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Effects of ethanol extract of the resin exudate of *boswellia dalzielii* hutch on pain in mice

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ABSTRACT

This study aimed to determine the analgesic properties and the acute toxicity of Ethanol Extract of the Resin Exudate of *Boswellia dalzielii* (EERBD) in mice animal model.

We used the writhing or acetic acid abdominal constriction, tail-immersion, and hot plate tests to assess the analgesic effect of EERBD at three doses (100, 200, and 400 mg/kg). To study the acute toxicity of EERBD, 24 female mice were divided into four groups (n=6) and were orally treated with EERBD at the doses of 0, 2000, 4000, and 5000 mg/kg, as per OECD (Organization for Economic Co-operation and Development) guidelines No. 420.

In the acetic acid-induced writhing reflex model, the EERBD ministration decreased the mean total number of writhes at the two doses (100 and 400 mg/kg), which were found highly significant ($P < 0.001$) compared to control group. In the tail immersion model, the EERBD administration at the dose of 400 mg/kg significantly increased the pain reaction time ($P < 0.001$ as compared to control) at 30 min, but another tested sample had no significant latency. In the hot-plate model, the drug extract created significant ($P < 0.001$) increase in the latency period compared to the control group at oral doses of 100 and 400 mg/kg when compared to initial time and control group (4.5 ± 1.29 s) with protective effect from 4.25 ± 1.50 s after 30 min. Administration of EERBD at the dose of 200 mg/kg showed no significant analgesic activity based on writing, tail immersion, and hot-plate tests. The extract did not show toxicity signs or death at dose of less than 5000 mg/kg per oral.

The results suggest that EERBD contain bioactive substances with analgesics effects; hence, it might be a better alternative to conventional drug therapy for pain management.

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Introduction

Pain isdefined as an unpleasant, emotional, and sensory experience associated with potential tissue damage (1). It usually signals a danger, from which should be escaped or avoided. Thus, it has a protective function and represents an adaptive response to harmful or potentially damaging stimuli. The limited efficacy and safety of therapeutic medications available to relieve painful conditions are the main causes of the increased prevalence of chronic and neuropathic pain (2). Nowadays, opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are widely and increasingly used to relieve pain throughout the world (3). However, the long-term use of the currently available therapies to alleviate pain result in serious side effects and low efficacy, especially for chronic diseases (4). However, long-term use of medications like NSAIDs could cause addiction or provoke gastrointestinal lesions or liver and renal failures (3, 5). Thus, finding novel medications to control pain is a rewarding endower. Researchers have always been interested in finding natural remedies with lasting and conclusive curative effects to treat human diseases and disorders. Africa is endowed with abundant plant life that serves as not only important sources of nourishment but also useful medicinal properties.

Boswellia dalzielii is a tree plant widespread in many African countries, particularly in Ivory Coast, Cameroon, Northern Nigeria, Burkina Faso, Togo, Benin, and Ghana. Its English name is Frankincense tree while the French calls it Arbre à encens (6). This tree has small fragrant and aromatic white flowers (7, 8). It is a very tall tree (can grow up to 13 m) with papery stem bark that releases resin exudate upon incision. The traditional healers used *B. dalzielii* for the treatment of rheumatism, pain, gastrointestinal disorders (7), tuberculosis (9), gingivitis (10), skin disorders, and nervous system disorders (11). The leaves and stem bark extract of the tree are used in the treatment of arthritis and other inflammatory diseases (12). The aqueous stem bark of *B. dalzielii* possesses anti-inflammatory effect, which may be related to anticholinergic mechanism (13). The resin is harvested by making a shallow incision in the trunk of the tree and removing a narrow strip of bark. The released milky sap clots on contact with air and can be picked up by hand. The resin extracted from *B. dalzielii* has traditionally been used in Ayurvedic medicine as an anti-arthritic, astringent, expectorant, and antiseptic agent (14). In India, the traditional Ayurvedic medical system refers to the use of the gum extracted from *Boswellia dalzielii*, which is recommended for arthritic and inflammatory conditions, and pulmonary diseases

(13). This study aimed to evaluate the analgesic effects and acute toxicity of Ethanol Extract of the Resin Exudate of *Boswellia dalzielii* (EERBD) in mice animal model. Although numerous studies have reported the medicinal values of this plant, so many issues have remained for further in-depth research. So far, there is no published report on the acute toxicity and analgesic effect of the EERBD.

Materials and methods

Plant collection

The resin of *B. dalzielii* was collected from Mandaka village, in Mokolo County, Far North Region, Cameroon, on December 22- 23, 2017. The plant material was authenticated by Pr. PM Mapongmetsem, a Botanist in the Department of Biological Sciences, Faculty of Science, University of Ngaoundéré, Cameroon and was confirmed at the Cameroon National Herbarium Yaoundé, Cameroon by comparing with voucher specimen (Number: 20532/SRF-CAM) has been deposited.

Preparation of the plant extract

One hundred gram of the resin of *B. dalzielii* was cold macerated in 95% v/v of ethanol for 24 h with intermittent shaking. The liquid ethanol extract was filtrated with No. 1 Whatman filter paper. After the extraction, the extract was sieved and filtrated. The filtrate was concentrated under reduced pressure in the oven at 55 °C through rotary vacuum evaporator. The percentage yield (w/w) of the ethanol extract was 60%. The ethanol extract was dissolved in 1% DMSO (dimethyl sulfoxide) to acquire the required concentrations for the experiments.

Experimental Animals

In the present study, young mice of both sexes weighing between 25 and 30 g were used. All animals, 6-8 weeks old, were acclimatized to room temperature (22– 25 °C), with relative humidity (30 – 70%) under 12:12 h light/dark cycle. They were giving food and drinking water ad libitum. The animals were obtained from LANAVET, Garoua, Cameroon. One week before the experiment, the animals were placed in clean cages in small groups of 6 mice per cage. This work was carried out in accordance with the Animal Ethical Committee of the Ngaoundere Regional Delegation of livestock; Fisheries and animal Industries Authority, Cameroon. Ethic number 075/16/L/RA/ DREPIA.

Drugs and Chemicals

All chemicals and drugs used in this experiment, such as acetic acid, ethanol, and DMSO were provided from Sigma (Yaoundé, Cameroon). The paracetamol was obtained from a local pharmacy (Ngaoundéré, Cameroon).

Acetic acid-induced writhing in mice

Acetic acid was injected intraperitoneally into the mice to induce pain (15). For this test, 30 mice of both sexes (25 – 30 g) were randomly divided into five equal groups (n=6) as follows. Group I, which is the vehicle control, was treated with 0.9% saline solution 10 ml/kg per oral. Group II received paracetamol (100 mg/kg), and groups III, IV, and V respectively received EERBD at doses of 100, 200, and 400 mg/kg suspended in 1% (w/v) DMSO in saline solution per oral. Thirty minutes after the administration of the extract or the standard drug (paracetamol), each mouse was intraperitoneally injected with 0.1 mL of 1% acetic acid. After a 5-min lag period, the number of writhes for each mouse was counted for 20 min. The results were evaluated by calculating the mean number of writhes per group and analgesic effect was expressed as the percentage decrease of contortions by using the following formula (16): % Inhibition = ((Nc - Nt) / Nc) × 100, where Nc refers to the number of contortions of the vehicle control (normal saline), and Nt denotes the number of contortions of the EERBD groups or the positive control (paracetamol) group.

Tail immersion test

The animals were divided into 5 groups (n=6) of group I to group V, as previously mentioned. For doing this test, the distal 5-cm part of the mouse tail was immersed in a beaker containing the heated water (temperature maintained constant at 54 ± 1°C) to study the reflex of the animal tail flick before and after the administration of the EERBD. The cut-off time of immersion set was 10 s to avoid tail tissue damage. The reaction time was measured 30 min before the experiment and then 30 min, 60 min, 90 min, 120 min, and 180 min after the treatment with paracetamol or EERBD.

Hot-plate test

The hot-plate test was used to determine the analgesic effect of the EERBD. For this experiment, mice were initially screened by placing them in a glass beaker setting on the surface of a hot plate (thermostatically maintained at 55 ± 1 °C). The cut-off time for hot-plate latencies was set at 15 s to avoid paw tissue damage. The time between the placement of animal on the hot plate and activities such as licking fore paws, holding the feet, shaking or jumping off the hot plate was recorded as the response latency (in seconds). The increase in latency (reaction) time denotes analgesic activity. The testing of response latencies was measured before the experiment and then 30 min, 60 min, 90 min, and 120 min after oral administration of the paracetamol, EERBD, or vehicle.

Acute oral toxicity test

The acute oral toxicity test was performed to evaluate the acute toxic effects or changes in the normal

behavior of the experimental animals. It was also used to find out the lethal dose (LD_{50}) of EERBD. The acute toxicity of EERBD was carried out in mice following the Organization for Economic Co-operation and Development (OECD) guidelines 420 for oral acute toxicity testing (17). A total of 24 female albino mice weighed 20 – 25 g were used for this test. The mice were fasted for 6 h before the drug administration until one hour after that. They were randomly divided into 4 groups with 6 mice per group. Group I was treated with 10 ml/kg of physiological solution. The EERBD was administered at different concentrations of 2000, 4000, 5000 mg/kg to the other three groups. All doses of standard drug, EERBD, or vehicle, were administered orally. The mice were observed individually after dosing at least once every 30 minutes, during the first 4 h, and then with particular attention during the first 24 h to detect changes in their behavioral pattern and signs of toxicity. At the end of 14 days observation period, the number of deaths was counted to determine lethal dose 50 (LD_{50}).

Statistical analysis

In this study, the results were presented as mean ± SEM (standard error mean). The obtained data were analyzed using the 1-way ANOVA followed by Dunnett's post hoc test (as appropriate) in SPSS version 16. A P level of less than 0.05 was considered significant in all tests.

Results

Mouse writhing test

Table 1 presents the EERBD effect at doses of 100 mg/kg and 400 mg/kg on writhing. The results indicate a significant inhibition of the writhing as 6.6 ±7.98 and 9.6 ± 5.02, respectively ($P < 0.001$). The standard drug, paracetamol, also induced protection (26 ± 9.52) against the contractions induced by 1% acetic acid. Mice given the 200 mg/kg of EERBD showed no significant changes in the number of abdominal writhing (26.8 ± 5.58). The analgesic effect of EERBD (at doses of 100 and 400 mg/kg) was higher than paracetamol (100 mg/kg).

Table 1: Effect of ethanol extract of the resin exudates of *Boswellia dalzielii* on writhing test

Groups	Dose	Number of writhing	% inhibition
Control	10 ml/kg	35 ± 4.94	/
Paracetamol	100 mg/kg	26 ± 9.52^{ns}	25.71
EERBD	100 mg/kg	6.6 ±7.98***	81.14
	200 mg/kg	26.8 ± 5.58^{ns}	23
	400 mg/kg	9.6 ± 5.02***	72.57

Values are expressed as mean ± SEM (n=6 animals per group). Control= Normal saline. Paracetamol =control positive. EERBD = ethanol extract of the resin exudates of *Boswellia dalzielii.* *** ($P < 0.001$),

Tail immersion analgesic test

Table 2 presents the results of the tail immersion test of groups of EERBD at doses of 100, 200, and 400 mg/kg, paracetamol (100 mg/kg) and vehicle control (10 ml/kg). The highest reaction time in response to a nociceptive stimulus (7.8 ± 0.84 s) belonged to EERBD group (100 mg/kg) 60 minutes after its oral administration compared to the control group. Paracetamol, the reference drug, also exhibited strong antinociceptive effect ($P < 0.001$) like EERBD at 100 mg/kg dose ($P < 0.001$) after 60 minutes compared to the control group.

Hot-plate analgesic test

Table 3 presents the results of the hot-plate test in groups of EERBD (100, 200, and 400 mg/kg), paracetamol (100 mg/kg) and vehicle control (10 mL/kg). According to Table 3, a significant increase in the activity is seen in the standard (paracetamol) group through the entire experiment duration. The activity of the 100 mg/kg p. o. reduces considerably nociceptive effects of the extract at 200 mg/kg p. o. were highly significant in the 60th min. However, the activity reduced as the observation time increased ($P > 0.05$ at the 30th, 90th, 120th, and 180th min). The EERBD groups at doses of 200 and 400 mg/kg did not show significant changes in the latency time. Administration of paracetamol (100 mg/kg) increased the latency time to thermal stimuli after 30 min compared with the vehicle control group (used as negative control).

Acute toxicity test

According to the acute toxicity test, no sign of toxicity or any abnormal behavior was observed after oral administration of the drug extract at the doses of 2000, 4000, and 5000 mg/kg. No mortality was observed in treated mice during the 14 days of the observation period. However, the mice, which received 4000 and 5000 mg/kg of drug extract showed a short period of lethargy and modification of peeling during the first 4 hours (the first day) of the observation period. The EERBD LD_{50} is higher than 5000 mg/kg of body weight so in the category of substances with low toxicity. These results indicated high margins of safety of this extract.

Discussion

Pain is a response to an external or internal stimulus of the thermal, mechanical, or chemical source (18). There are several tests to evaluate pain perception in the animal models. The first one is the acetic acid writhing test. It is a useful test to evaluate drugs with analgesic effect at a peripheral level of nociception (19). The intraperitoneal injection of acetic acid produces an abdominal writhing response and causes the release of chemical mediators such as serotonin, histamine, bradykinin, cytokines, prostaglandins E (PGE), PGF2α, and lipoxygenases (20). These mediators sensitize

Table 2 Effect of ethanol extract of the resin exudates of *Boswellia dalzielii* on tail immersion test

		Reaction time (s), Mean ± SEM					
Groups	Doses	Immediaely after drug administration	30 min after drug administration	60 min after drug administration	90 min after drug administration	120 min after drug administration	180 min after drug administration
Control	10 ml/kg	4.8 ± 0.84^{ns}	4.8 ± 0.84 ^{ns}	4 ± 1 ^{ns}	5.2 ± 1.92 ^{ns}	6.2 ± 0.84 ^{ns}	5 ± 1.58 ^{ns}
Paracetamol	100 mg/kg	5.4 ± 1.14 ^{ns}	7.2 ± 0.84 ^{ns}	7.8 ± 0.84***	7.8 ± 0.84 ^{ns}	8.6 ± 0.55 ^{ns}	8 ± 0.71 ^{ns}
	100 mg/kg	4.6 ± 2.07 ^{ns}	7 ± 2.12 ^{ns}	8 ± 1.58***	9 ± 1.58 ^{ns}	8 ± 2.35 ^{ns}	6.8 ± 1.64^{ns}
EERBD	200 mg/kg	4.4 ± 0.55 ^{ns}	4.8 ± 0.84 ^{ns}	5.8 ± 1.48 ^{ns}	6.2 ± 1.92 ^{ns}	7.8 ± 1.30 ^{ns}	8.4 ± 0.55 ^{ns}
	400 mg/kg	9.8 ± 1.30***	5.8 ± 0.84 ^{ns}	4.6 ± 1.14 ^{ns}	6.8 ± 1.64 ^{ns}	7.4 ± 1.52 ^{ns}	6.6 ± 1.67 ^{ns}

Values are expressed as mean ± SEM (n=6 animals per group). The obtained data were found and analyzed by 1-way ANOVA, followed by Dunnett's post hoc test. *** = very highly significant at P < 0.001 as compared to control, control= Normal saline, paracetamol =control positive, EERBD = ethanol extract of the resin exudates of *Boswellia dalzielii.*

Table 3 Effect of ethanol extract of the resin exudates of *Boswellia dalzielii* on the hot-plate test

		Reaction time (s), Mean ± SEM				
Groups	Doses	**0** min after drug administration	**30** min after drug administration	**60** min after drug administration	**90** min after drug administration	**120** min after drug administration
Control, 0.9% NaCl	10 ml/kg	2.5 ± 0.58	4.5 ± 1.29	5.5 ± 0.58	5.25 ± 0.99	5.75 ± 0.96
Paracetamol	100 mg/kg	1.25 ± 0.5***	1.25 ± 0.96***	5.25 ± 4.03^{ns}	3.75 ± 1,71***	3.75 ± 2.22^{ns}
	100 mg/kg	3.5 ± 1.29***	2.25 ± 0.96***	8.25 ± 10.3***	3 ± 3.37^{ns}	4 ± 1.83*
EERBD	200 mg/kg	3.75 ± 1.75***	4.25 ± 1.50***	9.25 ± 1.26*	1.5 ± 0.58^{ns}	7.5 ± 7.33^{ns}
	400 mg/kg	2.5 ± 1.91***	3 ± 1.41***	9.75 ± 1.50*	1.75 ± 0.50^{ns}	6 ± 2.58*

The results of 1-way ANOVA test followed by Post hoc (Dunnett's test).
* = significant at (P < 0.05) as compared to control. *** = very highly significant at P < 0.001 as compared to control. Control= Normal saline, paracetamol =control positive, EERBD = ethanol extract of the resin exudates of *Boswellia dalzielii.*

cholinergic and histamine nociceptors at the peritoneal level in response to painful stimuli that result in later and diffuse pain (19). The administered EERBD reduced the number of abdominal writhing contractions significantly (P < 0.001). The EERBD can exhibit its analgesic activity by the inhibition property of some phytochemicals found in the resin of *B. dalzielii* extract. These chemicals are capable of blocking prostaglandins pathway. So, the inhibition of these enzymes (COX-1 and COX-2) by the active gradients in the resin extract might reduce the production of prostaglandins and consequently the alleviation of pain (21). In this study, the EERBD demonstrated significant analgesic effect at 100, 200, and 400 mg/kg doses based on the tail immersion analgesic model two hours after administration (Table 2). The analgesic effect of EERBD was consistent from the time of administration to 30 minutes after it at 30 mg/kg according to the tail immersion analgesic test. In this work, we have demonstrated that the EERBD exhibited pain inhibition. The hot-plate test is an excellent indicator of potential pharmacological drug effect. We also used it to investigate the analgesic effect of EERBD. The test results revealed that the EERBD groups showed a longer latency time than the negative control group in a dose-dependent manner. The hot-plat test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models customarily

used for studying central nociceptive activity. The ethanol extract of *B. dalzielii* at a dose of 100 mg/kg must have a central effect. The protection from nociception exhibited by the EERBD and paracetamol may be executed through centrally modulating mechanisms involving opiate, dopaminergic descending noradrenergic and serotonergic receptor systems or maybe through the peripherally inhibiting mechanism of the prostaglandins, leukotrienes, and other endogenous substances that are key components of causing pain. The ability of the EERBD to increase the latency time is due to its effect on the peripheral or central nervous system by raising the threshold for pain and altering physiological response to pain. The inactivity of the 200 mg and 400 mg observed in both extracts could be attributed to their uncharacteristic inactivity at the supra-spinal level which is suggested to be common for the paw licking (22).

Despite the widespread use of medicinal plants in developing countries, few scientific data are available about their toxicity and adverse effects (23). Based on the study of acute toxicity of the EERBD after 14 days of oral administration, it did not have any mortality on the treated mice. Therefore, the extract was non-toxic at the graded doses (2000, 4000, and 5000 mg/kg) according to Lorke's lethal dose determination (24). The study of acute oral toxicity of EERBD showed a lethal dose 50 (LD_{50}) higher than 5000 mg/kg. According to the World Health Organization (WHO) and the scale of Hodge and Sterner (23), LD_{50} greater than 5000 mg/kg is considered as not toxic. The LD_{50} value also justifies the traditional use of this extract without any observed poisonous effect.

Conclusion

In conclusion, the oral EERBD administration showed a lethal dose 50 (LD_{50}) higher than 5000 mg/kg. Besides, our study results indicate that the resin exudate of *Boswellia dalzielii* has analgesic properties. Due to the presence of various chemical agents in the resin exudates of *Boswellia dalzielii*, it has the above mentioned pharmacological effects which support and validate its indications mentioned in folk medicine.

Authors' Contributions

This work was carried out by the collaboration of all authors. Jeweldai Vedekoi conceived and designed the study, wrote the protocol, and critically reviewed the manuscript. Sokeng Dongmo Selestin and Kamtchouing Pierre analyzed the data, wrote the draft, and contributed to the writing of the article. Koubé Juliette confirmed the results and conclusions, made the critical revisions and approved the final version. All authors reviewed and approved the final manuscript.

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Conflicts of interests

Authors declare that this work does not present any conflict of interests.

References

1. Senhadji L. Epidural analgesia for painless delivery. PhD thesis. University Ahmed Ben Bella D'Oran, Algerian Democratic and Popular Republic, 2018.
2. Walker CIB, Trevisan G, Rossatoetal MF. Antinociceptive effect of *Mirabilis jalapa* on acute and chronic pain models in mice. J Ethnopharmaco 2013; 149: 685–93.
3. Katzung B, Master S, Trever A. Basic and Clinical Pharmacology, Mc Graw Hill, 11th éd. New York, NY, USA; 2009.
4. Ghani A. Medicinal Plant of Bangladesh with Chemical Constituents and Uses, 2nd éd. Asiatic Society of Bangladesh, Dhaka, Bangladesh; 2003.
5. Naidoo V, Swan GE. Diclofenac toxicity in Gyps vulture is associated with decreased uric acid excretion and not renal portal vasoconstriction. Comp Biochem Physiol C Toxicol Pharmacol 2009; 149: 269–74.
6. Marc T, Gerard W, Denis L. Classification des anti-inflammatoires in Guide pharmacologie. 4th éd., Etudiants et professionnels paramédicaux; 2001.
7. Emmanuel AO, Adediji JA, Ehimigbai ARO. Histological effects of aqueous extract of *Boswellia dalzielii* stem bark on the testes of adult Wistar rats. J Pharm Sci Innov 2015; 4: 280–3
8. Burkill HM. Useful plants of West Tropical Africa. United Kingdom: White Friars Press Ltd 1985; 1: 300-1.
9. Uzama D, Gbubele JD, Bwai MD, Kabir MG. Phytochemical, nutritional and antimicrobial screening of hexane, ethyl acetate and ethanolic extracts of *Boswellia dalzielii* Leaves and Bar. Am J Biosci Bioeng 2015; 3: 76-9.
10. Kubmarawa D, Akiniyi JA, Okorie DA. Ethnomedicinal survey of the traditional medicine of Lala people of Nigeria. Int. J Med Plants Altern Med 2013; 1:39–57.
11. Henley-Smith CJ, Botha FS, Lall N. The use of plants against oral pathogens. Formatex 2013; 30: 1375–84.
12. Zerbo P, Compaore M, Meda NTR. Lamien-Meda A, Kiendrebeogo M. Potential medicinal plants used for by traditional healers in western areas of Burkina Faso. World J Pharmacol Pharm Sci 2013; 2: 6706-19.
13. Nwinyi FC, Binda L, Ajoku GA, Aniagu SO, Enwerem NM, Orisadipe A, Kubmarawe D, Gamaniel KS. Evaluation of the aqueous extract of *Boswellia dalzielii* Stem barks for antimicrobial activities and gastro intestinal effects. Afr J Biotechnol 2004; 3: 284-8.
14. Ben-Yehoshua S, Borowitz C, Hanus LO. Frankincense, myrrh and balm of Gilead: Ancient spices of southern Arabia and Judea. Horticultural Reviews, 39 1st Ed. Willy-Blackwell publisher, Isreal, 2012, 76 p.
15. Koster R, Anderson M, De-Beer EJ. Acetic acid for analgesic screen. Fed proc 1959; 18: 412-7.
16. Chatter R, Tarhouni S, Kharrat R. Screening of the anti-inflammatory and analgesic effect of seaweed in the Mediterranean Sea. Arch Inst Pasteur Tunis 2011;88:19-28.
17. OECD. OECD Guidelines for Testing of Chemicals: Acute Oral Toxicity – Acute Toxic Class Method. Test No. 423, Adopted 22nd March 1996, and Revised Method Adopted 17th December 2001, OECD, Paris. http://www.oecd.org/fr.

18. Fan S, Ali NA, Basri DF. Evaluation of analgesic activity of the methanol extract from the galls of *Quercus infectoria* (Olivier) in rats. Evid Based Complement Alternat Med 2014: 1- 6.
19. Yougbare-Ziebrou MN, Lompo M, Ouedraogo N, Yaro B, Guissoun IP. Antioxidant, analgesic and anti-inflammatory activities of the leafy stems of Waltheria indica L. (Sterculiaceae). J App Pharm Sci 2016; 6: 124-9.
20. Berkal G, Bouchama S. Phytochemical study and biological activities of a medicinal plant: Euphorbia characias L. Master's degree. Mentouri Constantine Brothers University, Algerian Democratic and People's Republic 2016.
21. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evid based Complement Alternat Med 2006; 3: 433–9.
22. Hodge HC, Sterner JH. Determination of substance acute toxicity by LD_{50}. Am Ind Hyg Assoc 1943; 10: 93–6.
23. Bhattacharya A, Agrawal D, Sahu PK, Kumar S, Mishra SS, Patnaik S. Analgesic effect of ethanolic leaf extract of *Moringa oleifera* on albino mice. Indian J Pain 2014; 28: 89–94
24. Lorke DA. A new Approach to practical Acute Toxicity testing. Arch Toxicol 1983; 54: 275–87.