

TOXICITY, ANALGESIC AND ANTI-PYRETIC ACTIVITIES OF METHANOLIC EXTRACT FROM *HYOSCYAMUS ALBUS*' LEAVES IN ALBINOS RATS

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ABSTRACT

Objective: The aim of this study was to investigate the toxicity; analgesic and anti-pyretic properties of standardized HA methanolic extract (HAMEOH) *in vivo*.

Methods: The acute toxicity study was performed on rats while adopting the OECD-420 Guidelines (fixed dose procedure). Assessment of analgesic activity was performed in rats with two analgesic models. One was acetic acid induced writhing response and the other formalin-induced paw licking. The anti-pyretic effect was tested by *Brewer's yeast* induced fever in rats.

Results: For the acute toxicity test, the higher dose administration of 2000 mg/kg bw. of *H.albus* did not produce any toxic signs or deaths in rats. There were no significant differences ($p>0.05$) in the body and organ weights between control and treated groups. The (LD50) of "*H.albus*" was higher than 2000 g/kg bw. In subacute toxicity study, no mortality and toxic signs were observed with the doses of 100 and 200 mg/kg bw. of extracts of for 28 consecutive days. These analgesic experimental results indicated that HAMEOH (100 mg/kg and 200 mg/kg) decreased the acetic acid-induced writhing responses and HAMEOH (100 mg/kg and 200 mg/kg) decreased the licking time in the second phase of the formalin test. Moreover, in the model of yeast induced elevation of the body temperature HAMEOH showed dose-dependent lowering of the body temperature up to 3h at both the doses these results obtained, were comparable to that of paracetamol.

Conclusion: The present findings indicate that the leaves of *Hyoscyamus albus* L. possess potent analgesic and antipyretic activity.

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

INTRODUCTION

Pain and inflammation are some of the most common manifestations of many diseases afflicting millions of people worldwide [1, 2]. Even though there are effective orthodox medicines used to alleviate these manifestations, traditional medicine practitioners in, mainly, developing countries have used herbal medicines to treat various ailments including pain and inflammation [3]. *Hyoscyamus albus* (HA) is an herbal medicine traditionally applied as a parasympholytic and nervous sedative. *Hyoscyamus albus* is grown on the Continent, particularly in France, and in the Indian subcontinent. It has petiolate stem leaves and the flowers have pale yellow, non-veined corollas.

They isolated the scopolamine and atropine and in addition to hyoscyne and the hyoscyamine, a new compound isolated from the leaves and the stems from *H. albus* were characterized like 2,3-dimethylnonacosane by spectral studies [4]. Also they announced the production of sesquiterpene-type phytoalexins with a vetispyradiene skeleton by *Hyoscyamus albus* hairy roots induced by methyl jasmonate (MeJA) [5,6]. This study was, therefore, intended to investigate the antinociceptive and antipyretic activities of methanolic extract from *H.albus*'s leaves in rats. The acute and subacute toxicity study of the plant were also carried out.

MATERIALS AND METHODS

Chemicals

Formaldehyde CH₂O (EDEN LABO), Acetic acid puriss glacial (Sigma-Aldrich), Indomethacin and acetylsalicylic acid (Sigma-Aldrich), were used in the present study.

Collection of plant material

The leaves of *H.albus* were collected from their natural habitat around Bouzina, Batna. This plant was identified by Dr. OUDJHIH, Laboratory of Botanic, Department of Agronomy, BATNA, ALGERIA. The leaves were dried under shade for 40 days at Room temperature, dried leaves parts were blended into fine powder and stored in the dark at a dry place.

Preparation of plant extract

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 Whatman filter paper. The solvents were then evaporated under reduced pressure (204 mbar) and controlled temperature (30 °C) using a vacuum rotary evaporator (Buchi Rotavapor).

Phytochemical screening

The phytochemical screening of HAMEOH was performed using standard method [7]. Phytochemical constituents such as phenolic compounds, terpenoids, saponins, alkaloids, steroids and tannins were qualitatively analyzed.

Animals

Wistar albino rats for either sex (140-170 g) procured for Research institute of both sexes were housed in separately in plastic cage at temperature of (23±2) °C and 50-55 % relative humidity, with a 12 light / dark cycle respectively before and during the experiment. Animals were allowed the access to standard pellet diet and water *ad libitum*.

Acute toxicity

The acute oral toxicity of methanolic extract from *H.albus* was evaluated in rats of either sex (150-170), as per the OECD guidelines 420 (OECD, 2001) where the limit dose of 2000 mg/kg body weight used [8]. Six female rats (140-160g body weight) were randomised into two groups viz., control and test groups. Control group received distilled water as vehicle at a dose volume of 10 ml/kg b. wt. whilst the test group received single oral dose of 2000 mg/kg b. wt. of HAMEOH (10ml/ kg b. wt. in distilled water) via gastric intubation. Observations were made and recorded at 30 min, 1, 2 and 4 hours and thereafter once a day for the next 14 days following vehicle or

HAMEOH administration for skin changes, morbidity, aggressivity, sensitivity of the sound and pain as well as respiratory movement. The LD50 was calculated using arithmetic method of Karbar.

Subacute toxicity

The plant extract at the dose of 100, 200 mg/kg body weight were administered orally to 3 groups of 6 rats (140-160g) (3 males + 3 females). Group I served as control and received distilled water as vehicle orally via gastric intubation at a dose volume of 10 ml/kg b. wt. Group II and III received HAMEOH at 100, and 200 mg/kg/day, p.o, respectively (10 ml/kg b. wt. in distilled water) to every 24 h for 28 days. The toxic manifestation such body weight, mortality, food and water intake was monitored. After 28 days all surviving animals were fasted overnight and anaesthetized with chloroform. The blood samples were collected for determining biochemical parameters. Animals were sacrificed after blood collection and the internal organs were removed and weighed to determine the relative organ weights and observed for gross lesions. The internal organs (liver, kidney, pancreas and heart) were preserved in 10% buffered formaldehyde solution for histological examination.

Biochemical estimations

Blood collected in heparin tubes and centrifuged at 3000 rpm for 10 min. The serum was analyzed for various parameters such as Aspartase amino Transferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Cholesterol, Triglyceride, Urea, Blood glucose, Creatinine, Bilirubin (Bil).

Histopathological study

Histopathological investigation of the organs was done according to the method described by Lamb [9]. The organ pieces (3 - 5 micro meters thick) were fixed in 10% formalin and washed in running water for 24 h. Samples were dehydrated in an autotechnicon and then cleared in toluene to remove absolute embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

Biological activities

Analgesic activity

a. Peripheral Analgesic Activity – Abdominal Constriction

Acetic acid induced writhing test was used for detecting peripheral analgesic activity using the method of Seighmund, E *et.al.*, 1957 [10]. The effect of different doses of the methanolic extract from of *H.albus*'s leaves on the number of writhes/stretching movement induced by 0.6% v/v of acetic acid (1ml/100g body weight) in rats. HAMEOH (100 and 200 mg/kg, p.o.) and reference substance (acetylsalicylic acid) were administered 30 minutes before the intraperitoneal administration of acetic acid. Control animals received 0.9% NaCl solution under the same experimental conditions. Number of writhes per animal was counted during a 15 min. series beginning 5 min after the injection of acetic acid. The number of writhing's and stretching's were recorded and permitted to express the percentage of protection using the rational formula. Percent protection = $(1 - V_c/V_t) \times 100$. Where:

Vt = Mean number of writhing in test animals

Vc = Mean number of writhing in control

b. Formalin Test

The method used in our study was similar to that described in the previous study [11]. Pain was induced by injecting 20 μ L of 5% formalin in distilled water in the subplantar of the right hind paw. HAMEOH (100 and 200 mg/kg, p.o.) was administered 60 min before formalin injection. Indomethacine (10 mg/kg, p.o.) was administered 30 min before formalin injection. The control group received the same volume of distilled water by oral administration. These rats were individually placed in a transparent cage. The time spent for licking and biting the injected paw, as the indicators of

pain, was recorded separately at 0–5min (first phase or neurogenic pain) and 20–30 min (second phase or inflammatory pain).

Antipyretic activity

The antipyretic activity of methanolic extract was evaluated using Brewer's yeast-induced pyrexia in rats [12]. Fever was induced in all rats by subcutaneous injection of 20 ml/kg body weight of 20% aqueous suspension of Brewer's yeast in normal saline. All test materials, vehicle control (distilled water), and reference drug paracetamol (150 mg/kg body weight) were administered orally 17 hours after the induction of fever. Rectal temperature of each rat recorded using thermal digital thermometer at 1, 2, 3, 4, 5 and 6 hr after the administration of the extract and reference drug.

Statistical analysis

The values were expressed as mean \pm 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

Phytochemical Screening

Phytochemical screening of HAMEOH demonstrated the presence of alkaloids, saponins, condensed tannins, and steroids. In addition, the presence of high content of total phenolic compounds in the HAMEOH, we demonstrated the presence of flavonoids (table 1).

Test of Toxicity

The results of this study are shown in Table 2. In this study, the oral administration of the methanolic extract of *H.albus* at all given doses (up to 2000 mg kg⁻¹) did not produce any visible sign of acute toxicity or instant death in rats tested during the period of observation. Regarding the subacute toxicity, no death was recorded during the treatment period either in the control or treated groups. The animals did not show any changes in general behavior or other physiological activities. The body and organ weights of rats, which received "HAMEOH" at 100, 200 mg/Kg (therapeutics doses of the drug) doses daily for 28 days, are given in Table 3. The results show that there were no significant differences (p>0.05) in the body and organ weights between control and treated animals. The biochemical profile of the treated and control groups are presented in Table 4. No statistically significant differences (p>0.05) were recorded in the most biochemical parameters analyzed after 28 days except for the urea and creatinine (p < 0.001) raised with groups treated with the (100 and 200 mg/Kg). Moreover, there was no effect on the levels of indicators of liver, kidney and pancreatic functions such as alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP) and Bilirubin. This result demonstrated that "*H.albus*" did not induce any damage to the liver, and was earlier confirmed by the histological assessment of these organs. But the renal histology demonstrated the presence a perivascular macrophage granuloma in groups treated with (100 and 200 mg/Kg) wich confirm the elevation of rate of urea and creatinine in biochemical analysis. Since examination of clinical signs plays major role in toxicological testing. Transaminases (AST and ALT) are good indicators of liver function and biomarkers to predict the possible toxicity of drugs [13] Therefore, ALT is more specific to the liver and is thus a better parameter for detecting liver injury [14]. Any elevation pertaining to these enzymes indicate their outflow into the blood stream due to damage in liver parenchymal cells. The Kidneys are highly susceptible to toxicants for two reasons; a high volume of blood flows through it and its ability to filter large amounts of toxins which can concentrate in the kidney tubules [15]. Again the elevation of blood urea and creatinine are also the good indicators for kidney disorders [16]. All the biochemical data were consistent with histological evaluation of the liver and the kidneys for 28 duration (Figure 1 and 2).

Table 1: Phytochemical constituents of methanolic extract from *H.albus*'s leaves

Sample	Phytochemical constituents	Result
HAmEoH	Alkaloid	+++
	Saponin	+++
	Flavonoid	++
	Tannins and polyphenolic compounds	++
	Terpenoids	+++
	Steroid	++

For saponins, +: 1-2 cm froth; ++: 2-3 cm froth; +++: >3 cm froth. For flavonoids, tannins, triterpenes, and steroids, +: weak colour; ++: mild colour; +++: strong colour. For alkaloids, +: negligible amount of precipitate; ++: weak precipitate; +++: strong precipitate.

Table 2: LD50 determination by arithmetic method of Karbar

Groups	Number of rats	No. of animals dead	Dose difference (a)	Mean mortality (b)	Probit (a×b)
Control group	6	0
<i>H.albus</i> 100 mg kg ⁻¹	6	0	100
<i>H.albus</i> 200 mg kg ⁻¹	6	0	100
<i>H.albus</i> 500 mg kg ⁻¹	6	0	400
<i>H.albus</i> 1000 mg kg ⁻¹	6	0	500
<i>H.albus</i> 2000 mg kg ⁻¹	6	0	1000

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

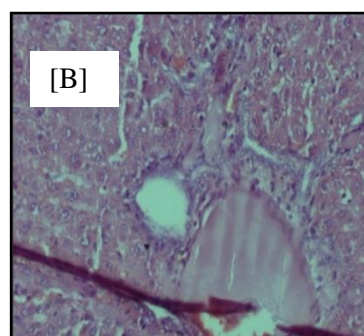
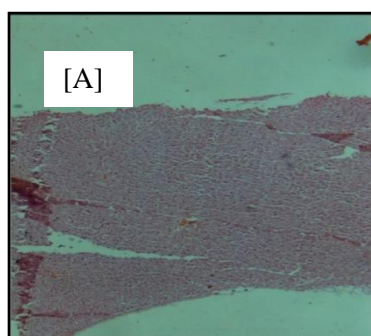
Control (2ml water/100g)	100 mg/Kg	200 mg/Kg
131.7 ± 1.52	132.3 ± 1.52	132.7 ± 2.51
175.0 ± 3.00	177.7 ± 2.88	175.7 ± 5.85
10.63 ± 0.57	10.67 ± 0.17	11.11 ± 0.63
1.20 ± 0.047	1.15 ± 0.055	1.073 ± 0.065
1.040 ± 0.079	1.14 ± 0.005	1.06 ± 0.065
0.82 ± 0.070	0.82 ± 0.026	0.85 ± 0.55

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

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

Parameters	Control group	HAmEoH treated 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
Bilirubin (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



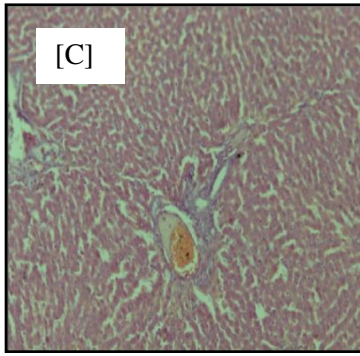


Fig.1 : Photomicrograph of Liver histology of treated and untreated albino rats with methanol extract of *H.albus's* leaves [A]control group, [B] methanolic extract treated rats [100 mg/kg body weight] : discrete (portal fibrosis + sinusoidal hemorrhage), [C] methanolic extract treated rats [200 mg/kg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

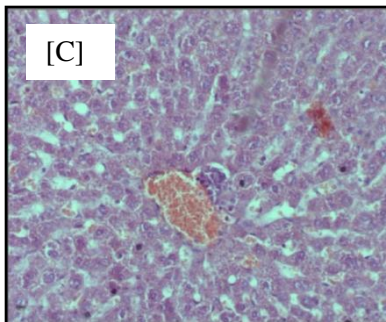
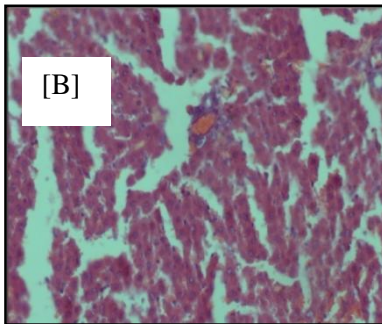
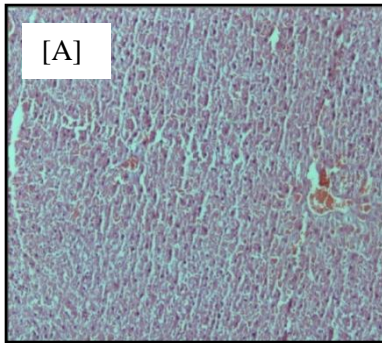


Fig. 2: Photomicrograph of Kidney histology of treated and untreated albino rats with methanol extract of *H.albus's* leaves [A]control group, [B] methanolic extract treated rats [100 mg/kg body weight] : perivascular macrophage granuloma, [C] methanolic extract treated rats [200 mg/kg body weight]: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

Biological activities

Analgesic activity

a. Peripheral Analgesic Activity – Abdominal Constriction

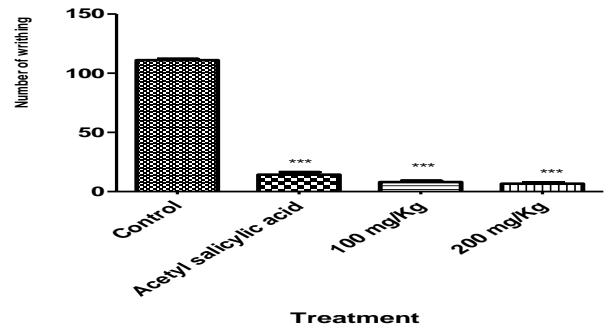


Fig. 3: Effects of methanolic extract of *H.albus's* leaves on acetic acid-induced pain in rats.

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

b. Formalin test

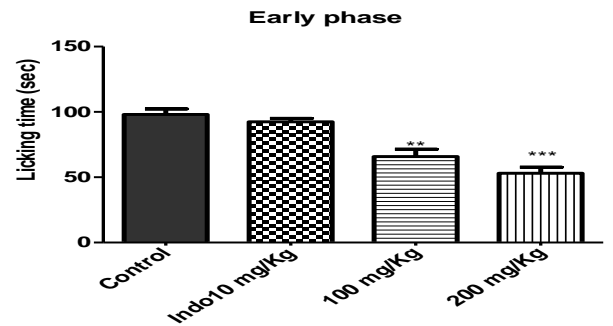


Fig.4: Analgesic effect of the HAMEOH and Indo on the early phase

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

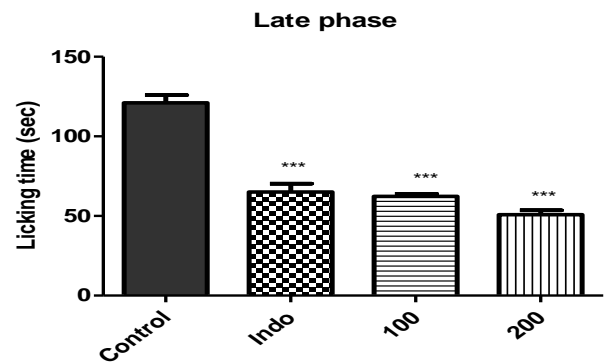


Fig. 5: Analgesic effect of the HAMEOH and Indo on the late phase

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

The oral administration of the methanolic extract of the leaves of *H.albus* in rat significantly ($P < 0.0001$) reduced in a dose-dependent manner the number of rat abdominal constrictions induced by acetic acid. The methanol extract at doses of 200 and 100 mg/kg showed a protective effect of 92.97 % and 94.00 %, respectively. The methanolic extract at 200 mg/kg exhibited significantly ($P \leq 0.05$) than the acetyl salicylic acid (87.09%) (Fig.3).

The formalin test is an applicable and reliable model of nociception. This method elucidates central and peripheral activities. The intraplantar injection of 20 μ L of formalin (5%) into the right hind paw generated a classical biphasic nociceptive response. As shown in Fig.4 and 5, HAMEOH (100 and 200 mg/kg) significantly and dose

dependently reduced the nociception in both the early and late phases. The methanolic extract significantly reduced the licking time at the first phase of observation by 32.85% and 45.91% at 100 and 200 mg/kg, respectively. Indomethacin did not show significant activity on this first phase. During the second phase of observation, the methanolic extract at the dose of 100 and 200 mg/kg showed a maximum protective effect of 48.55 % and 58.65 %, respectively while indomethacin significantly inhibited it by 46.28%. Diluted formaldehyde, injected subcutaneously into the rat upper lip, produces a nocifensive behavior (face rubbing) consisting in a biphasic response: a short-lasting response referred to as phase I, and a longer lasting phase, caused by inflammatory processes, called phase II [17].

Antipyretic activity

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

Treatment	Rectal temperature (°C) before and after 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 \pm SD. * $P \leq 0.05$, ** $P \leq 0.001$, *** $P \leq 0.0001$, when compared with the control values of corresponding hour.

Effect of methanolic extract of on yeast induced induced hyperpyrexia is shown in table 5. Subcutaneous injection of yeast suspension markedly elevated rectal temperature of rats after 17h of administration of yeast and the treatment with methanolic extract at 100 and 200 mg/Kg significantly decreased the rectal temperature in a dose-dependent manner. The result obtained from both the standard (paracetamol) and plant extract treated rats were compared with that of control and significant causes (** $P \leq 0.001$, *** $P \leq 0.0001$) reduction in the yeast induced elevated rectal temperature was observed from 3rd and 6th after treatment.

DISCUSSION

Flavonoids have been reported to exhibit antioxidant [18, 19], anti-inflammatory [20], and hepatoprotective [19] activities. Furthermore, condensed tannins have been suggested to possess free radical scavenging and antioxidant, anti-inflammatory, and hepatoprotective activities. Taking all these reports into consideration, it is plausible to suggest that the analgesic and antipyretic activities of HAMEOH involved, partly, synergistic action of alkaloids, flavonoids, condensed tannins, and saponins.

The acetic acid-induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity [21] because it is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods [22, 23]. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response [24]. The method has also been associated with prostanooids in general, for example, increased levels of PGE2 and PGF2 α in peritoneal fluids [25] as well as lipoxygenase products [26, 27]. These results strongly suggest that these extracts possess peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors or arachidonic acid pathways, involving cyclooxygenases and/or lipoxygenases. It has been suggested that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons to non steroidal anti-inflammatory drugs (NSAIDs) and opioids [28]. These neuronal fibers are sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and to narcotics and other centrally acting drugs [29, 30, 28]. Recently, it was found that the nociceptive activity of acetic acid may be due to the release of cytokines, such as TNF-C, interleukin-1N and interleukin-8, by resident peritoneal macrophages and mast cells [31, 28]. Pain sensation

in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [32] via cyclooxygenase (COX), and prostaglandin biosynthesis [33]. In other words, the acetic acid induced writhing has been associated with increased level of PGE2 and PGF2 α in peritoneal fluids as well as lipoxygenase products [25]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [34]. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [35]. Preliminary qualitative phytochemical screening reveals the presence of alkaloids, terpenoids, tannins, flavonoids & alkaloids. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins [35, 36]. There are also reports on the role of tannins in anti-nociceptive activity [37]. Besides alkaloids are well known for their ability to inhibit pain perception [38]. Aspirin (acetylsalicylic acid), the reference in the current study, offers relief from pain by suppressing the formation of pain substances in the peripheral tissues where prostaglandins and bradykinins are suggested to play an important role [38].

The biphasic component of formalin induced nociception reflects different underlying mechanisms; the first phase appears to be related to the direct chemical stimulation of nociceptive nerve endings [39], while the second phase depends on a combination of ongoing inputs from nociceptive afferents and, at least in part, of central sensitization [40]. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [41, 42]. The early phase is probably a direct result of stimulation of nociceptors in the paw and reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin, and prostaglandins [43] and at least to some degree, the sensitization of central nociceptive neurons [43]. Intraperitoneal administration of acetic acid or formalin mediates pain response through the release of inflammatory mediators, mainly prostacycline (PGI2) [42]. *H.albus* showed analgesic activity on both phases of the formalin test suggesting both direct analgesic effects on the nociceptor blockage and an inhibition of the synthesis and/or release of inflammatory

pain mediators such as PGs. Pyrexia or fever is caused as a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states; Normally the infected or damaged tissue initiates the enhanced formation of proinflammatory mediator's (cytokines like interleukin $1\beta, \alpha, \beta$ and TNF- α), which increase the synthesis of prostaglandin E₂ (PGE₂) near preoptic hypothalamus area and thereby triggering the hypothalamus to elevate the body temperature [44]. Yeast-induced pyrexia is called pathogenic fever and it's a etiology involves production of prostaglandins.

The effect of the drugs may be due to inhibition of prostaglandin synthesis [44]. In general non steroidal anti inflammatory (NAIDS) drugs produce their antipyretic action, through inhibition of prostaglandin synthesis within the hypothalamus [45]. Therefore it appears that antipyretic action of methanolic extract of *H.albus* may be related to the inhibition of prostaglandin synthesis in hypothalamus. Regulation of body temperature requires a delicate balance between the production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and drugs like paracetamol don't influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature [46].

CONCLUSION

In conclusion, the data obtained show that methanolic extract of *H. albus*'s leaves has both antinociceptive and antipyretic activities which may be produced by the plant inhibiting various chemical mediators including prostaglandins and bradykinin. The relatively high LD₅₀ value of 2000 mg/kg (p.o.) obtained for the plant shows that it may be safe in or non toxic to rat. The result obtained justifies the use of the plant species by traditional medicine practitioners in Africa for the treatment us parasympholytic. However, more studies are needed to further elucidate the mechanism of the antinociceptive and antipyretic actions of *H.albus*' leaves.

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