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ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF JATROPHA UNICOSTATA BALF, AS ENDEMIC PLANT IN YEMEN

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ABSTRACT

In the present study, we evaluate the analgesic activity of methanolic extract obtained from *Jatropha unicostata balf* leaves, using chemical and thermal models which will induce acute pain in mice and rats. This study was carried out by using sex Swiss mice (20-30gm) and wistar male rats (200-250gm).The methanolic extract was prepared by using maceration at room temperature (25°) over the period of 48 hours. The effect of methanolic extract of *Jatropha unicostata* was investigated for analgesic activity using acetic acid-induced abdominal writhings (Koster test) and Tail immersion method (Tail flick). The analgesic activity of methanolic extract of *Jatropha unicostata* at the dose of (100 and 200 mg/kg, p.o.) showed significant ($p \leq 0.001$) in reducing abdominal writhings when compared with control and standard drug (Aspirin, 200 mg/kg, p.o.). However this extract at the dose 200 mg/kg, p.o. showed significant ($p \leq 0.001$) central analgesic action when compared with control and morphine (5 mg/kg, S.C.) as reference drug. In conclusion, the methanolic extract of *Jatropha unicostata balf* produced significant analgesic activity in mice and rats.

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KEY WORDS

Medicinal plants, Jatropha unicostata balf, methanolic extract, acute toxicity, analgesic activity.

INTRODUCTION

The phytotherapy represents one of the most important fields of traditional treatment in Yemen especially in the Socotra Island. Thus, phytotherapy is practised by a large proportion of Yemen population for the treatment of several physical, physiological, mental and social ailments[1]. We selected this part of Yemen because this Island is undoubtedly a most precious natural asset not only for the republic of Yemen but also for the whole world. 273 plants of about 850 plants are considered to be endemic. The selection of this plants for evaluation was based firstly on the wild use of medicinal purposes and secondly on the occurrence as endemic plants on Socotra Island [1-3].

Jatropha unicostata is a species of plant in *euphorbiaceae* family. It is endemic to Socotra Island[3]. This plant is widely used in the traditional medicine for the treatment of severe wounds, acute pain, haemorrhage, inflammation, skin, eczema, tender gland, eye infection, chest pain, stomach pain, retching, vomiting and is used as a laxative and is vermifuge [1-3] .

The pharmacological activities already investigated on this plant include: anti-microbial effect, anti-viral effect against influenza virus type A and herpes simplex type1, anti-cancer and anti-protoscoleces [1-2, 4- 5].

The aim of the present study is to investigate the analgesic activity of *Jatropha unicostata* methanolic extract using chemical and thermal acute pain models in mice and rats.

MATERIALS AND METHODS

(i) *Plant materials:*

Fresh leaves of *Jatropha unicostata balf* (*euphorbiaceae*), were collected at dinghnne, Socotra Island in Yemen on January 8 2008 and identified by pr. Abdul karim Nasher and botanist of Socotra(Ahmed Slymane and Ahmed Aissa). A voucher specimen (N° RAB 76710) was deposited in the herbarium of botany department of scientific institute of Rabat.

(ii) Preparation of extracts:

Leaves of *Jatropha unicostata* were successively extracted with methanol by maceration at room temperature (25°) over the period of 48 hours. 500 g of plant material and one litre of methanol were used in the extraction. Methanol, containing the extract, was then filtered through Whatman paper and the solvent was vacuum distilled at 65c° in rotary evaporator. Final extract was a dark green semi-solid in percentage dray weight of 15%. This methanol extract was kept in deep freezer at -20 c° until use.

Animals:

Male Swiss mice (20-30g) (Offa-credo, France) and wistar male rats (200-250g) were used in the pharmacological tests, and females of the same strain mice in the LD_{50} calculation. The animals were acquired from the animal centre of Mohammed V-souissi University, medicine and pharmacy faculty-Rabat. All animals were kept in a room maintained under environmentally controlled conditions of 23 ±1C° and 12 h light-12 h dark cycle. All animals had free an access to water and standard food *ad libitum*. They were acclimatized at least one week before the experiments were started. The animals submitted to oral administration of the extracts or drugs were fasted for 18 h before the experiment (water was available).

(iv) Acute toxicity:

LD_{50} values were determined as described by Litchfield and Wilcoxon [6-7], six groups of mice of both sexes (n=10, 5 mal and 5 females), received single oral doses at different concentration (500, 1500, 2000, 2500, 4000 mg/kg, p.o.). The control group received only the distilled water. After a single dose administration, mice were placed in individual clear plastic cages and all animals were observed for possible mortality cases (24h) and behavioural changes followed by daily weight monitoring for 14 days.

(v) Analgesic activity

The evaluation of the analgesic activity of the methanolic extract of the leaves of *Jatropha unicostata* was carried out by using two different methods which used thermal stimuli [8] and chemical stimuli [9].

A: Acetic acid-induced writhing response in mice:

The method used in this test has been described by koster[9] .The total number of writhings following intraperitoneal administration of acetic acid solution (3% with 300 mg /kg, i.p.) was recorded over a period of 20 min, starting 5 min after acetic acid injection, the mice were treated with methanolic extracts of *Jatropha unicostata balf* (100 and 200 mg/kg) or standard drug (aspirin, 200 mg/kg p.o.), 30 min before administration of acetic acid. The number of writhings and stretching was recorded and permitted to express the percentage of protection using the following ratios (control mean-treated mean) x 100/control mean [10, 11].

B: Tail flick test:

The procedure is based on the observation that morphine like drugs are selectively prolonging the reaction time(in second) of the typical tail withdrawal reflex in rats induced by immersing the end of the tail about (4-5 cm) in warm water of 55 C° [12] . Morphine (5 mg/kg s.c), was used as positive control and *Jatropha unicostata balf* methanolic extract was administrated (100, 200 mg/kg; p.o.). The tail withdrawal reflex was recorded before and after 15, 30, 60 and 120 min following oral administration of the extract to different groups of six animals each.

(vi) Statistical analysis:

The results were reported as mean±S.E.M and analyzed by one-way ANOVA followed by student's t-test used for statistical evaluation. A value of $p< 0.05$ was considered significant.

RESULTS

Acute toxicity

Acute Toxicity Following oral administration of *Jatropha unicostata* methanolic extract at the dose 500mg/kg, neurological deficit and abdominal contraction were observed by 20-25min after the injection in 60% of mice. In addition to the abdominal contraction, the methanolic extract reduced spontaneous motor activity and exploratory behaviour. The mortality was recorded 24h after administration at the doses 1500, 2000, 2500 and 4000 mg/ kg. The severity of these effects was increased within 6h; and mortality was 100%. Boniface et al., have established a program that calculate the percentage of mortality according to the administration dose[13]. This calculation gives the following results:
LD_{50} = 1353 mg/kg, $1234<LD_{50}<1486$, with confidence limits of 95% (Table 1).

Table 1

Mortality according to the administration doses of Jatropha unicostata methanolic extract via oral route (p.o.). The methanolic extract of Jatropha unicostata was administered by oral route and all treated mice were carefully examined during 14 days to determine LD_{50} and any sign of toxicity n =10 per dose.

Dose (mg/kg)	% mortality N=10	LD_{50} (mg/kg)
500	10	LD_{50}= 1353mg / kg
1500	50	1234<LD_{50}<1486,with
2000	70	confidence of limits at 95%
2500	80	
4000	100	

Analgesic activity:

Acetic acid-induced writhing response in mice

In the acetic acid induced writhing test in mice, The Jatropha unicostata methanolic extract (100, 200mg/kg p.o.) demonstrated significant (p≤ 0.001) peripheral analgesic effect with an inhibition percentage of 37.55% and 64.40%, respectively, compared to Aspirin (50.1%) as positive control (Table.2.).

Table 2

The effect of Jatropha unicostata methanolic extract (JUME) on acetic acid induced writhes in mice. Data presented as mean ±standard error mean (M±S.E.M.) p≤0.001 evaluated by one-way ANOVA against control group. N=6: number of mice for each test. Standard drug- Aspirin 200mg/kg; Methanol extract100 and 200 mg/kg administrated by oral route (p.o.).

Treatment response	Dose (mg/kg, p.o.)	Number of writhes(per 20 min)	Inhibition of writhing (%)
Control	-	51.5±2.8*	-
Aspirin	200	25.66±2.9*	50.1
JUME	100	32.16±1.47*	37.55
JUME	200	18.33±1.03*	64.40

Tail immersion assay*:*

The anti-nociceptive activity of methanolic extract *of Jatropha unicostata* on the tail immersion test is shown in (Figure.1.). The methanolic extract (200 mg/kg, p.o.) significant (p≤O.001) increased the reaction time (7.11sec±O.15 second) at 45min in the thermal stimulus. Which comparable with morphine (5 mg/kg s.c), the reaction time was (7.49±0.25 sec) (p≤0.001).

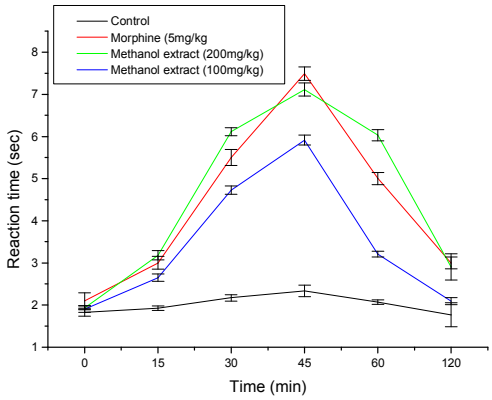

Fig.1.

Central Analgesic activity of Jatropha unicostata methanolic extract, by tail flick method. (n=6, values are expressed as mean± Standard error mean (M±S.E.M.) P≤0.001, evaluated by one-way ANOVA against control and reference (morphine) group.

DISCUSSION

Recently, many medicinal plants are largely used worldwide by the population and have proved to be a rich source of new active compounds, especially to treat pain and inflammation processes [14]. Pain is one of the classical signs of the inflammatory process in which sensitization of the nociceptors is the common denominator. This sensitization causes hyperalgesia or allodynia in humans, phenomena that involve pain perception, and is better described as nociception in animal models [15-17]. In general, acetic acid writhing tests are used to evaluate the compounds for peripheral anti-nociception activities [18]. Acetic acid injection produces peritoneal inflammation which triggers response characterized by writhing [10]. We analyzed the effect of different doses of methanolic extract from leaves of *Jatropha unicostata balf* for their acute toxicity and analgesic activities. We observed that, animals treated with *Jatropha unicostata* methanolic extract (100 and 200 mg/kg, p.o.), reduced significantly the number of abdominal writhing during 20 min in the peripheral analgesic ($p< 0.001$), while in the central analgesic at the dose 100 mg/kg, this extract don't exhibit significant analgesic activity when compared with control and morphine treated animal. By increasing the dose to 200 mg/kg, the methanolic extract of *Jatropha unicostata* produces significant ($p< 0.001$)central analgesic action, because this extract increase the reflex time of removal the tail of rats by inhibition the pain(7.11sec ±0.15 at 45 min), these results were compared with morphine (7.49sec±0.25), at the same time. Related studies have demonstrated that acetic acid indirectly induces the release of endogenous mediators of pain (such as prostaglandin, kinin, histamine, etc) that stimulate the nociceptive neurons, which are sensitive to non-steroidal anti-inflammatory drugs and opioïds [19-21]. These observations suggest that, the methanolic extract of *Jatropha unicostata balf* have a significant inhibitory activity in inflammation pain, and this activity may be related with the suppression of synthesis and/or release of endogenous pro-inflammatory substances. Some constituents of *Jatropha unicostata* have been identified, mainly sterols, ketosteroid, flavonoids (leutolin) and terpenoids [22, 2]. These chemical compositions were also identified in other plants extracts such as, *Stylosanthes fruticosa*[23], *Pistacia integerrima*[24-25], *Hedyotis puberula*[26-29] and *Argania spinosa*[30], that have

been provided to possess analgesic activity. Further chemical and pharmacological analysis of the extract will be conducted to isolate and characterize the active principles responsible for the analgesic effect.

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CONCLUSION

We conclude that, the methanolic extract of *Jatropha unicostata balf* is a notable, central, and peripheral analgesic activity

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