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Anti-inflammatory effect of *Capparis decidua* stems methanolic extract in acute and chronic inflammation models in rats

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ABSTRACT

Capparis decidua (Altondub) is used widely in folkloric medicine in Sudan due to its nutritional and medicinal values. In this study *C. decidua* stem methanolic extract was evaluated in acute and chronic inflammation models in rats. Carrageenan induced paw oedema was used as acute model to investigate the anti-inflammatory effect of *C. decidua* stem at a dose of 100, 200 and 400 mg/kg. Diclofenac sodium was used as standard drug. In chronic model, sterile cotton pellets (20mg) were surgically inserted subcutaneously under anesthesia in twenty rats to induce granuloma. *C. decidua* stem extract was tested at dose of 200 and 400 mg/kg, for 7 days, diclofinac sodium was also used as a reference drug. In acute model, oral administration of *C. decidua* stems extract at dose of 100, 200 and 400 exhibited a significant ($p<0.05$) dose dependent anti-inflammatory effect especially at 4th hours. The inhibition rates of paw oedema were 34.4, 44.4 and 65.3% respectively in stem extract and 82.0% in dichlofinac sodium. The high dose of stem extract (400mg/kg) was comparable to standard drug diclofenac sodium. In chronic model, the stem extract significantly reduced inflammatory oedema and masked the production of granulomatous tissue induced by cotton pellet granuloma. It is calculated that the *C. decidua* stem methanolic extract possesses potential anti-inflammatory effect in acute and chronic inflammation in rats. Further studies should be performed to explain the exact mechanism of *C. decidua* stems in inflammation.

Introduction:

Inflammation is a beneficial host response to external stimuli or cellular injury that triggers the activation of many of inflammatory mediators, accomplishing and restoring tissue structure and function. Although it is a useful response, prolonged inflammation can be harmful to the host, contributing to the pathogenesis of many disease states (Pascual and Glass, 2006). Chronic inflammation is a long lasting type changes that may persist for weeks, months or

even years and brought on by acute inflammation or immune maybe the result of an autoimmune disease (Whicher and Chambers, 1984).

Inflammation is accompanied by the release of various chemical mediators that are responsible for signs and symptoms associated with such conditions. Various anti-inflammatory agents are used to alleviate the inflammation and pain and other associated symptoms, most of them are synthetic drugs, associated with various side effects such as peptic ulcer and bleeding (Khuda *et al*., 2014).

In the folkloric medicine, various poly-herbal formulations are being prescribed for inflammatory conditions. Although these preparations have been claimed to have anti-inflammatory activity and some of the individual ingredients of the formulations have been shown to have anti-inflammatory activity (Bagul *et al*., 2005).

Capparis decidua (Forsk.) is an important shrubby plant of family Capparidaceae. It is reported to be used in curing various diseases and as food in various cultures (Azhar *et al*., 2017). The genus Capparis represents about 250 species of trees, shrubs and woody climbers. *Capparis decidua* (Forsk.) Edgew, is a branchy shrub, spinous up to 4 to 5 m height. It is well distributed in Pakistan, India, Arabian states and Tropical Africa including South Africa (Orwa *et al.,* 2009; Gupta, 2010). *C. decidua* is an important medicinal plant in Sudan and locally known as altundoub. The tree is typical of deserts and semi-deserts of northern and central Sudan, especially on sandy soils and in low rainfall savanna on clays spreading to the borders of Republic of Southern Sudan, sometimes mixed with *Acacia seyal* or *Balanites aegyptiaca* (Abdalrahman *et al*. 2016). The plant and its parts are widely used by traditional healers and tribal people in Sudan for curing variety of ailments. Paste of young leaves and branches are applied as plaster on boils and swelling, and as anti-inflammatory, astringent, stomachic, laxative, antidote for skin diseases (Al Yahya 1986; Atiqur *et al*., 2004). Decoction of fresh twigs is taken against jaundice and the fumigation of the stems is used as anti-rheumatic. The stems are also used as a poultice for swelling and joint pains and against head-ache (El Ghazali *et al*., 1994, 1997). The roots are used to relieve fever, rheumatism and jaundice (El Kamali and Elkhalifa, 1999). Pharmacological studies of plant for the exploration of biological activities play important part in science of traditional medicine. Different parts of the *Capparis spinosa* plant especially root bark and fruits have been used traditionally to cure various ailments (Al-Snafi, 2015).

Studies indicated that the *C. decidua* has significant pharmacological activities like treatment of hypercholesterolemia, anti-inflammatory, analgesic, antidiabetic, anti-microbial, anti-plaque, anti-hypertensive, anti-helminthic activities (Verma *et al*., 2011). Medicinal properties of plants are due many active compounds like alkaloids, glycosides, saponins, terpenoids, lactones, phenols and flavonoids (Meena *et al*., 2009).The plant is used widely in traditional medicine to treat a variety of conditions such as pain, cough and asthma heal (Verma *et al.,* 2011).

The aim of this study was to evaluate the anti-inflammatory effect of *C. decidua* stem extract using various experimental animal models.

Material and methods:

Plant material:

Capparis deciduas stems were collected from Arkaweit area in Khartoum state, Sudan. The plant material was identified by the Botanist in Medicinal and Aromatic Plant and Traditional Medicine Research Institute (MAPTMRI) National Center for Research (NCR), Khartoum, Sudan. The stems were then dried at room temperature and ground into powder.

Extraction of the plant

A known weight of *C. decidua* stems powder (1000g) were extracted by Soxhlet apparatus using methanol. The solvent was then collected and evaporated under reduced pressure using rotary evaporator apparatus (Harbone, 1984).

Experimental animals:

Wistar albino rats (100-150 g) of both sexes were used. The rats were purchased from Medicinal and Aromatic Plant and Traditional Medicine Research Institute (MAPTMRI) National Center for Research (NCR). They were kept in cages in the Laboratory Animals House, in the College of Veterinary Medicine, Sudan University of Science and Technology. The rats were maintained under standard environmental condition and provided with standard diet and water *ad labitum*. Rats were acclimatized for at least 7 day to the laboratory condition before experimentation.

Carrageenan induced paw oedema (for acute inflammation):

Anti-inflammatory effect of *C. decidua* methanolic extract was evaluated in albino rats of either sex (100 – 150 g) according to the method (Ramprasath *et al*., 2004).

Experimental design:

Twenty five albino rats were divided randomly into 5 groups of 5rats each.

Group 1: control; animals were administered distilled water only (10 ml/kg) used as vehicle.

Group 2: standard anti-inflammatory drug, rats were given orally diclofenac sodium at the dose of10 mg/kg.

Group 3: rats were treated orally with 100mg/kg of the *C. decidua* stems methanolic extract.

Group 4: animals were administered orally with 200 mg/kg of the *C. decidua* stems methanolic extract

Group 5: rats were given orally 400 mg/kg of the *C. decidua* stems extract.

Oedema was induced by injection of carrageenan (0.1 ml, 1% w/v in saline) into the sub planter tissue of the right hind paw after one hour of the administration of all treatments. The paw volume, up to the tibiotarsal articulation, was measured using a digital verniercalliper. The measure was determined at 0 h (before carrageenan injection) and 1, 2, 3 and 4 hours after carrageenan injection the % paw volume inhibition was evaluated using the following formula:

$$\% \text{ inhibition} = (Vf - VO) \text{ control} - (Vf - VO) \text{ treated} / (Vf - VO) \text{ control} \times 100$$

Where VO = paw volume before administration of carrageenan (i.e. Initial paw volume) and Vf = is the paw volume after administration of carrageenan

Cotton pellet granuloma (for chronic inflammation)

Twenty rats of either sex (100-150 g) were used. The animals were anaesthetized with Ketamine (10 mg/kg, i.p). The subcutaneous implantation of sterile cotton pellets (20 mg) was performed in lumbar region to induce chronic inflammation.

Experimental design

The animals were randomly divided into 4 groups of 5 rats each as follows.

Group 1: control, rats were given distilled water 10 ml/kg.

Group 2: standard drug, animals were administered orally diclofenac sodium at a dose of 10 mg/kg.

Group 3: animals were administered orally with 200 mg/kg of the *C. decidua* stems methanolic extract.

Group4: rats were given orally 400 mg/kg of the *C. decidua* stems methanolic extract.

The *C. decidua* stems methanolic extract and standard drug (diclofenac sodium 10 mg/kg) were administered orally for 7 consecutive days from the day of cotton pellet implantation. On the 7th day, animals were sacrificed by an over dose of chloroform anesthesia. The cotton pellets were removed surgically, dried at 60 °C for 24 hours until a constant weight were obtained and weighed. The increment in dry weight of pellets over 20 mg were taken as an index of granuloma formation.

Statistical analysis:

Data were expressed as the mean ± SEM. Differences between experimental groups were compared by one way analysis of variance (ANOVA). The results will be considered statistically significant when $P < 0.05$ (Gomez and Gomez, 1984).

Result:

Anti-inflammatory activity of *C. decidua* stems **methanolic extract on carrageenan induced paw edema:**

Control rats that received carrageenan at a dose of 0.1ml (1.0 w/v) showed significant increase in paw edema ($p<0.05$) compared with other groups. Diclofinac sodium used as standard drug was significantly decreasing the paw oedema. The inhibition percent was found 82% at 4th hour.

There were a significant ($p<0.05$) inhibition in paw oedema in rats treated with 200 and 400 mg/kg of *C. decidua* stems methanolic extract compared to control rats, especially at 4th hour. The inhibition rates were 44.5 and 65.3 respectively. The high dose was found to be the best in decreasing paw odema size.

Rat that received 100mg/kg of *C. decidua* stems showed significant decrease in paw oedema compared with control rats. However this group presented low anti-inflammatory activity compared to other test groups (Table 1).

Table (1):Anti-inflammatory activity of *C. decidua* stems methanolic extract on carrageenan induced paw oedema

Treatments	Increase in paw volume (mm) mean ±SE				Inhibition %			
	H1	H2	H3	H4	H1	H2	H3	H4
Control	1.57±0.03^{a}	1.64±0.03^{a}	1.56±0.03^{a}	1.50±0.03^{a}	-	-	-	-
DS 10 mg/kg	1.64±0.03^{a}	1.31±0.07^{c}	0.700±.03^{d}	0.27 ±0.09^{d}	6.78	19.96	55.14	82.00
CD 100 mg/kg	1.40±0.03^{b}	1.39±0.04^{b}	1.24±0.06^{b}	0.978±1.1^{c}	10.46	14.89	20.22	34.46
CD 200 mg/kg	1.44±0.03^{b}	1.45±0.05^{b}	1.09±0.03^{c}	1.44±0.03^{b}	8.17	11.61	29.43	44.52
CD 400 mg/kg	1.30±0.05^{c}	1.25±0.06^{c}	0.978±0.07^{d}	0.523±0.63^{c}	17.00	23.66	48.90	65.32

CD: *Capparis decidua*, DS diclofenac sodium; Values are expressed as mean ±SEM, means within the same column with different superscripts are significantly different at $p>0.05$ (n=5)

Anti-inflammatory activity of *C. decidua* stems on cotton pellet granuloma in rats

Diclofenac sodium used as a standard drug exhibited significant ($p<0.05$) inhibition of inflammatory oedema (44.1%) and granulomatous tissues induced by insertion of cotton pellets subcutaneously (44.4%)

Administration of *C. decidua* stems methanolic extract for 7days significantly masked the production of oedema and granulomatous tissue. The results were comparable to that produced by standard drag diclofinac sodium.

High dose (400mg/kg) of *C. decidua* stems methanolic extract exhibited potent activity compared to low dose (200mg/kg) in inhibition of granulomatous tissues (Table 2). The inhibition of eodema formation was found to be more effectively compared to inhibition of granulomatous tissues.

Table (2) Anti-inflammatory activity of *C. decidua* stems on cotton pellet granuloma in rats

		Increase in paw volume (mm)		
Treatments	Wet Wt.	Inhibition %	Dry Wt.	Inhibition %
Control	197.7±4.67^{a}		46.54±2.24^{a}	
DS 10 mg/kg	117..0±7.23^{b}	44.1	33.9±1.18^{b}	44.4
CD 200 mg/kg	111.1±18.97^{b}	34.3	39.5±3.60^{b}	28.7
CD 400 mg/kg	130.7±6.23^{b}	39.4	39.0±1.50^{b}	34.8

CD: *Capparis decidua*, DS diclofenac sodium; Values are expressed as mean ±SEM, means within the same column with different superscripts are significantly different at $p>0.05$ (n=5)

Discussion:

Capparis decidua is used in folkloric medicine in the treatment of various disorders including inflammation (Chishty and Bissu, 2014). In the present study; anti-inflammatory activity of methanol extract of *C. decidua* stems was investigated in acute and chronic models in rats.
Carrageenan induced paw edema as *in-vivo* model of inflammation was selected to assess the acute anti- inflammatory activity. This model has been extensively used to evaluate new anti-inflammatory plant extract (Sarika, 2012). Administration of Carrageenan in paw of rat induced acute inflammation characterized by swelling, redness, hotness, and pain. This is in agreement with the result of Almeida *et al.,* (2001). The initial phase of inflammation seen at the 1st hour in attributed to release of histamine, prostaglandin and serotonin (Saha and Ahmed, 2009).
Oral administration of *C. decidua* stems methanolic extract significantly reduced the size of paw oedema especially at 3rd and 4th hours compared to control group. The inhibition percent of *C. decidua* was (65.3%) at dose 400mg/kg at last hours and this result was comparable to Diclofinac sodium at 4th hours which produced 82% of inhibition. This confirms the model of Whiteley and Dalrymple (2001). This model has long been used to assess the anti-inflammatory properties of agents such as nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit prostaglandin production. This protocol is a method to elicit and measure carrageenan-induced foot pad edema. The carrageenin test was selected because of its sensitivity in detecting orally active antiinflammatory agent particularly in acute phase of inflammation (Di Rosa *et al.,* 1971).
The inflammatory granuloma is a typical feature of subacute inflammatory reaction (Spector, 1969) .The cotton pellet granuloma method has been widely used to assess the transudative, exudative and a proliferative phase of subacute inflammation. Most of the NSAIDs like diclofenac possess only slight inhibition on the granuloma formation. The steroidal drug on the contrary, exhibits profound reduction of the granuloma.
The cotton pellet granuloma method is widely used to evaluate the transudation and proliferation component in chronic inflammation (Paschapur *et al.,* 2009). In this study, subcutaneous implantation of cotton pellet in rat induced the formation of granular tissue. This is in agreement of the results of Sireeratawong, 2012; Afsar, 2013; Al-Snafi; Kumar *et al*., 2016; Patil and Patil, 2017: Antonisamy, 2019; Chandran, 2020).

The amount of granuloma formation was measured by weighing the dried pellet. Administration of ***C****. decidua* stem extract significantly ($p<0.001$) suppressed the formation of granular tissue, the inhibition was found to be similar in rat treated with400 mg/kg and the rats that treated with diclofenac sodium used as a standard drug. Oedema was also estimated by weighing wet pellets before drying of pellets.
The development of granuloma in rodents by cotton pellet represents a chronic inflammation model extensively used to assess the transudative and proliferative components of the inflammation. The weight of the cotton pellets corroborates with the amount of the granulomatous tissue (Guo *et al*., 2011). Based on this study, administration of the test compound was effective in lowering the weight of the cotton pellet. These data have suggested the anti-inflammatory effect of the test compound.
In conclusion results indicated that the methanolic extract of *C. decidua* stems possess significant anti-inflammatory activities in acute and chronic inflammatory models as a result of significant reduction of the size of paw oedema and granuloma formation also restricted. Further studies should be done to explain the exact phytoconstitutent (s) responsible for anti-inflammatory effect.

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