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Antimicrobial, Analgesic and Anti-inflammatory Effects of Euphorbia Helioscopia Plant (Al-jejan) Extracts

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ABSTRACT

The Antimicrobial, analgesic and anti-inflammatory activities of Euphorbia helioscopia (EH) aqueous and ethanolic extracts were evaluated. The effect of both extracts of EH was tested against some Gram positive, Gram negative and some fungi in vitro. Microtiter plates containing serial dilutions of each extract of EH were inoculated with the tested microorganisms and Microtiter MIC assay was used. The analgesic activity was evaluated against thermally and chemically induced pain in mice, using hot plate and acetic acid-induced writhing methods, respectively. The anti-inflammatory effect was investigated by induction of paw edema and swelling using formalin solution in rats. The results showed that aqueous and ethanolic extracts of EH exhibit effective antibacterial and antifungal activities. Both aqueous and ethanol extracts of EH in doses 250 and 500 mg/kg induced potent analgesic activity, in a dose-dependent manner, using both hot plate and writhing tests. Both extracts of EH induced a marked anti-inflammatory activity as it significantly reduced the paw edema and swelling induced by formalin in paw of rats. In conclusion, aqueous and ethanolic extracts of Euphorbia helioscopia produce antimicrobial, analgesic and anti-inflammatory effects. These results affirm the therapeutic uses of Euphorbia helioscopia in folk medicine for the treatment of bacterial infections, pain and inflammation.

Keywords: Euphorbia helioscopia; Antimicrobial; Analgesic; Anti-inflammatory.

INTRODUCTION

The use of herbal medicine represents a long history of human interactions with the environment. According to world health organization (WHO), more than 80% of the world's population depends upon traditional medicine for their primary healthcare needs. Medicinal plants contain a wide range of bioactive substances that can be used to prevent and treat many diseases. The most important of plant bioactive constituents are sterols, flavonoids, terpenes, diterpenes, diterpenoids, sesquiterpenes, and polyphenolic compounds (Olajide, 1999; Edeoga et al., 2005; Tao et al., 2008; and Saleem et al., 2014). Many researchers have shown an increasing interest in natural products from medicinal plants that can be used as therapeutic agents for various diseases (Hong et al., 2009 and Noor et al., 2013).

Euphorbia helioscopia (AL jejan sun spurge) Family Euphorbiaceae is a species of spurge native to most of Europe, northern Africa, and most of Asia (Zhang et al., 2012). It is an annual plant growing in arable land and disturbed ground. Previous studies revealed that different extracts of Euphorbia species have antifungal and anti-inflammatory (Uzair et al., 2009), antitumor (Yang et al., 2008 and Chen et al., 2012); antioxidant (Saleem et al., 2014); hypolipidemic (Cateni et al., 2014); antidiabetic (Uzair et al., 2009) and molluscicidal (Zhang et al., 2012) effects. Anthelmintic and antimicrobial (Lone et al., 2012 and Lone et al., 2013) activities of Euphorbia helioscopia were also reported. Euphorbia helioscopia has been used in folk medicine for the treatment of arthritis, hyperlipidemia, microbial infections painful inflammatory conditions, healing of wounds and gastrointestinal diseases (Cateni et al., 2014). The present study was performed to evaluate the antimicrobial, analgesic, and anti-inflammatory effects of Euphorbia helioscopia aqueous and ethanolic extracts.

Aspergillus fumigatus and Aspergillus niger). All broth cultures were freshly prepared, cultured in Muller Hinton broth and incubated at temperature of 37° C for 24 hours and the growth turbidity was matched to 0.5 McFarland turbidity tube. The plates were then incubated for 24 hours at 35°C. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that completely inhibited the growth of the microorganisms according to CLSI (2013).

Analgesic (Antinociceptive) Activity:

Acetic acid induced writhing test:

The peripheral analgesic activity (Non opiate activity) of Euphorbia helioscopia aqueous and alcoholic extracts was measured by acetic acid induced writhing test as described by Saha et al. (2007). Mice were treated orally with either 2% Tween-80, watery or alcoholic extract of Euphorbia helioscopia at doses 250 or 500 mg/kg or Diclofenac sodium (25mg/kg) as standard drug.

Radiant heat tail-flick method:

The central analgesic activity (opiate analgesic activity) of Euphorbia helioscopia aqueous and alcoholic extracts was studied by measuring drug-induced changes in the sensitivity of the pre-screened (reaction time: 2-4 sec) mice to heat stress applied to their tails as described by Janssen et al. (1963). Mice were pretreated orally with either saline solution (control group), or aqueous or alcoholic extract of Euphorbia helioscopia at dose of 250 and 500 mg/kg, respectively or Diclofenac sodium (25mg/kg) as standard drug.

Anti-inflammatory test:

In this experiment, formaldehyde-induced rat hind paw edema was used as the animal model of acute inflammation as described by Saha et al. (2007). Briefly, acute inflammation was produced by sub-plantar injection of 0.1 ml 2% formalin (w/v) into the right hind paw of rats. Rats were treated with Euphorbia helioscopia aqueous and alcoholic extract at doses of 250 and 500 mg/kg or Indomethacin (10mg/kg) as reference anti-inflammatory drug.

Statistical analysis:

Data were expressed as mean ± standard deviation (SD). Comparisons between groups were performed using one way ANOVA according to Snedecor and Cochran (1986). The difference between the control and

MATERIALS AND METHODS

Plant:

The studied plant material was the whole plant of Euphorbia helioscopia (Family Euphorbiaceae). The plant was collected from Kofraa Region in Hama, Syria. It was authenticated in Botany Department, Faculty of Agriculture, Homas University, Syria. The plant was air dried, grinded into fine powder by electric mixer and kept in a tightly closed glass container till preparation of watery and alcoholic extracts. A voucher sample is kept in the Department of Pharmacology, Faculty of Vet. Med., Cairo, Egypt.

Animals:

A total of 96 Wister adult male mice (20-25 g b. wt., 5-6 weeks old) and 20 Sprague Dawley rats (150-155 g b.wt. and 8 -9 weeks old) were used in this study. The animals were purchased from the Laboratory Animal Colony, Helwan, Egypt. Rats were housed under hygienic conditions at a temperature of 25 ± 2 °C with relative humidity 50–55% and on 12 hr light/12 hr dark cycles in the Animal House of Agricultural Research Center, Giza, Egypt. Basal diet and water were supplied to the rats ad libitum. Animals were acclimatized for 15 days before the start of the experiments.

Preparation of plant extracts:

Two hundred grams of fine powder of Euphorbia helioscopia were soaked in one liter of hot water at 70Ċ or one liter of 90% ethanol and kept in a refrigerator with daily shaking for 3 days. The liquid extract was filtrated using double layer of gauze. The liquid extract was concentrated for evaporation of solvent (water or ethanol) using Rotatory evaporator connected with an electric vacuum pump and metal water bath adjusted at 50°C. The remaining semisolid extracts after evaporation of the solvent were kept in refrigerator till use(Shalaby and Hamowieh, 2010).

Antimicrobial activity:

Microtiter plates containing serial dilutions of either watery or alcoholic extract of Euphorbia helioscopia were inoculated with the test organisms. These microorganisms included some Gram positive (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae and Enterococcus faecalis); some Gram negative (E. coli, Klebsiella pneumoniae, Salmonella typhimurium, Salmonella typhi and Pseudomonas aeruginosa) and some fungi (Candida albicans,

RESULTS

The results revealed that the aqueous and ethanolic extracts of Euphorbia helioscopia produce highly effective antibacterial activity against some Gram positive (Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus agalactiae and Enterococcus faecalis); some Gram negative (E. coli, Klebsiella pneumoniae, Salmonella Typhimurium, Salmonella Typhi and Pseudomonas aeruginosa) and some fungi (Aspergillus fumigatus and Aspergillus niger), but not against Candida albicans. The Minimum inhibitory concentrations (MICs) are recorded in Table (1).

Data in Table (2) showed that oral administration of Euphorbia helioscopia aqueous and alcoholic extract to mice in doses of 250 and 500 mg/kg b. wt, respectively caused significant ($P < 0.05$) decreases the number of acetic acid-induced abdominal writhes and stretches as compared to the normal (2% Tween-80) control group. The protection percents produced by Diclofenac sodium (reference drug), aqueous and alcoholic extracts of Euphorbia helioscopia against abdominal writhing and stretches were 69.73, 48.02 and 55.92%, respectively.

The latency times following oral administration of Euphorbia helioscopia aqueous and alcoholic extracts in doses of 250 and 500 mg/kg b.wt to mice were 10.0± 0.60 and 12.5 ± 0.70 seconds, respectively. The latency time was 14.0 ± 0.3 seconds for Diclofenac sodium (reference drug) versus to 7.4 ± 0.1 seconds for 2% Tween-80 control group. The percentages of protection were 35.14 and 68.91% following oral administration of aqueous (250 mg/kg) and alcoholic (500 mg/kg) extract of Euphorbia helioscopia, versus to 89.18% for Diclofenac sodium as recorded in Table (3).

Oral administration of Euphorbia helioscopia aqueous and alcoholic extract in doses 250 and 500 mg/kg to rats with experimental edema in paw significantly decreased the volume of edema in rat paw at 3,6 and 12 hr post-administration when compared with the negative control group (2% Tween 80) and standard anti-inflammatory (Indomethacin , 10 mg/kg) as recorded in Table (4).

Table (1): Minimum inhibitory concentrations (MICs) of aqueous and ethanol extracts of Euphorbia Helioscopia against some microorganisms in vitro.

Bacterial species	Minimum inhibitory concentration MIC (µL/ml)	
Gram positive	Aqueous extract	Ethanolic extract
Staphylococcus aureus	> 256	16
Staphylococcus epidermidis	> 256	32
Streptococcus agalactiae	32	64
Enterococcus faecalis	32	64
Gram Negative		
E. coli	16	8
Klebsiella pneumoniae	> 256	32
Salmonella Typhimurium	4	128
Salmonella Typhi	32	256
Pseudomonas aeruginosa	16	16
Fungi		
Candida albicans	No inhibitory effect	No inhibitory effect
Aspergillus fumigatus	32	16
Aspergillus niger	68	34

Table (2): Analgesic (antinociceptive) effect of the aqueous and alcoholic extracts of Euphorbia helioscopia (EH) against acetic acid (2%) induced - abdominal writhes in mice. (n = 5, Mean ± SD)

Groups	Analgesic effect	
	Number of writhes per30 minutes (Mean ± SD)	Protection (%)
Normal Control (2% Tween-80)	76.0 ± 0.95[a]	----
Diclofenac sodium (25 mg/kg)	23.0 ± 0.25[d]	69.73
EH aqueous extract (250 mg/kg)	39.5 ± 0.60[b]	48.02
EH alcoholic extract (500 mg/kg)	33.5 ± 0.40[c]	55.92

Means in the same column with different superscript letters are significant atP < 0.05 using one way ANOVA test.

Table (3): Effect of aqueous and alcoholic extracts of Euphorbia helioscopia (EH) on latency time of the radiant heat tail flick in mice. (n = 5, Mean ± SD)

Groups	Latency time (Sec.) (Mean ± S.D.)	Protection (%)
Normal Control (2% Tween-80)	7.4 ± 0.1[d]	——
Diclofenac sodium (25 mg/kg)	14.0 ± 0.3[a]	89.18
EH aqueous extract (250 mg/kg)	10.0 ± 0.6[b]	35.14
EH alcoholic extract (250 mg/kg)	12.5± 0.7[c]	68.91

Means ± SD in the same column with different superscript letters are significant at P<0.05 using one way ANOVA test.

Table (4): Anti-inflammatory effect of aqueous and alcoholic extracts of Euphorbia helioscopia against formalin- induced paw edema in rats. (n= 5, Mean ±SD).

Groups	Mean ± S.D. of rat's paw thickness (mm) at				
	1 hr	2 hr	3 hr	4 hr	6 hr
Normal (2% Tween-80)	2.1±0.55[a]	2.0±0.61[a]	2.0±0.61[a]	1.9±0.65[a]	1.8±0.27[a]
Formalin 2% sol.	4.4±0.55[b]	4.4±0.55[c]	5.4±0.49[c]	5.0±0.55[c]	4.8±0.57[c]
Indomethacin (10 mg/kg)	3.9±0.55[b]	3.4±0.42[b]	2.7±0.45[ab]	2.6±0.42[ab]	2.8±0.27[b]
EH aqueous extract (250 mg/kg)	4.0 ±0.71[b]	3.6±0.42[b]	3.4±0.82[b]	3.1±0.65[b]	3.2±0.45[b]
EH alcoholic extract (500 mg/kg)	4.1 ±0.31[b]	3.7±0.22[b]	3.2±0.25[b]	3.2±0.35[b]	3.0±0.25[b]

Means in the same column with different superscript letters are significant at p< 0.05 using one way ANOVA test.

DISSCUSION

The present study revealed that Euphorbia helioscopia (EH) aqueous and ethanolic extracts possessed highly effective antibacterial and antifungal activities using Microtiter MIC assay. The reported antibacterial activity of Euphorbia helioscopia in this study was similar to that previously reported by Natarajan et al. (2005) and Lone et al. (2013). The antifungal activity of Euphorbia helioscopia was in accord with that reported by Shunyi et al. (2006) and Uzair et al. (2009). Moreover, Ramezani et al. (2008) reported that Euphorbia helioscopia extracts have considerable antiviral activity.

Pains and inflammations are frequently accompanying the degenerative diseases such as cancers, rheumatoid arthritis, and vascular disease which cause disaster to the patient.

Non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressant drugs have been used usually in the relief of inflammatory diseases by the people all over the world (Bacchi et al., 2012). However, these drugs were often associated with severe adverse side effects. Many natural bioactive substances derived from medicinal plants and marine organisms were considered as the effective and safe for the treatment of various diseases including degenerative diseases characterized with pain and inflammation (Sheir et al., 2001).

The results of the present study denoted that Euphorbia helioscopia (EH) extracts produced an analgesic activity as measured by writhing tests and radiant heat tail flick method in mice. The reported analgesic effect of EH, in this study, was similar to that previously demonstrated by Saha et al. (2007); Uzair et al., (2009) and Chen et al. (2012). Moreover, the present study showed that the thermal tail-flick method was more sensitive than the chemical writhing test by acetic acid. This finding agreed with that previously reported by Shanmugasundaram and Venkataraman (2005) when compared between the two tests for studying analgesic activity of Hygrophila auriculata extract and reported that thermal hot plate method was more sensitive than the chemical writhing test by acetic acid.

The mechanism of analgesic activity of Euphorbia helioscopia (EH) extract could be due to its bioactive substances which raised pain threshold by depressing pain receptors centrally (Gawade, 2012). A second possible mechanism of analgesic effect of Commiphora molmol might be due to an inhibition of release of prostaglandins (PGs) which are mediators that produce a wide variety of effects including pain and peripheral inflammation (Goodwin, 1991). Accordingly, Euphorbia helioscopia extract might produce analgesic effect via both peripheral and central mechanisms.

Inflammation is a local response of living mammalian tissues due to an injury or irritant chemical substance as formalin solution. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent the components of inflammation (Purushoth et al. 2012).

Euphorbia helioscopia (EH) aqueous and ethanol extracts induced an anti-inflammatory effect evident by the decrease in thickness of paw edema induced by formalin in rats. The anti-inflammatory of EH was in accord with that reported by Uzair et al. (2009); Lone et al. (2013) and Cateni et al. (2014). The mechanism of anti-inflammatory activity of EH could be due to an inhibition of inflammatory mediator prostaglandins (PGs). This explanation was confirmed by the finding of Lone et al. (2013) who reported that Euphorbia helioscopia significantly decreased the levels of inflammatory factor PGE2 in the edema paw tissue at the fourth hour after formalin injection. In addition, it is well known that non-steroidal anti-inflammatory (NSAID) drugs act by reducing the formation of prostaglandins (Bacchi et al., 2012).

In conclusion, Euphorbia helioscopia exhibits antimicrobial, analgesic, and anti-inflammatory activities. Therefore Euphorbia helioscopia may be beneficial for patients who suffer from microbial infections, pain and inflammation. These results may support the fact of the traditional uses of Euphorbia helioscopia plant for the treatment of microbial infections, pain and inflammation.

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الملخص العربي

التأثيرات المضادة للميكروبات والمسكنة للألم والمضادة للالتهاب لخلاصات نبات ايوفوربيا هيلوسكوبيا (الجيجان)

أستهدف هذا البحث دراسة التأثيرات المضادة للميكروبات والمسكنة للألم والمضادة للالتهاب لخلاصات نبات ايوفوربيا هيلوسكوبيا (الجيجان). وتم دراسة التأثير المضاد للميكروبات معمليا ضد بعض الميكروبات موجبة الجرام وسالبة الجرام وكذا بعض الفطريات بتحديد اقل تركيز يسبب تثبيطا لنمو الميكروب. وتم دراسة التأثير المسكن للألم بواسطة إحداث ألم بمؤثر كيميائى بحقن حامض الخليك بالتجويف البريتونى فى الجرذان وإحداث ألم حرارى بغمر ذيل الجرذ فى ماء ساخن حتى يجذب الجرذ ذيله. وتم فحص التأثر المضاد للألتهاب بطريقة إحداث اوديما (ورم) بحقن محلول الفورمالين بكمية 0.1 ml من تركيز 2 % وذلك فى باطن القدم الخلفية. واشارت النتائج ان الخلاصة المائية والكحولية لنبات ايوفوربيا هيلوسكوبيا لها تأثيرات مفيدة كمضادة للميكروبات وكمسكنة للألم وكمضادة للالتهاب. وتؤكد نتائج هذه الدراسة حقيقة استخدام نبات ايوفوربيا هيلوسكوبيا فى الطب الشعبى لعلاج الاصابات الميكروبية وحالات الألم والالتهاب. وبمزيد من الدراسة يمكن ان نواة لدواء من اصل نباتى.