Original Article

Study The Pharmacological Activity Of Methanolic Extract Of Cercis Siliquastrum L. Leaves On Animal Model

Asmaa M H Al Ali[1*], Faten S Mounce[2], Nadheerah F. Neamah[3]

[1]pharmacology &Toxicology Department, College Of Pharmacy, University Of Basrah, Iraq. Email: Asmaa.Hussein@Uobasrah.Edu.Iq
[2]pharmacology &Toxicology Department, College Of Pharmacy, University Of Basrah, Iraq. Email: Faten.Mounce@Uobasrah.Edu.Iq
[3]pharmacology &Toxicology Department, College Of Pharmacy, University Of Basrah, Iraq. Email: Nadheerah.Neamah@Uobasrah.Edu.Iq

Corresponding Author: Asmaa M. Al Ali Phd

[1]pharmacology &Toxicology Department, College Of Pharmacy, University Of Basrah, Iraq. Email: Asmaa.Hussein@Uobasrah.Edu.Iq
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Abstract

Cercis siliquastrum (Judas tree) is a commonly used traditional medicine in Iraq with drug discovery potential. The aim of this study was to investigate the in vivo acutes toxicity, analgesic activity by acetic acid induced writhing model, Anti-inflammatory by Carrageenan induced paw edema method, gastrointestinal tract (GIT) motility by charcoal meal protocol, antidepressant activity by using forced swimming test (FST) model of methanolic extract Cercis siliquastrum L. leaves (MECSL). Differenet concentration of MECSL curde extract was used (50 mg/kg, 150 mg/kg, 300 mg/kg. The results revealed that Cercis siliquastrum methanloic extract is dose dependent. Crude MECSL extract proved a strong analgesic, anti-inflammatory, and properties.

Keywords: Analgesic; *Cercis* siliquastrum; Anti-inflammatory; Gastrointestinal; Antidepressant activity

INTRODUCTION

Plants supply both calories and medically and therapeutically essential substances as well as useful bio-activates [1]. Medicinal plants have not only molecules or constituents that have medical benefits, but also serve as precursors to those that can be used to synthesize new pharmaceuticals. For phytotherapy, some applications were used to herbal medication or medicinal use where the plants or rudimentary ingredients are taken in the form of phytomedicinals or applied to the body to be therapeutic and medicinal for different ailments [2]. There are various sections of plants in phytotherapy, including leaves, stems, flowers, fruit, or entire plants, as well as intact plants. Folk medicine is focused on medicinal plants and their derivatives; these are the core elements of the medicinal culture [3]. There is an almost endless amount of different medicinal flora on Earth, and huge asset in the development of herbal medicine. Up to thirty-four of the world population depends on herbal medicines, since they have no harmful side effects, and can be found in abundance in nature. Herbal remedies are known to have been used for medicinal purposes for 5,000 years, based on information gleaned from the Sumerians who lived in southern Mesopotamia around 3,000 B.C.C. Some research on phytomedic medications has shown the use of these medications to have been practiced for between sixty and eighty thousand years in Iraq and China [4]. Primitive forms of drugs discovered indicate that medicine is thousands of years old.

There has long been a need for the use of medicinal plants in agriculture, pharmaceuticals, and now they are also important in the pharmaceutical and food industries [5]. Regardless of advances in synthetic chemistry and analytical methods, phytochemicals also loom in the cure and research of disease and the discovery of new medicines [6]. Using new methods, several medications have been developed to cure certain ailments, and are regularly used, which leads to microbes being resistant and associated side effects. For any prescription that has to be paid for, traditional medications are much more costly and average citizens cannot afford it. A natural product use for nature-based medicine is increasing, and more more in general to plant-based medicine. Worldwide, over four billion people use phytomedicals as a complementary medicine. The primary importance of plant-based phytotherapy has been accepted by WHO [7]. There has been a huge push in the contemporary era to test current medications on the curing features of medicinal plants because of their effectiveness, with fewer side effects, and lower cost. On a wide scale, medicinal plants serve as a valuable sources of raw material for pharmaceutical industries to produce pure medicines [8]. Natural cures, in long-term illnesses, have been shown through experimental study.

MATERIALS AND METHODS

Plant Collection and Extraction

The *Cercis siliquastrum* L. leaves was collected from folk medicine market in Al Ashar , Basrah, Iraq, in January 2021 and was identified b, Plant Taxonomist,

The plant materials that were obtained were shade dried at room temperature. The dried leaves of the plant were ground into powder using an electronic grinder. The powder (2kg) was soaked in industrial grade methanol (5L) at room temperature for 15 days with intermittent shaking. After 15 days, it was filtered with a colorless white thin fabric, and the methanol soluble residue collected was condensed with a rotary evaporator at 40 ºC [8], yielding 30 g of crude methanolic extract of *Cercis siliquastrum* L. leaves (MECSL). MECSL crude extract was used for a vaious of biological activities (in-vivo).
Animals collection and acclimation

Acute toxicity
The acute toxicity of crude MECSL extract of leaves was calculated using doses ranging from 500 mg/kg (body weight of mice) to 2.5 gm/kg. The mice were divided into groups and given varying amounts of plant crude extract. The negative control group received purified water at a dosage of 10 ml/kg [9]. The animals were held under surveillance for 24 hours after being given test doses. The animals were monitored for acute toxicity effects for the first four hours. After 24 hours, the percentage of deaths was estimated.

Analgesic activity (Acetic acid induced writhing)
Crude MECSL extract for analgesic effects has been screened. BALB/C mice of 18-22g body weights were chosen for this reason [10]. The animals were categorized into five groups (n=6). The negative and positive control sets in Category I and II respectively served. Normal saline was given as a dosage of 10ml/kg (Body Weight) at Group I and 10mg/kg (Body Weight) for Group II at Diclofenac Sodium. The food was supplied according to recommended instructions, but 2 hours before the start of the activities was stopped supplies of food. A dosage of 50, 100 and 200mg/kg of the MECSL extract was given to the remaining groups, III, IV and V. (Body weight). 1 percent acetic acid was given to both groups intra-peritoneally after 30 minutes of the above therapies. The abdominal writhings (constrictions) began to count for the next ten minutes after 5 min of acetic acid injection. The analgesic effect (percentage) was determined using the following formula.

$$\text{Analgesic effect} = 100 - \frac{\text{Number of writhing in tested animals}}{\text{Number of writhing in control animals X 100}}$$

Anti-inflammatory activity (Carrageenan induced paw edema)
The crude MECSL extract had an anti-inflammatory effect screened. BALB/C mice with body weights of 25-30g were selected for this reason. This species have been classified into five classes. There were 6 mice (n=6) in each group of species. Groups I and II have been both used as negative and positive controls [11]. Diclofenac sodium was administrated in Group I at a dosage of 10 ml/kg (body weight) and in a group II dose of 10 mg/kg (body weight). The remaining classes, i.e. III, IV and V, were administered to the crude MECSL extract with 50, 150 and 300 mg/kg (body weight). 1% of carrageenan was injected into each mouse's right hind paw sub plant tissue after 30 min from the above-mentioned treatments. The effect of antiinflammation was calculated by Plethysmometer (LE 7500 plan laboratory S.L) 5 hours after Carrageenan was injected (0, 1, 2, 3, 4 and 5 hours). The percentage inhibition of edema was determined using the formula given below.

$$\% \text{ Inhibition} = \frac{A - B}{B} X\ 100$$

Where the edema size of negative control represented by A, while **B** as paw edema of tested group.

Gastrointestinal tract (GIT) Motility (Charcoal meal protocol)
The crude MECSL extract has been tested to influence the motility of GIT. The selection of the BALB/C mice was 25-30 g. The species were classified into five groups [7]. Classes I and II was both positive and negative. Normal saline was administered in group 1 at a dosage of 10 ml/kg while castor oil (0.1 ml/kg) was administered in group II as normal. The remaining classes, III, IV and V received the raw MECSL extract at 50, 150, and 300 mg/kg in an i.p pathway, respectively [12]. The suspension of charcoal (water) at a dosage of 0.2 ml was applied to each mouse after 15minutes of the above-mentioned treatments. After 30 minutes of charcoal therapy animals were then destroyed by cervical dislocation. By dissecting the small intestine and by measuring GIT motility percent with a formula given below, the charcoal movement in the small intestinal area was extracted.
Percent Motility = 100 - Distance covered / total length of intestine × 100.

Antidepressant Activity (Using forced swimming test (FST) model)
In order to test an antidepressant effect of MCSLE forced swimming test (FST) has been used. For this reason, a bathtub with water measuring 42 x 19 x 19 cm was used. An animal was classified into five classes. There were 6 mice (n=6) in each group of animals. Group I and Group II were both negative and positive. The usual 10 ml/kg dose of saline was given in Group I while the 15 mg/kg dose of fluoxetine was given in Group II via i.p route. A dosage of 50, 100 and 150 mg/kg was given to the remaining classes III, IV and V. The day before the experiment was carried out, both animals were learned to swim in the water bath. The water temperature was held at 25 ± 2 ºC [13]. The animals were correctly managed and the laboratory setting recommended (sound proof and red dim light) was in compliance with the recommended procedure. The above mentioned doses of crude extract and medicine were used by both types. Every mouse was bare to

a bath for swimming after 30 minutes and allowed to swim for 360 s, during the last 240 s, though stillness has been seen.

RESULTS AND DISCUSSION

Acute toxicity

The crude MECSL extract has been checked with various doses as mentioned in table 1 for its toxic effect. For that function, BALB/C mice have been used. The mice were analyzed 24 hours after test sample administration and were observed for a gross reaction. No adverse effect was found within the first 4 hours at a dosage of 900 mg/kg and no death was caused after 24 hours by the test study. 100% of the animals died at a 1 gm/kg dosage. So we found that up to a dosage of 900 mg/kg the test sample is healthy. After confirming the safety profile of the crude MECSL Extract, different in-vivo experiments were carried out.

Table 1: Acute toxicity of crude MECSL extract

Treatment (crudeMECSL) (ml or mg/kg)	No. of Animal aliveafter 4hrs	No. of Animal aliveafter 24hrs	% Death after 4hrs	% Death after 24hrs
Normal Saline 10	All alive	All alive	--	--
500.0	All alive	All alive	-	--
600.0	All alive	All alive	--	--
700.0	All alive	All alive	--	--
800.0	All alive	All alive	--	--
900.0	All alive	All alive	--	--
1.0	All alive	none alive	--	100

Analgesic activity of crude MECSL extract of roots

The crude MECSL extract shows a drop in the mean writhing in the various test groups at different doses by i.p. route (50 mg/kg, 100 and 200 mg/kg) as seen in table 2. The medium writhing was 55.00 ± 1.79 in saline treated community. Different test doses of MECSL extract were 10.86% (50mg/kg), 40.66. 24.17% (150 mg/kg) and 59.11% (300 mg/kg) for the writhing inhibitory effect produced. The dose-dependent effect of blunt MECSL. Diclofenac sodium (standard drug) provided a maximum inhibition of a 10 mg dose of 73.43 percent, more than the largest crude MECSL dose (300 mg/kg).

Table 2: Analgesic Activity of crude MECSL extract

S.No.	Treatment	Dose ml or mg/kg	No. of writhing (10 min) (Mean+SEM)	% inhibition of writhing
1	Normal saline	10	55.00 ± 1.79	-
2	Crude MECSL	50	52.23 ± 1.50	10.75*
		150	43.67 ± 1.58	24.17 **
		300	24.33 ± 1.39	59.11 **
3	STD (Diclofenacsod.)	10	14.50 ± 1.20	73.43 ***

The studies discovered that the analgesic activity of the crude MECSL extract. Permeability is a popular, efficient, and easily quantifiable approach for describing the peripheral effect of plant extracts and other pharmaceuticals [11]. It is believed that intestinal constriction is caused by local receptors (i.e., peritoneal) it is also said that there has been a rise in the number of nociceptors in the peritoneal tissue. Generally, if you add acetic acid to the synthesis of prostaglandins like PGF2α and PGE2 and lipoxygen derivatives are thought to produce more in the peritoneal fluids, the peritoneal fluid will become more painful. The compounds formed by the COX pathway are known as phospholipids of aridonsaccharides which are found in inflamed abdominal tissue and act as cyclo-phenols. Spasms, because people who experience discomfort when in the abdominal cavity due to a spasm of peritonitis are more vulnerable to these types of chemicals. The ability to wriggle may be related to suppression of prostanoids, which is thought to inhibit pain at the periphery [9]. It is hypothesized that the peripheral analgesic affects may be brought on by reduced synthesis of prostaglandins or a diminished supply of prostaglandins. Crude MECSL developed abdominal muscle relaxant in the form of analgesic chemicals, demonstrating a pain-relieving process as a side effect.

Anti-inflammatory activity of crude MECSL extract

Antioxidant activities of the crude MECSL (50, 100 and 200mg/kg) were measured. It can be found in the table 3. the MECSL (400mg per kg) was found to provide the greatest anti-inflammatory response after four hours Of the previous doses, at 5 hours there was a 44.54% inhibition of edema inhibition for the 1st, 14% edema inhibition at the 2-hour mark, and a 15% edema reduction, and the 2nd and 3rd hours. Approximately 150 mg/kg of MESL of the extract produced an anti-inflammatory activity at 3, 4, and 5 hours after injection. In anti-inflammatory doses, the safety was about three-and-and-a-a-half, four, and four-and-a-a-half percent. The .highest percentage of edema suppression (1505%) was detected at the 4-hour plateau time point. The anti-inflammatory activity of the norm (diclofluamide sodium, 57.49%) was stronger

than that of the higher and lower doses of crude MECSL (0.5% and 0.100% and 0.200% concentrations), suggesting a need for fine-tuning of the processing conditions in order to enhance it..

Table 3: Anti-inflammatory activity of crude MECSL (50, 150 and 300 mg/kg) in comparison with Diclofenac as a reference drug

Treatment	Dose ml or mg/kg	NPS	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour
Saline	**10**	0.1683± 0.10	0.2132 ± 0.13	0.2323 ± 0.24	0.2540 ±0.10	0.2660 ±0.28	0.2520 ±0.27	0.2333±0.20
Diclofenac	**10**	0.1833± 0.07	0.2228 ±0.07	0.1610 ±0.29*	0.1510 ±0.04 **	0.1187 ±0.10**	0.1023 ±0.21 **	0.1033±0.21 **
Crude MECSL extract	**50**	0.1700± 0.21	0.2175 ±0.24	0.2261 ±0.26	0.2440 ±0.31	0.2387 ±0.17*	0.2215±0.24 *	0.2200± 0.21 *
	150	0.1817± 0.31	0.2166 ±0.18	0.2047 ±0.16	0.1920 ±0.25 *	0.1737 ±0.22 **	0.153 ±0.32**	0.1450±0.19 **
	300	0.1917± 0.27	0.2190 ±0.28	0.2030 ±0.19	0.1947 ±0.22 *	0.1720 ±0.27 **	0.1423±0.27**	0.1317±0.15 **

*** p < .001; ** p < .01; * p < .05

There was a dose-dependent response to the anti-inflammatory action of the blunt MESL extract. It can be concluded from the data provided above that the plant had strong anti-inflammatory properties when tested on carrageenan-induced inflammation. Pregnancy outcomes in rats given 150 mg/kg or 300 mg/kg of MEC reported significantly better findings at 3rd, 4th, and 5th hours, respectively, compared to those of the control group. Our preliminary test doses (50 and 150 mg/kg) were highly important, as well [14]. As we learned from earlier research on its anti-inflammatory benefits, this behavior is substantiated by this plant's traditional use. potential. Effects on chronic inflammation Carrageenan mediated inflammation (emmolecular inflammation) was shown to have an anti-inflammatory effect. The active compounds in crude MEC extract may have an anti-inflammatory function, which requires more research to identify the specific chemicals. Umbeline, which is contained in the extract of Ferula, had the in-vivo anti-inflammatory properties, according to the in vivo research. This brings into line with our previously determined results about the existence of anti-inflammatory properties in this herb [10].

Gastrointestinal tract (GIT) Motility activity of crude MECSL extract

GIT testing on motion was performed on the crude MESL extract. Effects are seen in Table 4, which show that a dose-dependent effect was present in the MECSL extract. For a 50 milligram/kilogram dosage, the percent GIT motility was found to be 46.21%, but at the 150 milligram/kilogram dose, it dropped to 38.59%, and for the 300 milligram/kilogram dose, it decreased to 31.98% The number of leukocytes (white blood cells) in the control Saline population was 48.37% As can be seen from the data, the potency of crude MECSL decreases in a nonlinear fashion. The greatest reduction in GIT motility occurred when 200 mg/kg of MECSL was administered.

It is one of the most well-known ways of determining gut transit time Assafiduous means assafide,and is known to be a helpful herb for antispasmodic treatment of disease. There is an improvement in intestinal muscle contraction with the help of the MECSL as well as acetylcholine is available to M3 receptors located in the small intestine, leading to relaxation [15]. The use of antispasmodics is to reduce the signs and symptoms of irritable bowel syndrome. GIT motility decreases, it may be used for the treatment of diarrhea, and for stomach spasm, it's helpful.

Table 4: Effect of crude MECSL extract on GIT Motility

Treatment	Dose mg or ml/kg	Mean Total length of intestine (cm)	Mean Charcoal movement (cm)	% GIT Motility
Normal saline	10	57.51±1.128	28.32±0.8829	49.38 ± 1.249
Crude MECSL	50	56.10±1.221	25.82±0.6019	46.22 ± 1.135*
	150	55.53± 0.728	21.51±0.6181	38.58 ± 0.845**
	300	58.64±0.928	18.82±0.7022	31.97 ± 1.362**

Antidepressant activity of crude MECSL extract

There was a preliminary test to determine antidepressant activity of the crude extract from MECSL. Table 5 shows that no antidepressant activity was seen for any of the test doses. At the higher dosage, the immobility of the animals increases. The forced swimming test (FST) is often used in animal studies to test antidepressant function. If the water displacement increases, it is believed that antidepressants are having an effect. If water displacement decreases, it is thought to be due to central nervous system (CNS) depressants [16]. In terms of antidepressant medications, the animal's exhibit signs of being exhausted, sleep deprived, and sad, which reflect that they are fatigued. When patients with these signs, the most common type of depression is present. Our testing determined that the plant extract had the CNS (central nervous system) depressant (negative) effect, not the antidepressant (positive) effect. The immobility of MECS, which is correlated with sedative and tranquilizing effects, may be because of the drug's CNS depression.

Table 4: Antidepressant activity of crude MECSL extract and fluoxetine as positive control

Treatment	Dose mg or ml/kg	Immobility time (s)
Saline	10	131 ± 0.75
Crude MECSL extract	50	142 ± 1.13
	150	160 ± 1.17
	300	174 ± 0.70
Fluoxetine	15	32 ± 0.35

CONCLUSION

Cercis siliquastrum L. is a well-known remedy used ethnomedicinally for all these conditions; it has been known to be helpful in the treatment of cough, asthma, stomach complaints, constipation, and angina pectoris. To cite some aspects of the ethnobotanical uses of *C. siliquastrum* leaf components in scientific studies, crude methanolic extract is explored and validated using both in-vivo results. Crude MECSL extract demonstrated a strong analgesic, anti-inflammatory, and properties. There was an inhibitory effect on the ability of research animals to reproduce in the crude MECSL extract. The plant couldn't find any antidepressant action in the plant extract.

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