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RESEARCH ARTICLE

Acute Toxicity and Analgesic Activity of the Aerial Parts of *Ajuga iva* (L.) Schreb. Grow in East of Algeria

Nadia ZEGHAD[*], Ejaz AHMED[**], Aicha MADI[***], Sihem HALMI[****], Abdelmalik BELKHIRI[*****]

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SUMMARY

Ajuga iva (L.) Schreb.is a medicinal plant belongs to the Lamiaceae family. In view of its medicinal importance, the present research was focused on the determination of the acute toxicity and the analgesic properties of the aerial part hydroalcoholic extract of Ajuga iva L. by in vivo approach in rats using acetic acid, hot plate and tail immersion methods, the extract was used at the doses of 1.0, 2.0 and 3.0 g/kg while acetylsalicylic acid was used as a standard reference drug (0.1 mg/kg). In the acetic acid-induced model, the plant extracts and the reference drug significantly (p<0.0001) reduced the abdominal writhing in rats when compared to the control group, by increasing the percentage inhibition of writhing in a dose dependent manner. In the hot plate and tail immersion models, the extract like the acetyl salicylic acid showed high analgesic activity in a dose dependent manner and significantly (p<0.0001) increasing the pain reaction time. These results suggest significant analgesic potential of Ajuga iva (L.) Schreb. which may act through both peripheral and central mechanisms.

Key Words: *Ajuga iva (L.) Schreb., Acute toxicity, analgesic activity, acetic acid, hot plate, tail immersion.*

Cezayir'in doğusunda yetişen Ajuga iva (L.) Schreb Toprak Üstü Kısımlarının Akut Toksisitesi ve Analjezik Aktivitesi

ÖZ

Ajuga iva (L.) Schreb., Lamiaceae familyasına ait tıbbi bir bitkidir. Tıbbi önemi göz önüne alındığında, mevcut araştırma, asetik asit, sıcak plaka ve kuyruk daldırma yöntemleri kullanılarak sıçanlara in vivo yaklaşımla, Ajuga iva (L.) Schrebtoprak üstükısmı hidroalkolik ekstresinin analjezik özelliklerinin ve akut toksisitenin belirlenmesi üzerine odaklanmıştır. Ekstre, 1.0, 2.0 ve 3.0 g / kg dozlarında, asetilsalisilik asit ise standart bir referans ilaç (0.1 mg / kg) olarak kullanıldı. Asetik aside bağlı modelde, bitki ekstreleri ve referans ilacı önemli ölçüde (p<0.0001) sıçanlarda abdominal kıvrımı, kontrol grubuna kıyasla, writhing oranının doza bağlı bir şekilde arttırarak azaltmıştır. Sıcak plaka ve kuyruk daldırma modellerinde, asetil salisilik asit gibi ekstre, doza bağımlı bir şekilde yüksek analjezik aktivite gösterdi ve ağrı reaksiyon süresini önemli ölçüde arttırdı (p <0.0001). Bu sonuçlar Ajuga iva (L.) Schreb.'in hem çevresel hem de merkezi mekanizmalar yoluyla etki edebilen önemli analjezik potansiyelini ortaya koymaktadır.

Anahtar Kelimeler: *Ajuga iva (L.) Schreb., Akut toksisite, analjezik aktivite, asetik asit, sıcak plaka, kuyruk daldırma.*

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[*] ORCID: 0000-0003-4619-7358, Laboratory of pharmacology and toxicology, Faculty of Natural Sciences and Life, University Constantine 1- Algeria
[**] ORCID: 0000-0003-0432-8831, Department of Botany, Faculty of Sciences, PMAS-Arid Agriculture University Rawalpindi, Pakistan
[***] ORCID: 0000-0003-4374-6534, Laboratory of pharmacology and toxicology, Faculty of Natural Sciences and Life, University Constantine 1- Algeria
[****] ORCID: 0000-0001-6010-6040, Laboratory of pharmacology and toxicology, Faculty of Natural Sciences and Life, University Constantine 1- Algeria
[*****] ORCID: 0000-0003-0429-2795, Laboratory of pharmacognosy, Department of pharmacy, University Constantine 3- Algeria

[*] Corresponding Author; Nadia ZEGHAD
Tel: +213 698 979 736
E-mail: zeghadnadia@umc.edu.dz

INTRODUCTION

Ajuga iva (L.) Schreb. locally named "Chendgoura", belongs to the Lamiaceae family. It grows in period from spring to late summer (between May and June). It is widely distributed in the Mediterranean region: Southern Europe and Northern Africa, from Morocco to Egypt. The aerial part of the plant is used as an infusion or decoction in Algerian traditional medicine to treat different diseases like diabetes (Ziyyat et al., 1997) and infection fungal diseases (Mouheb et al., 2018). Most of the recent investigations of this medicinal plant have been related to their health-beneficial properties. The traditional use of the *Ajuga iva* was also supported by the isolation and the identification of several potential bioactive components. The pharmacological activity of this herb is mainly associated to the flavonoids and polyphenols contents in the aerial parts including fruits (Diafat et al., 2016). Several biological studies have confirmed the pharmacological properties of *Ajuga iva* ; these include hypoglycemic, anti-inflammatory, analgesic, anti-arthritis, antipyretic, hepatoprotective, antibacterial, antifungal, antioxidant, cardiotonic, and antimalarial properties (Khanavi et al., 2014).

Therefore, little is known about potential toxicity of this medicinal plant, the aim of this study was to evaluate in rats, acute toxicity and analgesic effects of oral administration of hydroalcoholic extract of the aerial parts of *Ajuga iva* (L.) Schreb.

MATERIAL AND METHODS

Plant material

Aerial parts of *Ajuga iva* (L.) Schreb.were collected from "Ain Smara-Constantine", eastern of Algeria, in March 2016. Plant materials (aerial parts) were dried under laboratory conditions. Medicinal plant was identified by Professor Laouar Hocine (University of Setif). A voucher specimen AJ016-AS was deposited in the herbarium of the Department of Plant Biology and Ecology, Faculty of Natural Sciences and Life, University Constantine 1, Algeria.

Phytochemical analysis

Phytochemical analysis of hydroalcoholic extract of *Ajuga iva* implicated qualitative determination using standard reactions of the following biological active groups (Kokate, 1997; Harborne, 1998): phenolic compounds, flavonoids, tannins, alkaloids and saponins.

Extraction process

The extraction process was carried out according to previous work (Babero et al.,2008; Ma et al., 2009). Dried aerial parts (25g) was finely ground using an electrical blander and immediately extracted by maceration in methanol / water (70/30) assisted by ultrasound for 30 minutes at room temperature. The extraction was repeated twice under identical conditions. The filtered extracts were combined and evaporated in a rotary evaporator under vacuum at low temperature (<40°C) to afford crude extracts which were subjected immediately to lyophilization. Freeze dried plant material (21.41mg/100g dried plant material) were kept under low temperature (-25°C) until required for further experiments.

Experimental animals

Young albino male Wistar rats, weighing between 218-236 g and aged (4-5) weeks were used for the studies. The animals were kept in standard polypropylene cages at room temperature (24 ± 2°C) in a 12 hrs light/dark cycle. They were allowed free access to standard pellet diet and water *ad libitum,* and were allowed to acclimatize to the laboratory conditions for seven days before the beginning of the experiment. The study was carried out following the guidelines of the principals of Laboratory Animal Care "Guide for the Care and Use of Laboratory Animals" (Protocol no:2016-AA10). The Committee of the National Center for Biotechnology Research (Constantine-Algeria) agreement has been obtained before the experiments.

Acute toxicity

After depriving animals from food overnight, they were weighed and randomly distributed into 5 groups of six animals each (one control group and four treated groups). Rats from the four treated groups received serial diluted doses (0.5-2.5-5.0-10.0g/kg) as previously reported (El Hilaly et al., 2004; Diafat et al., 2016). Samples were dissolved in distilled water and animals were fed by oral gavages using a specially designed mice needle. Animals' observationhas been done in the half hour, then periodically during the first 24 hours and once daily over 14 days after the

start of the experiment. Death or changes in general behavior and other physiological activities were noted (Shah Ayub et al., 1997; Bürger et al., 2005). Rats of all groups were weighted on days 7th and 14th. At the end of the experiment, animal were scarified and their internal organs including heart, liver, kidneys, lungs, spleen were examined and visually checked for any abnormality (Thanabhorn et al., 2006). No further investigation was carried out because it was been finding no alteration or changes in the appearance of examined organs.

Procedure for testing analgesic activity

Analgesic activity was measured by three different experiments: chemical induced writhing, hot-plate and tail-immersion assays as previously reported (Uma Shankar et al., 2010). The peripheral analgesic effect of the extracts was evaluated by chemical induced writhing test (Koster et al., 1959; Taber et al., 1969; Singh et al., 1996), while the involvement of central mechanisms was studied by using the hot-plate and tail-immersion tests. These latter assays are known to activate supraspinal and spinal nociceptive pathways (Wani et al., 2012), respectively. Tests were conducted after 24 hours of food depriving, healthy animals were then weighed and randomly distributed into groups of six animals each ($n = 6$).

Acetic acid-induced writhing test

The method described initially by Collier *et al* (1968), was conducted as follows; the writhing was elicited by an intraperitoneal (*i.p*) injection of 1 % acetic acid aqueous solution. Animals (6 per groups) were pretreated with hydroalcoholic extract of *Ajuga iva* (1.0, 2.0 and 3.0 g/kg, *per os*, single dose) reconstituted in distilled water, and acetylsalicylic acid (standard drug, 0.1 g/kg, *per os*). Then they were allowed to adapt for 60 min before the intraperitoneal (*i.p*) injection of acetic acid aqueous solution. The number of writhes was counted for each rat during 20 minutes. The Index of Pain Inhibition (IPI) was expressed by the present formula ratio (1):

$$IPI = \frac{Nc - Nt}{Nc} x100 \qquad (1)$$

Where: ***N*c** represents the number of writhes observed for control group, and ***N*t** : number of writhes in tested groups (*Ajuga iva* (L.) Schreb.extract, acetylsalicylic acid).

Central analgesic activity

Central analgesic activity was monitored using two standard mechanical methods, the hot plate and tail immersion tests.

Hot plate test

Hot plate test was performed according to the method described before (Taber et al., 1969) and adapted as follows. Five groups of mice (6 mice per group) were used. They received, one hour before testing *Ajuga iva* hydroalcoholic extract at different concentrations (1.0, 2.0 and 3.0 g/kg, single dose, *per os*), distilled water (control), and acetylsalicylic acid as anti-inflammatory drug (0.1g/kg, *per os*). Animals were placed on a heated surface of a hot plate maintained at 55.0 ±0.5°C. The pain threshold is considered to be reached when the animals licked their hind paws or jumped out (Naveed et al., 2012).

Tail immersion test

Tail immersion test was performed according to the method described before and adapted as follows. Five groups of mice (6 mice per group) were used. They received, one hour before testing, *Ajuga iva* hydroalcoholic extract at different concentrations (1.0, 2.0 and 3.0 g/kg, single dose, *per os*), distilled water (control), and acetylsalicylic acid as anti-inflammatory drug (0.1g/kg, *per os*). The lower portion of the animal tail was immersed gently in a hot water bath maintained at 55.0 ±0.5°C. Within a few seconds the mice reacts by withdrawing the tail. The reaction time it takes to animal to withdraw its tail is recorded (using a chronometer), after administration of treatments (Hoque et al., 2011).

Statistical analysis

The results of pharmacological testing were expressed as mean ±SD and analyzed by the test of *Tukey* (*HSD)* to determine the level of significance. A value of $P<0.05$ was considered to be significantly. The results obtained were compared to the control group. Statistical analyses were performed using *XL Stat* version.

RESULTS

Phytochemical analysis

Preliminary phytochemical screening of hydroalcoholic extract of *Ajuga iva L.* revealed the presence of various bioactive components among which flavonoids, phenolic compounds and tannins. The results of phytochemical tests has been summarized in (Table

1).

Table 1. Phytochemical analysis of hydroalcoholic extract of *Ajuga iva* (L.) Schreb.

	Flavonoids	Phenolic compounds	Tannins	Saponins	Alkaloids
Extract of *Ajuga iva L.*	+++	+++	+++	-	-

Noticeable presence (+++), moderate presence (++), traces (+), absence (-)

Acute toxicity

In acute toxicity studies, hydroalcoholic extract of *Ajuga iva* showed zero mortality within 24 h at all tested dose levels. There were no visible signs of acute toxicity up to 10.0 g/kg within 14 days observation. According to some references, substance that presents LD_{50} higher than 5.0 g/kg administered by oral route can be considered practically non toxic (Collier et al., 1968). In the absence of reagents and adequate equipments for the microscopic analysis of the organs, the study was concentrated only on the macroscopic aspect. The macroscopic examination performed later on the main organs (heart, liver, kidneys, lungs, spleen) also revealed that there was no abnormality.

Analgesic activity

Peripheral analgesic activity

The hydroalcoholic extract of *Ajuga iva* used orally at different doses (1.0, 2.0 and 3.0 g/kg body weight) demonstrated significantly analgesic activity ($p<0.0001$) in a reverse dose dependent manner (Table 2) by reducing the number of abdominal writhing; 62.87%, 80.51% and 94.97% respectively as compared to control group. However, the percentage inhibition of pain by 0.1 g/kg body weight of acetyl salicylic acid (reference drug) was found to be 78.34 % compared to the control. The analgesic effect of *Ajuga iva* extract showed no significant difference at the dose of 2.0 g/kg compared to the group treated by the drug reference.

Table 2. Antinociceptive effect of *Ajuga iva* (L.) Schreb. extract and acetylsalicylic acid on acetic acid-induced pain in rats.

Groups	Dose (g/kg)	Number of writhings* (IPI%)
Control (distilled water)	-	95.50 ± 5.32$^{\blacksquare}$ (-)
Ajuga iva L.	1.0	35.50 ± 3.08$^{\#\blacksquare}$ (62.87%)
	2.0	18.67 ± 1.88$^{\#}$ (80.51%)
	3.0	4.83 ± 2.58$^{\#\blacksquare}$ (94.97%)
Acetylsalicylic acid	0.1	21.50± 4.46 $^{\#}$ (78.34%)

* Values are expressed by mean ± SD (*Tukey HSD-test, n =6*); $^{\#}P<0.0001$:vs. control group; $^{\blacksquare}P<0.0001$ vs. acetylsalicylic acid (standard drug) treated group; IPI: Index of Pain Inhibition (%).

Central analgesic activity

Hot plate test

Results of hot plate test are presented in Table 3 for the hydroalcoholic extract of *Ajuga iva* (L.) Schreb.. The extract of aerial part was found to exhibit a dose dependent increase in reaction time when compared to the control ($p<0.0001$) at all doses and to standard drug ($p<0.001$).

Tail immersion test

The antinociceptive activity by oral administration of the hydroalcoholic extract of aerial part of *Ajuga iva* taken at different doses, showed dose dependent characteristic (Table 3). All doses increased significantly ($p<0.0001$) the reaction time compared to the control group.

Table 3. Central analgesic activity of *Ajuga iva* (L.) Schreb. extract, measured by hot plate and tail immersion tests

Compound and Plant extract	Dose (g/kg)	Reaction Time (RT) (seconds) *	
		Hot plate test	Tail immersion test
Control (distilled water)	-	1.79 ± 0.20■■	1.98 ± 0.31■■
Ajuga iva L.	1.0	3.77 ± 0.39#■■	3.99 ± 0.18#
	2.0	6.65 ± 0.44#■	6.35 ± 0.44#■
	3.0	14.62 ± 0.74#■■	10.13 ± 1.14#■■
Acetylsalicylic acid	0.1	5.43 ± 0.43#	4.72±0.86#

* RT in second expressed as mean ± SD, *Tukey (HSD)-testn=6*; # *P < 0.0001*: compared to control group;

■*P < 0.001*,■■ *P < 0.0001*: compared to acetylsalicylic acid treated group.

DISCUSSION

Three different analgesic testing methods were employed in the present study aimed to identify possible peripheral and central effects of the hydroalcoholic extract of aerial part of *Ajuga iva* (L.) Schreb.

The hydroalcoholic extract of *Ajuga iva* at all doses used (1.0, 2.0 and 3.0 g/kg) as well as standard drug (acetyl salicylic acid) (0.1 g/kg) demonstrated a prolongation of the hot plate and tail immersion latency time than the control group in a dose related manner. Therefore, by considering several reportsand our current results, the antinociceptive activity of the extract of *Ajuga iva* , is likely to be mediated centrally.

The hydroalcoholic extract of *Ajuga iva* showed also significant analgesic effect at all dose levels compared to the reference drug acetyl salicylic acid against acetic acid induced pain in rats. Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting in release of free arachidonic acid from tissue phospholipid via cyclooxygenase and prostaglandin biosynthesis (Banibrata et al., 2015). The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Banibrata et al., 2015). Results of the present study show that the hydroalcoholic extract of *Ajuga iva* produced significant analgesic effect, which may be due to the inhibition of the synthesis of the arachidonic acid metabolite.

Phytochemical analysis of the hydroalcoholic extract of *Ajuga iva* revealed the presence of tannins, phenolic compounds and flavonoids. It is well established that polyphenols such as tannins have anti-nociceptive activity (Arvind et al., 2011). It is also reported that flavonoids such as rutin, quercetin and luteolin produced significant antinociceptive activity (Küpeli et al., 2007).

CONCLUSION

The study demonstrated that the hydroalcoholic extract of aerial part of *Ajuga iva* exhibited significant analgesic activity, which may be mediated via peripheral and central mechanisms in acetic acid, hot plate and tail immersion models. The potency of the present extract can be improved by the purification of crude extract or by the isolation of pure constituents responsible for the activity from them which needs further studies in advanced level.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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