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Research Article

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Screening of analgesic and anti-inflammatory activities for two Libyan medicinal plants: *Helianthemum lippii* and *Launaea residifolia*

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ABSTRACT

Natural products are often a source for bioactive compounds which have great potential for developing novel therapeutic agents. In this study, two Libyan medicinal plants Helianthemum lippii (H. lippii) and Launaea residifolia (L. residifolia) were collected from El-Jabel El- Garbi (Gharian) in the Spring season (2010). They were extracted successively by using microwave technique with three different solvents of different polarities. The analgesic activity of these plant extracts was evaluated using the hot-plate method and the anti-inflammatory activity was evaluated using Carrageenen-induced paw edema method. The methanol and chloroform extracts exhibited significant analgesic activity at the doses tested while the petroleum ether extracts of both plants did not show any significant effect. In addition, the anti-inflammatory activities of various extracts showed a significant percentage inhibition of paw edema for ***H. lippii*** *extracts in methanol and chloroform but not in petroleum ether. Moreover, the results exhibit different percentage inhibitions of paw edema for L. residifolia extracts in methanol, chloroform and petroleum ether. The analgesic and anti-inflammatory effects produced by the extracts may be attributed individually or collectively to the flavonoids and tannins. H. lippii and L. residifolia can be introduced as new plant sources for analgesics and anti inflammatory agents. The methanolic and chloroform extracts of both plants showed a significant analgesic activity due to an increase in the reaction time* ***(p<0.05)*** *in comparison to controls, where codeine was used as standard analgesic drug. The petroleum ether extracts did not show any activity as analgesic. The anti-inflammatory activity was also evaluated using the Carrageenan-induced paw edema method, the methanolic extract of H. lippii* ***(53.42%)*** *exhibit activity comparable to that of aspirin standard anti-inflammatory drug* ***(62.28%)*** *with no significant difference, while the petroleum ether extract of L. residifolia* ***(31.65%)*** *exhibited a moderate anti-inflammatory activity* ***(p<0.01)*** *in comparison with aspirin as a standard. The chloroform extract of H. lippii, the methanol and chloroform extracts of L. residifolia exhibited a weak inhibitory effect on paw edema volume with percentage inhibition of* ***(23.60%, p<0.05)****,* ***(16.45%, p>0.05)*** *and* ***(14.56%, p>0.05)*** *respectively compared to control.*

Key words: *Helianthemum lippii, Launaea residifolia*, analgesic activity, anti-inflammatory activity.

INTRODUCTION

Natural products from animals or plants provide a source for bioactive compounds and have the potential for developing some novel therapeutic agents. There has been an ever growing interest of drugs originating from plants which have been found to form an important class for disease control. Inflammation is a pathophysiological reaction

that occurs in tissues when they are exposed to injury and this reaction leads to accumulation of plasmatic fluid and blood cells in injured tissues. At the same time these processes help the body in the protection against infection, burns, toxic chemicals, allergens or other noxious stimuli. These complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1,2]. According to International Association for the Study of Pain (IASP) pain has been defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage"[3]. Most of the synthetic analgesics and anti-inflammatory drugs that are currently in use cause potential toxic effects, for example the risk of gastrointestinal bleeding is significantly associated with use of non-steroidal anti-inflammatory drugs (NSAIDs) and respiratory depression and possibly dependence related to opioids [4,5]. Drugs currently used for management of pain or inflammation exhibit toxic side effects upon chronic use. Consequently, attempts have been made to study promising plant ingredients which may lead to develop novel or safer drugs [5-6]. The present study of *Helianthemum lippii* (*H. lippii*) and *Launaea residifolia* (*L. residifolia*) was carried out to evaluate and study some of their contents against pain and inflammation.

EXPERIMENTAL SECTION

Plant materials

Whole aerial parts of the plants were collected from the area of El-Jabel El- Garbi (Gharian) in Libya during the Spring season (2010) and sent to the herbarium of the Botany department Faculty of Sciences, Tripoli University, Tripoli, Libya for plant identification and authentication. The plants were dried in shade and grind into coarse powder.

Preparation of plant extracts

Plant ingredients were extracted using a microwave closed extraction system (milestone start E 2450 MHz, Italy) the microwave power adjusted to 500W, with three different solvents with different polarities. Five grams of each plant powder were extracted for 10 minutes using petroleum ether, or 15 min chloroform or 20 min methanol. The six crude extracts were then dried and stored at -20°C.

Phytochemical screening

The phytochemical screening of *H. lippii* and *L. residifolia* was performed according to standard literature methods in which the extracts were exposed to different reagents to identify the primary metabolites, like carbohydrates (Molisch reagent test), Proteins (Biuret test) and the secondary metabolites such as alkaloids (Mayer's test), flavonoids , terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones(Borntrager's test) [11, 12].

Animals

Swiss-albino mice obtained from the animal house of National Medical Research Center (NMRC), Zawia, Libya, aged 4-5 weeks, average weight 20 - 40 g were used for the experiment. The animals were kept in our animal facility at 22 °C ± 1°C under 12 h light/ dark cycle and were fed with standard laboratory chow and water. The study was conducted in accordance with the nationally accepted guidelines for laboratory animal use and care which was approved by Animal Ethical Committee (NMRC35/2009).

Analgesic activity

Hot-plate method was used in the current study [13,14], where forty eight fasted male albino mice with freely access to water were randomly selected and divided into seven groups (6 mice in each). All groups were treated intraperitoneally, and each group received a particular treatment *i.e*. control (0.2 ml normal saline), positive control (Codeine 5mg/kg) and test samples groups received (500 mg/kg of the plants extracts). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 ^{0}C. A cut off period of 30 seconds was observed to avoid damage to the paw. Reaction time is the interval between dropping the animals onto the surface of hot plate until they licked their fore or hind paws, or jumped. This time was recorded at zero time and 60 and 120 min after extracts administration.

Anti-inflammatory activity

Carrageenan-induced mice paw edema model was used in this study [15], where forty eight fasted male albino mice with freely access to water were used and divided into four groups (6 in each). All groups were treated intraperitoneally. The first group was subjected to 0.5 ml normal saline (negative control), the second group was treated with standard Aspirin 100 mg/kg (positive control), while the test groups were treated with (500 mg/kg) of extracts. The acute inflammation was induced by the sub-plantar administration of 0.1 ml of 1% carrageenan in the right paw, and the paw volume was measured at 0' and 3' hrs after carrageenan administration using a

plethysmometer (Ugo Basile-Italy). The anti-inflammatory effects of plant extracts under investigation were calculated by the following equation [16]:-

Anti-inflammatory activity (%) = (1-D/C) × 100

Where D represents the percentage difference in paw volume after drugs administration, C represents the percentage difference of volume in the control groups.

Statistical Analysis

Values for analgesic and anti-inflammatory activities were expressed as "mean increase in latency after drug administration ± SEM" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ± SEM". The significance of difference between means was determined by one-way analysis of variance (ANOVA) and values of $p < 0.05$ and $p < 0.01$were considered as significant and highly significant respectively.

RESULTS

Phytochemical screening

The phytochemical screening of *H. lippii* and *L. residifolia* revealed that, the main active constituents of *H. lippii* were polyphenols includes; flavonoids, tannins, glycosides, simple phenolics, free reducing sugars and saponines, while free anthraquinones, steroids, terpenoids and alkaloids were absent. The main active constituents of *L. residifolia* were polyphenols includes; flavonoids, tannins, glycosides simple phenolics, free reducing sugars and saponines, steroids and terpenoids, while free anthraquinones and alkaloids were absent.

Analgesic activity

The results in table 1 demonstrated that the methanol and chloroform extracts exhibited a significant analgesic activity at the doses tested while the petroleum ether extract did not show any significant effect. The analgesic activities are comparable with the reference analgesic agent (codeine) used in the present study with significant increase in the reaction time **($p < 0.05$)** for the two plants under investigation in comparison with the control group.

Table 1: Effect of *H. lippii* and *L. residifolia* extracts on response times in the hot-plate test

Treatment groups		Doses (mg/kg)	Mean Reaction Time (in sec)		
			0 Min.	60 Min.	120 Min.
Control		-----	4.98 ±0.92	5.63 ± 2.07	5.33 ± 1.18
H.lippii	methanol	500	4.46± 0.65	12.03±3.04*	17.86±3.27*
	chloroform	500	4.70± 0.64	16.46±3.00*	11.73±1.99*
	petroleum ether	500	5.85 ±0.53	6.26±1.00	6.53±0.65
L. residifolia	methanol	500	5.46±0.47	10.30±0.45*	15.96±1.28*
	chloroform	500	4.06±0.27	10.46±3.11*	15.07±2.23*
	petroleum ether	500	5.30±0.37	5. 20±2.35	6.80±2.34
Codeine		5	4.85±1.16	7.25±0.04*	7.02±0.015*

*Results are means ±S.E.M. and data are evaluated by using one-way analysis of variance *p <0.05 n=6.*

Table 2: The anti-inflammatory activities of *H.lippii, L. residifolia* extracts on Carrageenan-induced paw edema

Treatment		Dose mg/kg	Paw edema volume(µl) (% Inhibition)
Control		-------	98.75±3.54 (0.0%)
H.lippii	methanol	500	46.00±2.30**(53.42%)
	chloroform	500	71.80±9.05*(23.60%)
	petroleum ether	500	106.16±7.57 (0.0%)
L. residifolia	methanol	500	82.50±2.50**(16.45%)
	chloroform	500	84.37±2.13*(14.56%)
	petroleum ether	500	67.50±4.78**#(31.65%)
Aspirin		100	37.25±3.30**(62.28%)

*Results are means ±S.E.M. and data are evaluated by using one-way analysis of variance *p <0.05, **p<0.01 vs Control, # p < 0.01 vs standard aspirin, n = 6/group.*

Anti-inflammatory activity

The anti-inflammatory activities of various extracts of *H. lippii* and *L. residifolia* were assessed by carrageenan induced paw edema method. Table 2 shows the different percentage inhibition of paw edema for ***H. lippii*** extracts in methanol, chloroform and petroleum ether with **53.42 %**, **23.60 %,** and 0.0 % respectively. In addition, Table 2 also

shows the percentage inhibition of paw edema for *L. residifolia* extracts in methanol, chloroform and petroleum ether with **16.45 %**, **14.56 %**, and **31.65 %** respectively.

DISCUSSION

The results of the preliminary phytochemical screening of the *H. lippii* revealed the presence of flavonoids, tannins, glycosides, simple phenolics, free reducing sugars and saponines, while that of ***L.*** *residifolia* revealed the presence of flavonoids, tannins, glycosides simple phenolics, free reducing sugars, saponines, steroids and terpenoids. The Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported (18); hence the analgesic and anti-inflammatory effects produced by the extract may be attributed individually or collectively to the flavonoids and tannins.

The methanol and chloroform extracts of *H. lippii and L. residifolia* showed significant increase in latency time to heat stimulus as compared with control group, also **Codeine** produced analgesia and induced an increase in time latency of pain (table 1). The hot plate induced pain test was performed in order to determine whether the analgesic activity of the extracts was caused by central or peripheral mechanisms, where the hot plate test is believed to show the involvement of central mechanisms [18]. However the analgesic activity that exhibited by methanol and chloroform extracts of both plants is related to the polar and semi polar constituents (tannins, flavonoids and saponins) as reveled in the literature, as well as the non polar constituents (steroids and terpinoids) have no analgesic effect.

Inflammation is a response of living tissue to injuries that involve activation of various enzyme, mediators release, cell migration, tissue breakdown and repair [19]. The present study shows the anti-inflammatory activity of the methanol, chloroform and petroleum ether extracts of both *H. lippii* and *L. residifolia* on experimental models. Carrageenan induced paw edema is suitable experimental animal model for evaluation anti- edematous effect of natural products [20]. Vinegar *et al.* reports that the carrageenan induced paw edema takes place in three phases, in the first phase (1 hr after carrageenan induce) involves the release of serotonin and histamine from mast cells, in second phase (2 hrs) is provided by kinins and the third phase (3 hrs) is mediated by prostaglandins, the cycloxygenase and lipoxygenase products [21].

As shown in the results mentioned above, methanol and chloroform extracts of *H. lippii* and the methanol, chloroform and petroleum ether extracts of *L. residifolia* decreased the oedema formation compared to the control (table 2), therefore the anti-inflammatory effect of *L. residifolia* petroleum ether extract might be related to steroids and terpenoids which are absent in *H. lippii.*

The aqueous fraction of the methanol extract of *H. lippii and* ***L. residifolia*** significantly inhibited carrageenan induced paw edema. Aspirin is cycloxygenase inhibitor and it inhibits prostaglandin synthesis and somewhat cycloxygenase-2 selective. The methanol extract has activity which is comparable to aspirin can be related to inhibition of cycloxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan paw edema as reported in the literature [22]. It has been reported that carrageenan induce rat paw edema *via* release of histamine and serotonin from mast cells [23].

CONCLUSION

It can be concluded that both *H. lippii* and *L. residifolia* have analgesic and anti-inflammatory activity against hot-plate induced pain and carrageenan induced paw edema in rats. These activities may be due to their contents individually or collectively. This study demonstrates the efficacy of both plants as analgesic and anti-inflammatory agents. However, further study is required to determine the constituents responsible for the analgesic and anti-inflammatory activities. Finally, *H. lippii* and *L. residifolia* can be introduced as new plants sources for analgesics and anti inflammatory agents.

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