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Analgesic effects of *Arum maculatum* plant extract in rats compared to other routine analgesics

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Due to low side effects and costs as well as proper adaptation to body's physiology, there is a global trend in using herbal medications. To investigate the analgesic effects of *Arum maculatum* plant extract in rats via an experimental study, 60 healthy male Wistar rats were divided into 6 equal groups including 3 plant extract groups, a negative control with normal saline and 2 positive control groups with morphine and diclofenac Na injections. Morphine, diclofenac or different dosages of extract were injected subcutaneously into the left sole of rats via insulin syringes. Two hundred microliters of 1% formalin were then subcutaneously injected into the left paw of each rat for pain induction. The duration of the left paw licking was measured just after formalin injection (phase I) and 20 min after the injection (phase II). Finally, the mean duration of paw licks was compared between treatment and control groups. The shortest duration of licks was found in the morphine injected group (30.6 ±1.9 and 37.2±1.1 seconds at phase I and II, respectively). Compared to diclofenac injection, plant extract of 500 and 750 mg/kg reduced the licks duration significantly during both phases ($p<0.0001$). The lick durations, after the injection of 500 and 750 mg/kg extracts, were almost the same as that in the morphine injected group during phase I, but took longer during phase II. Rats in the 750 mg/kg extract injected group, compared to those in the 500 mg/kg extract injected group, showed a significantly shorter lick duration during phase II ($p<0.0001$), but not phase I ($p=0.708$). Following further investigations in human, *A. maculatum* plant extract can be introduced as a natural and better analgesic substitution for diclofenac Na which has the same effect as morphine during phase I.

Key words: *Arum maculatum*, morphine, diclofenac Na, formalin, Iran.

INTRODUCTION

There is a global trend for the use of plant based medications rather than those with chemical bases. Annual expenditures on complementary and alternative medicine are high in the United State and other countries

(Davis et al., 2012). About 4.2 billion dollars were expended for plant based drugs in the USA in 2001 which shows the importance and trend of using these kinds of medications in this country (Marcus and Grollman, 2002). Different kinds of plants are used for different purposes in various parts of the world. Many studies have been performed on medicinal characteristics of different plants globally and have reported an appetence among people to use herbal based drugs (Joao, 2005; Rios and Recio, 2005; Hua-Bin et al., 2008; Abebe, 2002; Gurib-Fakim, 2006; Estomba et al., 2006). The current study aimed to investigate the analgesic effects of *Arum maculatum* in rats and compared its effect to the current medications used for this purpose.

The plant *A. maculatum* is a common woodland plant species of the Araceae family which has been applied for medical purposes traditionally in some parts of the world. It is widespread across temperate Northern Europe and other parts of the world and is known by an abundance of common names including wild arum, lords and ladies, devils and angels, cows and bulls, cuckoo-pint, Adam and Eve, bobbins, naked boys, starch-root and wake robin (Wikipedia, 2014). *A. maculatum* is also one of the most common causes of accidental plant poisoning based on attendance at hospital A and E departments. All parts of the plant can produce allergic reactions in many people and the plant should be handled with care. The root of the cuckoo pint, when roasted well, is edible and it is utilized like Salop (a working class drink popular before the introduction of tea or coffee). If prepared incorrectly, it can be highly toxic so should be prepared with due diligence and caution (Wikipedia, 2014).

Pain which is measured as an outcome of this study is a protective mechanism of body which is created during tissue damages and causes a body reaction against pain stimulant. Three different stimulants including mechanical, thermal and chemical can cause pain. Fast pain is due to mechanical and thermal stimulants; however, slow pain is due to all three pain stimulants (Gayton, 2007).

METHODOLOGY

Extract preparation

The samples of *A. maculatum* plant were collected from hillside of Zagross Mountains in Ilam province, West of Iran and were confirmed by the Pharmacy Faculty of Mashhad and the Agricultural Faculty of Koln in Germany. Areal parts of the collected plants were cleaned and dried at room temperature and then powdered. A total of 125 g of the powdered plant was then added to 1000 ml boiled water and after 15 min, the boiled mixture was filtered twice and dried, using Buchner filter cone and filtering paper, resulting in a dense extract (72%). The filtered solution was kept at the -20°C and using physiologic saline solution, the different dosages of the extract were prepared.

Animals

Sixty healthy male rats (weights 200 to 250 g) received from Pasteur Institution of Iran were divided into 6 equal groups. Animals were kept at the same luminosity conditions and at 20 to 22°C and were freely accessed to food and water. Rats in all 6 groups, including 3 extract injected groups (250, 500, and 750 mg/kg), a negative control group injected with normal saline (10 mg/kg) and 2 positive control groups injected with morphine (10 mg/kg) and diclofenac Na (10 mg/kg). All rats were subcutaneously injected at the sole part of their left feet. Morphine and diclofenac ampoules tested in this study were produced by Taulid Daru Company in Iran. According to the rats' weights, the proper dosages of plant extracts, normal saline, morphine or diclofenac were injected via insulin syringes. In order to induce pain, 200 µl formalin (1%) was applied subcutaneously at previous injection sites (left sole) 30 min after drug injections. The durations of the left paw licks (as a marker of pain) were measured just after formalin injection (phase I of pain) and 20 min later (phase II of pain). Finally, the mean durations of licks were compared between the treated and control groups.

Data were analysed using SPSS version 13.0, and the mean and standard deviation of the time consumed for licking among different groups were compared by independent T-test or ANOVA accordingly. A p-value less than 0.05 was considered as significant. This research was conducted in accordance with the Principles of Laboratory Animal Care (NIH Publication, 1985) and was approved prospectively by Ethics Committee of Ilam University of Medical Sciences.

RESULTS

Rats in the N/S group showed the longest lick durations at both phases I and II (59.9 ±3.9 and 120.7±2.4 s, respectively) and the shortest duration was seen in the morphine injected group (30.6 ±1.9 and 37.2±1.1 s at the phase I and II, respectively). This means that N/S created pain in rats and due to lack of analgesic injection in this group the longest duration of licking was obtained. Groups injected with plant extract of 500 and 750 mg/kg, had a significantly lower mean duration of licks at both phases I and II ($p<0.0001$) compared to the group injected with diclofenac. The lick durations in the groups injected with plant extract of 500 and 750 mg/kg were almost the same as those obtained in the morphine injected group in phase I, but had a discrepancy and longer duration in phase II. The group injected with 750 mg/kg of extract showed a significantly shorter lick duration in phase II, but not in phase I ($p=0.708$), compared to the extract injected group of 500 mg/kg ($p<0.0001$) (Tables 1 and 2). Comparisons between the treated and control groups have been shown in Tables 1 and 2 and Figure 2, indicating a significant analgesic effect of *A. maculatum* plant in this study.

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Table 1. Comparison between analgesic effects of different dosages of *A. maculatum*plant extract and other analgesics and their difference significance in phase I.

Compared extract dosage (mg/kg)	Comparison of variables	Mean licking (s)	P-value	Significant
250	Extract vs. normal saline	47.9 vs. 59.9	0.0001	Yes
	Extract vs. morphine	47.9 vs. 30.6	0.0001	Yes
	Extract vs. diclofenac	47.9 vs. 37.8	0.0001	Yes
	Extract vs. extract 500	47.9 vs.30.9	0.0001	Yes
	Extract vs. extract 750	47.9 vs.31.0	0.0001	Yes
500	Extract vs. normal saline	30.9 vs. 59.9	0.0001	Yes
	Extract vs. morphine	30.9 vs. 30.6	0.692	No
	Extract vs. diclofenac	30.9 vs. 37.8	0.0001	Yes
	Extract vs. extract 750	30.9 vs. 31.0	0.708	No
750	Extract vs. normal saline	31.03 vs. 59.9	0.0001	Yes
	Extract vs. morphine	31.03 vs. 30.6	0.596	No
	Extract vs. diclofenac	31.03 vs. 37.8	0.0001	Yes

Table 2. Comparison between analgesic effects of different dosages of *A. maculatum*plant extract and other analgesics and their difference significance in phase II.

Compared extract dosage (mg/kg)	Comparison of variables	Mean licking (s)	P-value	Significant
250	Extract vs. normal saline	81.9 vs. 120.7	0.0001	Yes
	Extract vs. morphine	81.9 vs. 37.2	0.0001	Yes
	Extract vs. diclofenac	81.9 vs. 79.5	0.01	Yes
	Extract vs. extract 500	81.9 vs. 78.9	0.0001	Yes
	Extract vs. extract 750	81.9 vs. 53.3	0.0001	Yes
500	Extract vs. normal saline	78.9 vs. 120.7	0.0001	Yes
	Extract vs. morphine	78.9 vs. 37.2	0.0001	Yes
	Extract vs. diclofenac	78.9 vs. 79.5	0.0001	Yes
	Extract vs. extract 750	78.9 vs. 53.3	0.0001	Yes
750	Extract vs. normal saline	53.3 vs. 120.7	0.0001	Yes
	Extract vs. morphine	53.3 vs. 37.2	0.0001	Yes
	Extract vs. diclofenac	53.3 vs. 79.5	0.0001	Yes

DISCUSSION

Analgesics with chemical bases show a lot of complications and there are global attempts to find a natural base component to substitute these kinds of medications (Ferda et al., 2009; You et al., 2006; Joao, 2005; Rios and Recio, 2005; Hua-Bin et al., 2008; Abebe, 2002; Gurib-Fakim, 2006;Estomba et al., 2006; Asadollahi et al., 2010). Due to the traditional use of *A. maculatum* as a topical analgesic medication in some areas, different dosages of this plant were prepared in this study and by an experimental method were investigated among different groups of rats. The results were then compared with some routine pain relievers. Formalin, used as a pain inducer in this study, creates two premature (phase I) and dilatory responses (phase II). The premature response is related to the activation of type C fibres and dilatory response is related to the inflammatory reactions of peripheral tissues as well as operational variations of spinal cord (Adeyemi et al., 2004; Avallone et al., 2000; Olaleye et al., 2000). In the current study, all dosages of *A. maculatum* plant extract showed a better effect compared to normal saline in both phases of pain.

A. maculatum plant extract at the dosage of 250 mg/kg showed a lower effect than diclofenac at both phases of pain; however, increasing the dosage of this extract to 500 mg/kg, produced a significantly better analgesic effect than diclofenac in phase I, but with the same effect in the phase II. On the other side, increasing its dosage to 750 mg/kg, resulted in a significantly better painless

Figure 1. Natural picture of *A. maculatum* plant.

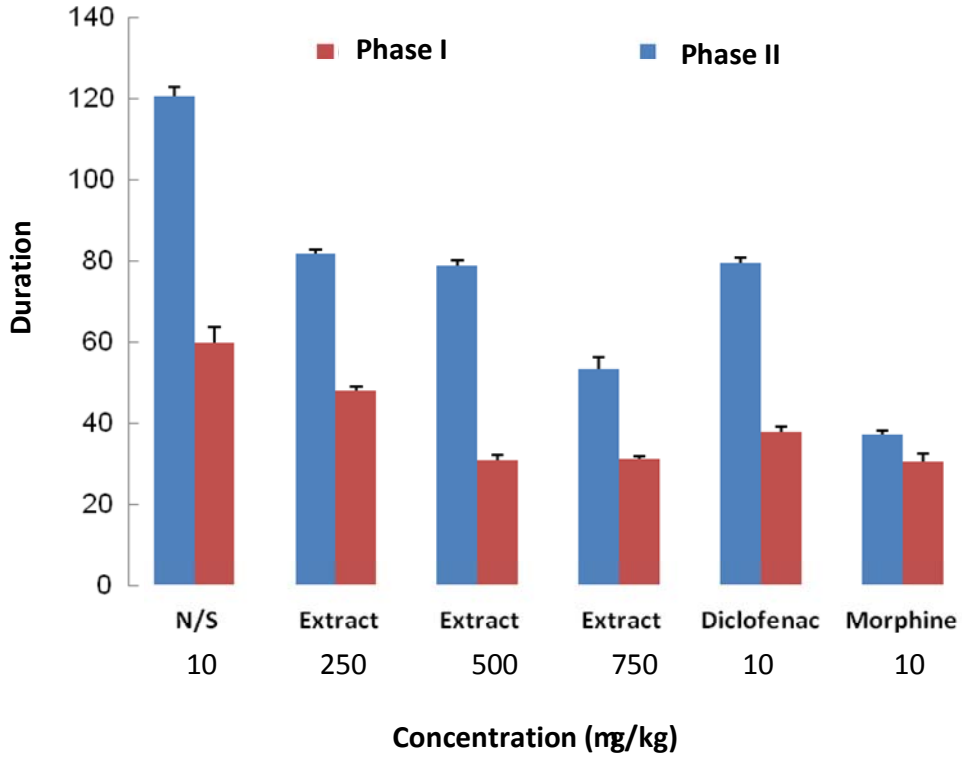

Figure 2. Mean licks duration of left sole by rats in different groups at phase I and II of pain. N/S: Normal saline, Bars = standard deviations.

effect of the plant extract compared to diclofenac in both phases I and II ($p<0.0001$). The analgesic effects of the *A. maculatum* extract, seems to be due to a cholinergic mechanism (Olaleye et al., 2000; Paulino et al., 2003) which is in accordance with the findings of the other reports reports which is considered a central analgesic effect for this plant (Ferda et al., 2009). The analgesic effect of the *A. maculatum* extract at the dosages of 500 and 750 mg/kg was better than that in diclofenac and almost similar to that in morphine at phase I. However, at phase

II, the analgesic effects of the plant extract was higher than diclofenac and lower than morphine.

No similar reports were found in the literature to be compared with the analgesic effects of *A. maculatum* extract, and therefore, the results of this study were compared to the analgesic reports for other plants. A study performed in Ghazvin (Iran) in 2001, compared the analgesic effects of *Matricaria camomile* plant with aspirin (300 mg/kg) and morphine (2.5 mg/kg). The results showed that *M. camomile* extract, at the dosage of 200 mg/kg, had a significantly better effect than aspirin and a relatively similar effect as morphine (Haidari et al., 2001). Since the dosage used for morphine at the mentioned study was 4 times lower than that used in the present study (2.5 mg/kg vs. 10 mg/kg), it seem that the analgesic effects of the *A. maculatum* extract is better than that of the *M. camomile* plant. Another study from Nigeria in 2009 compared the analgesic effects of Mimosaceace plant extract with 10 mg/kg of morphine in rats and reported that this plant had a painless effect, but with a much lower efficiency than morphine (Okunrobo et al., 2009). It can be concluded that, due to the higher efficiency of the *A. maculatum* extract than NSAIDS and a relatively similar effect as morphine, it has a stronger analgesic effect than Mimosaceace plant extract. Mohajjel et al. (2008) in Tabriz investigated the analgesic effects of *Erica arborea* plant extract in rats and compared their results to the analgesic effects of 10 mg/kg morphine. A 10 mg/kg dosage of the plant extract showed a significant analgesic effect, with a much lower painless efficiency compared to morphine. Therefore, a better or at least, a similar analgesic effect can be considered for *A. maculatum* plant extract compared to *E. arborea* plant extract. Adeyemi et al. (2004) in another study, investigated the analgesic effects of *Acanthus montanus* and compared their results to the effects of morphine (10 mg/kg) and reported a moderate analgesic effect, at the dosages of 100 to 400 mg/kg for *A. montanus* plant extract, which however, was less efficient than morphine. Since the morphine dosages used in Adeyemi et al. (2004) study was the same as that in this present study, their results were similar.

Conclusion

A. maculatum plant extract showed a better analgesic effect than diclofenac Na at both phases of pain and had the same effect as morphine at phase I. By further investigations, this natural analgesic medication could be introduced as a good substitution for diclofenac as a pain reliever in human.

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Abbreviations

AM, *Arum maculatum*; **Na,** Sodium; **N/S,** normal saline.

Conflict of interest

Authors declare no conflict of interest.

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