	6	0	No	No	No
H.albus 100 mg /kgb.w	6	0	100	No	No
H.albus 200 mg /kgb.w	6	0	100	No	No
H.albus 500 mg /kgb.w	6	0	400	No	No
H.albus 1000 mg /kgb.w	6	0	500	No	No
H.albus 2000 mg /kgb.w	6	0	1000	No	No

Table 3: Effect of methanolic extract from *H.albus*'s leaves on body and organ weights (g) of rats in subacute toxicity

	Control (2ml water/100gb.w)	100 mg/Kgb.w	200 mg/Kgb.w
Body weight			
Initial	131.7 ± 1.52	132.3 ± 1.52	132.7 ± 2.51
Final	175.0 ± 3.00	177.7 ± 2.88	175.7 ± 5.85
Organweight			
Liver	10.63 ± 0.57	10.67 ± 0.17	11.11 ± 0.63
Kidney	1.20 ± 0.047	1.15 ± 0.055	1.073 ± 0.065
Lungs	1.040 ± 0.079	1.14 ± 0.005	1.06 ± 0.065
Heart	0.82 ± 0.070	0.82 ± 0.026	0.85 ± 0.55

Data are expressed as mean ± s.d. (n=6). No statistical difference (p>0.05) between control and HAMeOH groups (p>0.05)

The biochemical profile of treated groups with HAMeOH and the control group are shown in Table 4. No significant difference detected (P> 0.05) in the biochemical parameters analysed after 28 days compared to the negative control, only the elevation of urea and creatinine (P < 0.001) was revealed in the groups treated with HAMeOH (100 and 200 mg / kgb.w). There was no effect on liver levels of indicators such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin (Bil). These results demonstrate that " *H.albus* " do not cause liver damage which was confirmed by histological evaluation of this organ. But renal histology showed the presence of perivascular macrophage granuloma in treated groups with(100 and 200 mg / kgb.w) that is consistent with the biochemical tests which resulted in the elevation of urea and creatinine. Our results are consistent with our histological evaluation, histopathological results of the liver and kidney are presented in (Figure 1 and 2).

Table 4: Effect methanolic extract from *H.albus*'s leaves on biochemical parameters of rats in subacute toxicity (mean ± SEM, n= 6)

		HAMeOH treated group	
Parameters	**Control group**	**100 mg/kg**	**200 mg/kg**
Glucose (g/L)	1.21±0.23	1.21±0.11	0.87±0.13
Urea (g/L)	0.36±0.05	0.79 ±0.11**	0.83±0.08**
Creatinine (mg/L)	6.73±1.04	21.99 ±0.46**	29.98±2.16**
Cholesterol (g/L)	0.77±0.09	0.79±0.10	0.66± 0.17
Triglycerides (g/L)	0.60±0.18	0.63±0.11	0.60 ± 0.07
SGOT (U/L)	138.7±3.16	148.0±7.70	137.0±5.71
SGPT (U/L)	37.00±2.16	35.75±1.25	31.25±2.50
ALP (U/L)	261.5±11.09	250.98±20.20	266.5±18.27
Biliribin (mg/L)	0.85±0.08	0.55±0.11	0.69±0.21

Values expressed as mean ± STD; Significance with Tukey's test following one way ANOVA is evaluated as *p < 0.05 and **p < 0.001 vs control group. SGOT –Serum Glutamate oxaloacetate transaminase; SGPT – Serum Glutamate pyruvate transaminase, ALP- Alkaline

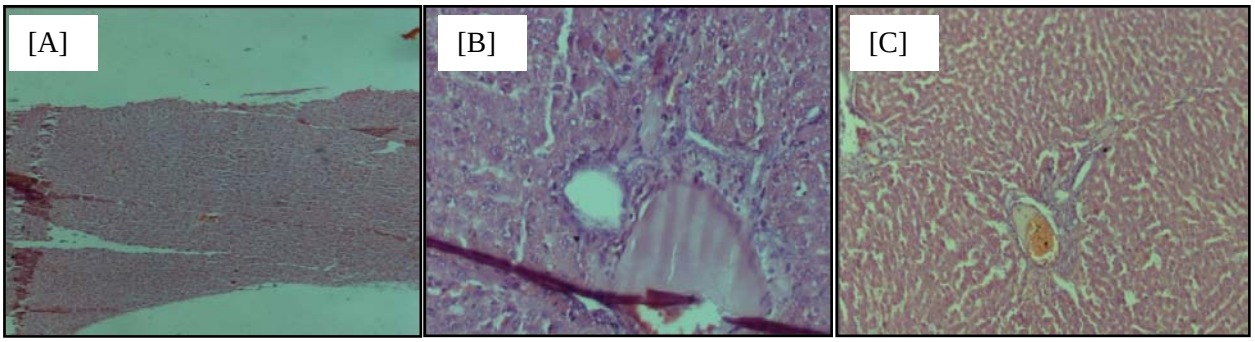

Fig.1: Photomicrograph of Liver histology of treated and untreated albino rats with methanol extract of *H.albus's* leaves [A]control group, [B] HAMeOH [100 mg/kg b.w]: discrete (portal fibrosis + sinusoidal hemorrhage), [C] HAMeOH[200 mg/kg b.w]: discrete portal fibrosis. Liver sections stained with haematoxylin and eosin (90 X).

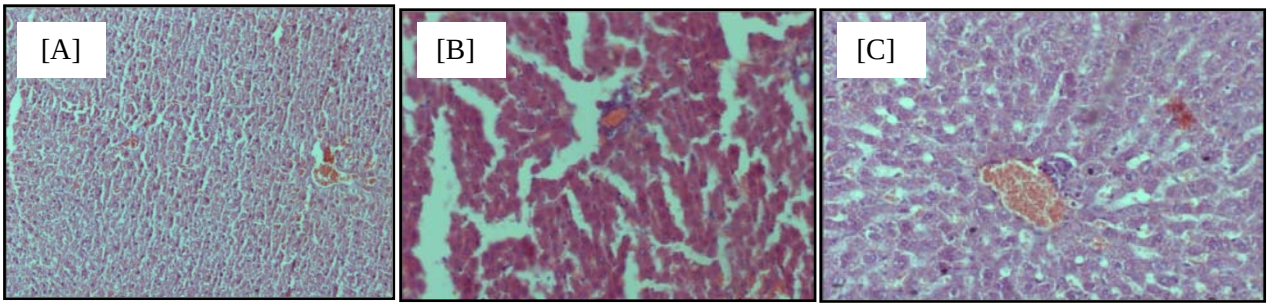

Fig. 2:Photomicrograph of Kidney histology of treated and untreated albino rats with methanolic extract of *H.albus's* leaves[A]control group, [B] HAMeOH [100 mg/kg b.w]: perivascular macrophage granuloma, [C] HAMeOH [200 mg/kg b.w]: perivascular macrophage granuloma. Kidney sections stained with haematoxylin and eosin (90X).

Biological activities

Analgesic activity

a. Peripheral Analgesic Activity – Abdominal Constriction

Each bar represents the mean ± SEM of 6 animals; ***P* <.0001 statistically significant compared to their respective control The oral treatment with HAMeOH reduced significatively (P <0.0001) and dose dependent the number of abdominal constrictions induced by acetic acid. The methanol extract with doses 100 and 200 mg / kg b.w showed a protective effect of 92.97% and 94.00% respectively. The concentration 200 mg / kg b.wof methanolic extract remove pain significantly (P≤ 0.05) relative to the acetyl salicylic acid (87.09%) (Fig.3).

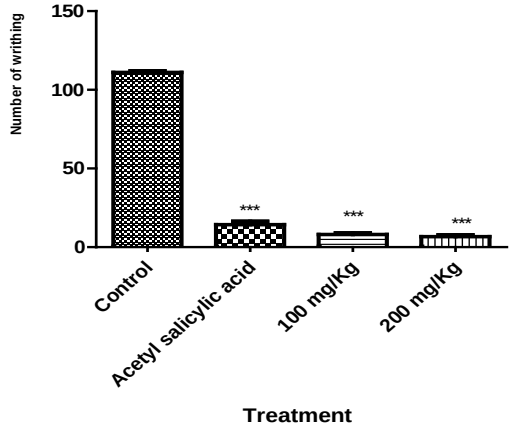

Fig. 3: Effects of methanolic extract of *H.abus'*sleaveson acetic acid-induced pain in rats.

b. Formalin test

Each bar represents the mean ±SEM of 6 animals; ***P* <.0001 statistically significant compared torespective control. The subcutaneous injection of solution formalin 5% into the right hind paw of the rats caused a biphasic nociceptive response. As shown in Fig.4 and 5, HAMeOH (100 and 200 mg / kgb.w) reduced significantly and dose- dependent manner the nociception in both phases (early and late phase). HAMeOH has significantly reduced the licking time in the first phase with an activity of 32.85% and 45.91% with doses 100 and 200 mg / kg b.wrespectively, knowing that indomethacin did not inhibit pain significantly during this phase.

During the second phase, the both doses of (100 and 200 mg / kgb.w) of HAMeOH were showed a maximum protective effect of 48.55% and 58.65%, respectively, whereas indomethacin showed an inhibitory effect 46.28%.

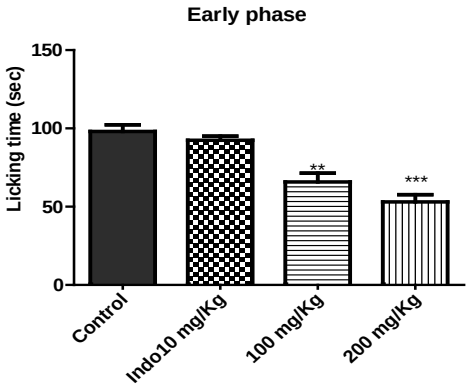

Fig.4: Analgesic effect of the HAMeOH and Indo on the early phase.

Each bar represents the mean ±SEM of 6 animals; ***P* <.0001 statistically significant compared torespective control.

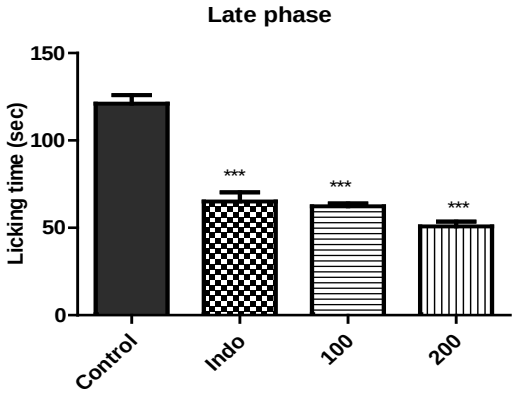

Fig.5: Analgesic effect of the HAMeOH and Indo on the late phase.

3.2. Antipyretic activity

Table 5: Effect of Methanolic extract of of*H.albus'*sleaves on yeast induced pyrexia in rats.

	Rectal temperature (°C) before and after treatment						
Treatment	**17h**	**1h**	**2h**	**3h**	**4h**	**5h**	**6h**
Control Vehicle	36.43±0.05	38.56±0.02	38.95±0.08	38.63±0.11	38.43±0.05	38.57±0.15	38.47±0.05
HAMeOH (100mg/Kg)	36.33±0.30	38.63±0.15	38.62±0.36	38.13±0.05**	38.07±0.05*	37.90±0.17**	37.50±0.10***
HAMeOH (200mg/Kg)	36.43±0.15	38.37±0.03	38.74±0.10	37.90±0.20***	37.33±0.15***	37.10±0.10***	37.13±0.15***
Paracetamol (2mg/Kg)	36.37±0.11	38.38±0.22	38.65±0.02	38.03±0.11**	36.67±0.15***	36.57±0.05***	36.10±0.10***

Each value represents Mean ±SD. **P*≤ 0.05, ***P*≤0.001, ****P*≤0.0001, when compared with the control values of corresponding hour.

The effect of HAMeOH on yeast-induced fever is shown in (Table 5). Subcutaneous injection of the suspension of yeast caused the rise of rectal temperature in rats after 17 hours from the administration of the suspension. The treatment with HAMeOH (100 and 200 mg / kgb.w) decreased significantly (P≤ 0.05) and with dose -dependent manner the rectal temperature. The reduction of elevated rectal temperature induced by this yeast was observed from the 3rd hour after treatment in groups treated with either the extract or paracetamol.

DISCUSSION

The evaluation of the acute toxicity is the first step in the toxicological assessment of unknown substances. The researcher found that the index of acute toxicity is the LD50[11]. The

classification of orally toxic substances was done by the organization for Economic Cooperation and Development (OECD, Paris, France) [12] according to this classification: very toxic when the extract is inferior than 5 mg / kg; toxic (> 5 < 50 mg / kgb.w); harmful (> 50 < 500 mg / kg b.w) and non-toxic (> 500 < 2000 mg / kgb.w). The LD50 was found higher than 2000 mg / Kg in the methanol extract of *H.albus,* is an indication that the extract is relatively non-toxic by the oral route.

For subacute toxicity, 1/20 th and 1/10 th of safe dose were selected as therapeutic doses wich are 200 mg / kg and 100 mg / Kgb.w After 28 days of treatment, the biochemical analyse showed no liver toxicity. The transaminases AST and ALT are biomarkers for predicting the toxicity of extracts opposite the liver and their elevation indicate a malfunction of the liver,and the elevation of urea and creatinine suggests kidney toxicity [13]. Histopathological study showed the presence of perivascularmacrophagegranulomain the kidneys that is a sign of inflammation in the kidney that explain the high level of urea and creatinine in the serum. The phytochemical screening of HAMeOH revealed the presence of alkaloids, terpenoids, flavonoids and saponins which may be responsible for the inhibition of pain.

It is therefore possible that the analgesic and antipyretic activity is due to the synergistic effect of these various substances. The method of acetic acid is the most responded in evaluating of peripheralantinoceptive activity [14].This method is characterised by the increasing of PGE2 and PGF2a and lipoxygenases in peritoneal fluid which stimulates nociceptive neurons [15]. These fibers are sensibles to non-steroidal anti-inflammatory drugs (NSAIDs) and nactroticsand sensible to other compounds of central action[16]. suggested that HAMeOH inhibited the cyclooxygenase and lipoxygenase pathways and subsequent synthesis of prostaglandin so; it is a preferable agent for inhibiting peripheral mechanism of pain and our results are similar with which find by ***Senatos et al.*** 1997[16]. The formalin test causes the creation of both early and late phases. Early phase (neurogenic phase) is characterised by activation of C-fibers [17] and a delayed phase is due to the release of mediators such as serotonin, histamine, bradykinin and prostaglandins initiated by the C-fibers [18].The central compoundsacting inhibit both phases (early and late phases) while peripherally acting compounds inhibit only delayed phase which due bythe inhibition of the prostaglandin synthesis [19].

HAMeOH showed an analgesic effect in the two phases which confirms the blocking of the nociceptorsin addition, inhibition the formation of inflammatory mediators. Pyrexia or fever is a phenomenon caused by infection, tissue damage or transplant rejection [20].Brewer's yeast inducing fever is a phenomenon called pathogenic fever and it is due to the production of prostaglandin in the brain [20; 21].

Antipyretic agents inhibit fever, either by inhibiting the synthesis of prostaglandin E2 in the brain by inhibiting the expression of cycloxgénase 2 or by inhibiting the synthesis of interleukin - 1 α [22]. The results showed that HAMeOH inhibited the fever induced by Brewer's yeast, this is may be due to the presence of flavonoids and saponins. Flavonoids are known for their antipyretic effects by supressionTNF- α [23]. Even alkaloids are known for their antipyretic effects [24].

CONCLUSION

In conclusion, our results showed that the methanol extract of *H. albus's* leaves has an anti nociceptive and antipyretic effect and these effects are due to inhibition of the synthesis of mediators especially prostaglandin. The value of the LD50 is greater than 2000 mg / kg b.wand this dose is tolerated to rats. These results may justify its use in traditional medicine as a nervous sedative. But deep studies are needed to elucidate the mechanism of action of the extracts and substances responsible for these effects.

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