Plant Archives Vol. 20, Supplement 1, 2020 pp. 2808-2811 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETERMINATION OF THE ED50 AND ANTINOCICEPTIVE ACTIVITY OF MAHALEB CHERRY (*PRUNUS MAHALEB* L.) IN MICE

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Abstract

Herbs are the main part of traditional medical and dental practice. *P. mahaleb* L., a source of compounds useful for pharmaceutical industries. Aim of research to evaluate the antinociceptive effect of *P. mahaleb* L. Forty albino mice were used. *P. mahaleb* L. powder was given for animal orally at dose of (1, 5, 3, 12, 24) mg/kg. The antinociceptive effect was estimated by Hot-plate test. Median effective dose (ED_{50}) of *P. mahaleb* L., determined by using up & down method. Difference between groups were done by one way analysis of variance and the least significant difference test (LSD). *P. mahaleb L.* at (3, 12, 24) mg/kg produce analgesic effects and increased in threshold of pain between pre and post treatment following 10 minute in comparison with 1.5 mg/kg at (6±0.0) second and control at (7.0 ± 0.0) second group respectively. The analgesic effect of *P. mahaleb* L. after 20 minutes of administration significantly difference between pre and post treatment in dose (12, 24) mg/kg (10 ±1.15) (12 ±1.1) sec respectively in comparison with other lower doses (1.5, 3) mg/kg and control. *P. mahaleb* L. not produce a dose dependent analgesic effect in mice. The pain reaction time was increased after 2 min and the maximum analgesic effect after 10 min of oral *P. mahaleb* L. administration in dose (3, 12, 24) mg/kg and duration of analgesia last from 2 min to 20 min. *P. mahaleb* L. possess analgesic activity, it can be useful as a good natural source of compound in pharmaceutical industry.
***Keywords*:** *Prunus mahaleb* L., Analgesic, Albino Mice, ED_{50}.

Introduction

Some herbs have been accepted as an accessible and cost-effective therapeutic agents. These days, the general population is becoming gradually more aware with the over prescription and mishandling of conventional drugs (Yadav *et al.*, 2019). Medicinal herbs have a great indications in medicine and dentistry and pharmaceutical industries (Alma *et al.*, 2004; Özgül-Yücel, 2005; Alma *et al.*, 2007). The *P. mahaleb* L. tree is a member of Rosaceae family, subfamily prunoidae. It contains small amount of cyanogenic glycosides and also coumarin derivatives have been found (Aydin and Konak, 2002). The genus of Prunus from the family Rosaceae included more than 400 species (Third, 1976). In Arabia, mahaleb has been used for stimulation of sensory tissues and relief of pains arising from internal organ diseases (Moreno *et al.*, 1996; Aydin and Konak, 2002). In Arabia, seeds are employed as sedative and protection purposes (Al-Said and Hifnawy, 1986). The plant is used as a flavoring material and used in the food industries (Marcos and El-Dakhakhany, 1962). The *P. mahaleb* L. seeds contain herniarin, coumarin, dihydrocoumarin and amygdaline. Furthermore, it contains fatty oil and proteins (Favre-Bonvin *et al.*, 1968; Al-Said and Hifnawy, 1986; Mariod *et al.*, 2009). *P. mahaleb* L. is available as seed or its powder (Sezik and Basaran, 1985). It is ability to act as analgesic agent has not been investigated yet (Ferramosca *et al.*, 2019). This is the first time for in vivo study of analgesic effect of *P. mahaleb* intake. The aim of this study was to determine the ED_{50} with antinociceptive activity of Mahaleb Cherry (*Prunus mahaleb* L.) in mice.

Material and Methods

Plant material

P. mahaleb fruits powder was purchased from local market and dissolved in distilled water to be given for animal orally (p.o).

Animals

Forty healthy adult albino male mice (28 ± 5 g) were involved. They were kept under standard environmental conditions 23±2 ºC and normal laboratory environment. All animal experiments were carried out as per protocol approved by the Committee. Collage of Dentistry, University of Mosul, Iraq.

Experiment 1: Determination the *P. mahaleb* ED50 using Up and Down method

Exp. (1): Five mice were included for the median effective analgesic dose (ED_{50}) determination of *P. mahaleb* using up & down method.

Hot-plate test was used for every mouse previous to treatment to calculate threshold of pain and following 2 min of *P. mahaleb* intake by 3mg /Kg as a start dose depending on our polite study. The decrease and increase was in a constant dose of (1 mg/Kg). Median analgesic dose of *P. mahaleb* L. was evaluated on animals. Mice were positioned on a hot-plate at 55±1 ºC. Evaluation of the response latency (analgesic activity) was on the principle of either fore or hind paw lick or jump reaction, after positioned lying on the hot-plate. The ED_{50} of drug was determine according to Dixon Table (Dixon, 1980).

Exp. 2: Determination of pain reaction time and duration of analgesia:

Healthy albino mice (male and female) of 20-30 gm weight were selected for the experiment. They were randomly divided into five groups, 7 mice / group and received their doses as follow:

Group1: Control group normal saline 0.9%mg/kg I.P.
Group 2: *P. mahaleb* L. 1.5 mg/kg orally
Group 3: *P. mahaleb* L. 3 mg/kg. I.P.
Group 4: *P. mahaleb* L. 12 mg/kg. I.P.
Group 5: *P. mahaleb* L. 24 mg/kg. I.P.

The reaction time of pain and latency "licking paw or jumping" was confirmed before and (20, 40, 60, 80, 100 and 120 min.) after intake of *P. mahaleb* to evaluate the dose-response and time effective curves. The long duration of latency times and the values of the control was used for comparative assessment. The percentage of antinociceptive Maximal Possible Effect (MPE) was calculated from the formula: (Giusti *et al.*, 1997)

$$\%\ \text{MPE} = \frac{\text{Test latency - predrug latency}}{\text{Cut off time - predrug latency}} \times 100$$

MPE: Percentage of antinociception maximal possible effect.

Test latency: sec after drug treatment.

Predrug latency: Sec before drug treatment at zero time.

Cut off time: 30 second.

Statistics

Results were presented as mean ± SD, differences between test groups were analyzed by ANOVA) and then by LSD. It is significance at $p \leq 0.05$.

Results

Determination of ED_{50} of *P. mahaleb* L. for antinociceptive effect in mice:

Median analgesic dose of *P. mahaleb* L. were evaluated using Hot-plate technique : Mouse was placed on this device at 55±1^{0}C. The response time was estimated on the principle of jump reaction or y fore and/or hind paw lick, after hotplate attachment. The ED50 of *P. mahaleb* as analgesic were measured according to Dixon Table (1).

Determination the pain reaction time and duration of analgesia:

Maximum antinociceptive effect (MPE) in hot plate device test in mice was increased from zero in control group to (8.5, 26.9, 32, 11.5)% after 2 min respectively according to dose increased (1.5, 3, 12, 24) mg/kg (Figure 1). The (MPE) reach the highest value after 10 min and last for 20 min in doses (12, 24) mg/kg. (Table 2)

Table 1: Determination of ED_{50} of *P. mahaleb* L. for antinociceptive effect in mice.

Variable	Result
ED_{50}	3.295 mg\kg IP
Range of the doses used	2-4 mg\kg IP
Initial dose	3 mg\kg IP
Last dose	3 mg\kg IP
Number of mice used	5 (XOOXX)
Increase or decrease in the dose	1 mg\kg IP

Table 2 : Effect of *P. mahaleb* L. in different doses on antinociceptive maximum effect (MPE) % in hot plate test in mice

Doses mg\kg	2 min	10min	20 min	30 min	40 min
Control	0%	0%	0%	0%	0%
1.5	8.5%	0%	0%	0%	0%
3	26.9%	30.7%	11.5%	1.82%	1.28%
12	32%	28%	28%	1.3%	0%
24	11.5%	30.7%	30.7%	3.8%	0%

In the present study, the analgesic effect for *P. mahaleb* L. was evaluated through pain reaction induced by hot-plate. Oral administration of *P. mahaleb* L. at (3, 12, 24) mg/kg produce analgesic effects and increased in pain threshold between pre and post treatment after 10 minute in comparison with 1.5 mg/kg at (6±0.0) second and control at (7.0 ± 0.0) Sec group respectively (Table 3). The analgesic effect of *P. mahaleb* after 20 minutes of administration significant difference between pre and post treatment in dose (12, 24) mg/kg (10 ±1.15) (12 ±1.1) sec respectively in comparison with other lower doses (1.5, 3) mg/kg and control (Table 3).

Table 3 : The pain reaction time of *P. mahaleb* L. in mice

Time / Dose	2min	10 min	20 min	30 min	40 min
Control	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
1.5	8 ±0.5	6±0.0	6 ±0.0	6 ±0.0	6 ±0.0
3	11±1.15	12±1.1*	7 ±0.5	4.3 ±0.3	4 ±0.3
12	13±1.7 *a	12±1.1*	10 ±1.15*ab	5.3 ±0.3	4 ± 0.4
24	7 ±0.5 abc	12±1.1*	12 ±1.1*ab	5 ±1.7	4 ±0.0

* significant (control) at p≤0.05;
a: significant (mahaleb) at 1.5 mg/kg at p≤0.05.
b: significant (mahaleb) at 3 mg/kg at p≤0.05.
c: significant (mahaleb) at 12 mg/kg at p≤0.05.

In present study, systemic oral administration of tested doses of *P. mahaleb* L. not result in a dose dependent analgesic effect in mice experienced test of hot-plate. The pain reaction time was increased after 2 min and the maximum analgesic effect after 10 min of oral *P. mahaleb* L. administration in dose (3, 12, 24) mg/kg and duration of analgesia last from 2 min to 20 min (Figure 1)

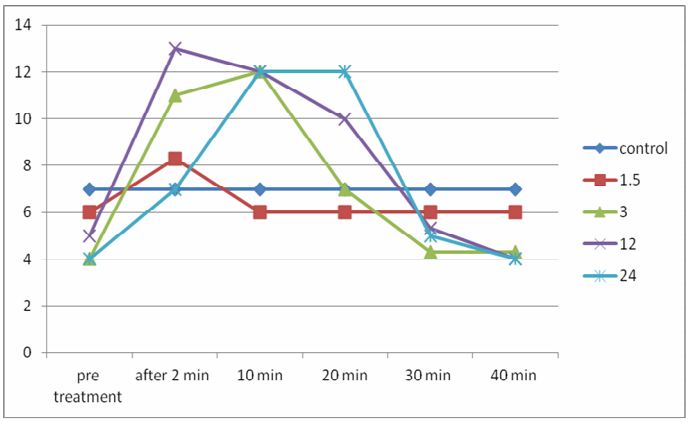

Fig. 1 : Duration of analgesic activity of *P. mahaleb* L. (1.5, 3, 12 and 24)

Discussion

Pain can be resulted by many factors which need medical and dental treatment including pain killers, herbal medicines are one of these. They are part of many traditional theraputic practice (Abolhassanzadeh *et al.*, 2016). Several strategies have been realized to found a new treatments for pain management, including synthesis and improvement of drugs that target pain resolution mechanisms endogenously and they simultaneously adjust multiple pathophysiological mechanisms that trigger pain (Moreno *et al.*, 1996; Aydin and Konak, 2002). Drugs that act on oxidative stress are one of them. In rodent models of peripheral pain, reactive oxygen species are elevated in dorsal root ganglia neurons and in glia and immune cells (Marcos and El-Dakhakhany, 1962; Moreno *et al.*, 1996). Reactive oxygen species enhance hyper excitability of dorsal root ganglia neurons through several mechanisms, including mitochondrial bioenergetics disruption, which cause energy production and ion homeostasis impairment and leads to degeneration of nociceptors. These reactive species also indirectly induce neuronal hyperexcitability by over expression of neuroinflammatory mediators (Al-Said and Hifnawy, 1986; Moreno *et al.*, 1996; Mariod *et al.*, 2009). Therefore, restoring redox balance has the ability to resolve various pain mechanisms. Many attempts have been done to pharmacologically control oxidative stress in chronic pain conditions; to date, none are in clinical use. Supplementation of antioxidants to control pain has some difficulties due to unfavorable pharmacokinetics and pharmacodynamics, and also because multiple antioxidants are required to return normal homeostasis by collaboratively catabolizing reactive oxygen species (Al-Said and Hifnawy, 1986; Moreno *et al.*, 1996). For this reason, studies have been focused on the transcription nuclear factor erythroid 2-related factor 2 (NFE2L2; Nrf2). Li *et al.* (2019) Plants are good sources of antioxidant which have a role against the action triggered by ROS. Resistance to biotic stress, antioxidant capacity, and anti-inflammatory effects and of *P. mahaleb* were recently confirmed by cell-based assay in vitro studies (Gerardi *et al.*, 2015; Ferramosca *et al.*, 2019; Gerardi *et al.*, 2019). Both free radicals and antioxidants have damaging effects on the viability of organisms. They can result in multiple diseases knowledge about the activity of antioxidants against free radicals in the nervous system throughout pain is deficient. The application of antioxidants like *Prunus mahaleb* L. increases ability of antioxidation reaction and improve the protection against the pain (Rokyta *et al.*, 2003; Bermúdez-Soto and Tomás-Barberán, 2004; Ou *et al.*, 2012). In this study *Prunus mahaleb* L. showed moderate analgesic effect in experimental animal which could be due to moderate antioxidant potential and anti inflammatory activities of this plant (Ou *et al.*, 2012). In all animal models of acute mechanical and neuropathic pain, like the animal model of the present study, the hold back stress is present in most times. The stress reactions provoked by pain so, it is very hard to distinguish the pain from stress contributions in painful status (Tezcan *et al.*, 2003; Li *et al.*, 2019). A study carried out to evaluate the antinociceptive effect of herbal drug (silymarin) and some medicinal plants like curcumin, *Ginkgo biloba*, essential oil, and wheat germ, it was found that it has significant antinociceptive properties which is probably mediated via anti inflammatory effect and antioxidant effect. Sahib (2011); Niazi *et al.* (2019) From other point of view, analgesic effect produced by *P. mahaleb* L. intake result, at least, by the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (NFE2L2; Nrf2). Pathways which regulate the antinociceptive effect by suppressing the inflammatory response through regulating the expression and phosphorylation of nuclear factor (NF)-κB, a known inflammation associated sensor and by attenuation of several underlying pronociceptive mechanisms (Ferramosca *et al.*, 2019; Lan *et al.*, 2019; Li *et al.*, 2019). Studies intended at improved considerate of Nrf2- mediated effects obtained from *Prunus mahaleb* L. are going in progress. Its combination with analgesics could normalize the oxidative stress suggesting that the antioxidants during pain management can be used to reduce the analgesics required dose avoiding the harm of reactive oxygen species on pain. Antioxidants, when properly used are able to decrease the sensation of pain (Rokyta *et al.*, 2003).

Conclusions

P. mahaleb L. possess analgesic activity. It can be employed as natural compound available for pharmaceutical industry. Though, exact substances responsible for the detected effects need to be recognized, isolated then compared with that of the basic extract and further experiment on structure clarification and recognition of them are needed.

Acknowledgment

The authors are very appreciative to the University of Mosul, College of Dentistry for their help to develop and improve the quality of this work.

Conflict of interest

All authors state that they have no known competing financial attention or personal relations that could influence the work of this manuscript.

Source of funding

Ministry of Higher Education, University of Mosul, College of Dentistry, Department of Basic Science.

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